An International Journal of The American Phytopathological Society

Phytopathology

VOLUME 102, NUMBER 7 (SUPPLEMENT) JULY 2012

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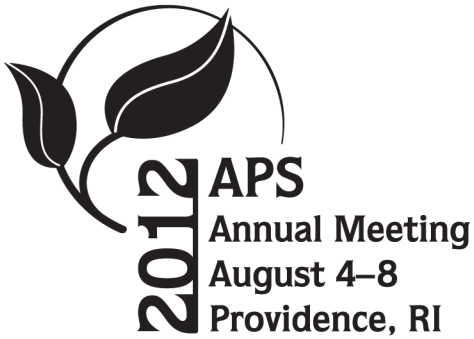


2012 APS Annual Meeting Abstracts of Presentations

Abstracts submitted for presentation at the APS Annual Meeting in Providence, Rhode Island, August 4–8, 2012 (including abstracts submitted for presentation at the 2012 APS Northeastern Division Meeting). The abstracts are arranged alphabetically by the first author's name. Recommended format for citing annual meeting abstracts, using the first abstract below as an example, is as follows: Abbas, H. D., Shier, W., Weaver, M. A., and Horn, B. W. 2012. Detection of aflatoxigenic *Aspergillus flavus* contamination of coconut (*Cocos nucifera*) nutmeat (copra) using ammonia treatment. (Abstr.) Phytopathology 102(Suppl. 4):S4.1. http://dx.doi.org/10.1094/PHYTO-102-7-S4.1

Detection of aflatoxigenic *Aspergillus flavus* contamination of coconut (*Cocos nucifera*) nutmeat (copra) using ammonia treatment
H. D. ABBAS (1), W. Shier (2), M. A. Weaver (1), B. W. Horn (3)
(1) USDA-ARS BCPRU, Stoneville, MS, U.S.A.; (2) University of Minnesota, Minneapolis, MN, U.S.A.; (3) USDA-ARS, National Peanut Research Laboratory, Dawson, GA, U.S.A.
Phytopathology 102:S4.1

For many crops government regulations define mycotoxin contamination levels that represent the primary determinants of quality, value and possible uses of crops. Quality can be raised in some crops by lowering the mycotoxin level through removal infected products. In the case of copra, the dried nutmeat of the coconut, hand sorting to remove *Aspergillus flavus*-contaminated copra is an effective remediation strategy. However, typically only about one third of *A. flavus* contaminants in plants are aflatoxigenic, so it would be useful to have a method to visually distinguish aflatoxigenic from non-aflatoxigenic *A. flavus* contamination of copra. We have applied to copra the cultural method for identifying aflatoxigenic *A. flavus* in which ammonia exposure is used to raise the pH, changing the color of anthraquinone pigments associated with the aflatoxin biosynthetic pathway from yellow to a much more visible red. Aflatoxigenic *A. flavus* was readily differentiated from non-aflatoxigenic *A. flavus* on copra by the appearance of red color after ammonia exposure, particularly along break lines. These studies suggest ammonia exposure would be a useful addition to aflatoxin remediation practices in copra and possibly other crops.

Long-term crop rotations suppress soybean sudden death syndrome in Iowa
N. ABDELSAMAD (1), G. C. Mbofung (1), A. E. Robertson (1), M. Liebman (1), L. F. Leandro (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.1

Sudden death syndrome (SDS) of soybean caused by *Fusarium virguliforme* is one of the most damaging diseases of soybean in the US. Management of SDS relies on selection of resistant varieties, cultural practices to improve soil drainage, and avoiding planting in cool, wet soils. The effectiveness of crop rotation for SDS management is not well understood. In this study, the effect of long-term crop rotations in reducing SDS was studied in 2010 and 2011, in a field trial established in Iowa since 2002. The trial included three rotation treatments: corn-soybean, corn-soybean-oat/red-clover, and corn-soybean-oat/alfalfa-alfalfa. SDS incidence and severity, root rot and root growth, yield, and *F. virguliforme* and soybean cyst nematode (SCN) populations in soil were assessed. In both years, the 2-year rotation showed greater (P<0.001) SDS incidence and severity, and lower yield, compared to the 3- and 4-year rotations. Roots from the 2-year rotation showed more severe rot and reduced growth (P<0.05) compared to the longer rotations. SCN populations did not differ among rotations. Quantification of *F. virguliforme* in soil using real-time PCR suggested a greater pathogen population in the 2-year rotation compared to the 3-year rotation, but the mechanisms behind the reduction in SDS need further clarification. These findings suggest that long-term crop rotations may offer an alternative management practice for reducing risk of SDS.

Identification of quantitative trait loci for partial resistance to *Phytophthora sojae* in soybean
N. ABEYSEKARA (1), R. Matthiesen (1), S. Cianzio (1), M. Bhattacharrya (1), A. Robertson (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.1

Stem and root rot caused by the oomycete pathogen *Phytophthora sojae*, is an economically important disease of soybean across the world. The disease is managed primarily by planting cultivars with single-gene mediated resistance. However, this type of resistance is becoming ineffective due to the emergence of new pathogen races. Partial resistance (PR) or field tolerance to *P. sojae*, which is polygenic in nature, has also been reported in soybean. It provides broad-spectrum, low level of root resistance against all physiological races of the pathogen. Therefore, incorporation of PR into soybean cultivars would provide a more durable form of disease management. The main objectives of this study were to identify molecular markers linked to quantitative trait loci (QTL) for PR to *P. sojae* (PRPS), and to validate the rice method as a more objective screening method for PR. Two recombinant inbred line populations were developed by crossing the plant introduction, PI399036, with two germplasm lines, AR2 and AR3. PI399036 carries high level of PRPS. AR2 and AR3 show low PRPS but carry genes for resistance to iron deficiency chlorosis. Both populations were advanced to the F7 generation and screened for PRPS using the rice method. Roots of both the *P. sojae*-infected and uninfected plants were evaluated 30 days after planting using a WinRhizo root scanner. Dry root and shoot weights were also obtained to quantify the levels of PR in the populations. Data analysis is on progress and the mapping data will be presented.

http://dx.doi.org/10.1094/PHYTO-102-7-S4.1

A novel *Marafivirus* from *Ranunculus repens*

N. Abou Ghanem-Sabanadzovic (1), A. Lawrence (2), S. SABANADZOVIC (3)
(1) Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Mississippi State, MS, U.S.A.; (2) Institute for Imaging and Analytical Technologies, Mississippi State University, Mississippi State, MS, U.S.A.; (3) Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 102:S4.2

Creeping buttercup plant (*Ranunculus repens*), displaying mosaic symptoms, collected during a survey on plant viruses present in the Great Smoky Mountain National Park was submitted to lab analyses in order to characterize the putative agent. Electron microscope observations of partially purified preparations indicated the presence of putative empty and intact isometric particles. Nucleic acid extracts from this sample reacted positively in RT-PCR when tested with degenerate primers for viruses belonging to the family *Tymoviridae*. Initial BLAST analyses indicated the presence of a novel virus, which prompted further characterization. Polyadenylated viral genome, of 6.6 kb in size, contains a single open reading frame potentially coding for a 237 K polyprotein with signature motifs of the replication polyprotein and viral coat proteins. In phylogenetic analyses, performed on viral RdRp and CP, the virus grouped with marafiviruses. In-depth comparisons of overall polyprotein sequences and several domains showed that this virus, for which we propose the name *Ranunculus mosaic virus*, is a new member of the genus *Marafivirus* as it shares limited identies with known marafiviruses.

Plants vs. pathogens: *VEGEVADERS*—A game of infiltration and detection

B. E. Adams (1), J. P. LaFountain (1), C. W. Collmer (2), M. Lindeberg (3), A. COLLMER (3)
(1) Wells College, Aurora, NY, U.S.A.; (2) Wells College, Ithaca, NY, U.S.A.; (3) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.2

The VEGEVADERS game simulates the battle between biotrophic pathogens and the two-layered immune system of plants, which is comprised of microbe-associated molecular pattern (MAMP)-triggered immunity (MTI) and effector-triggered immunity (ETI). Players in groups of up to six (three pathogens versus three plants) play with cards denoting “features” (pathogen MAMPs), “detectors” (plant pattern recognition receptors), “disablers” (pathogen effectors), and “alarms” (plant R proteins) to experience co-evolutionary dynamics and costs, benefits, and vulnerabilities associated with MTI, ETI, and different disease management strategies. “Force of nature” wild cards introduce lessons about the impact of weather, cultural practices, advanced plant breeding technologies, and other external factors. Variants of the game are designed to work at multiple educational levels, from high school biology to graduate plant pathology. The game is being developed with support from NSF Plant Genome Research Program grant IOS-102564 and is being deployed through the Cornell Institute for Biology Teachers and the Pseudomonas-Plant Interaction website High School Connect web links (http://www.pseudomonas-syringae.org/Outreach/HSC-newhome.htm). Computer, mobile device, and web-based versions are also in development. The design of the game and experiences from play testing in various educational settings will be presented.

Reproducing bacterial blast of sweet cherry in the field and identifying new treatments for managing the disease

J. Adaskaveg (1), H. FORSTER (1), L. Wade (2)
(1) University of California, Riverside, CA, U.S.A.; (2) Arysta LifeScience, Roseville, CA, U.S.A.
Phytopathology 102:S4.2

Bacterial blossom blast of cherry and other stone fruits is one phase of bacterial canker caused by *Pseudomonas syringae* pv. *syringae* or to a lesser extent by *P. syringae* pv. *morsprunorum*. Blast is associated with wetness and cold temperatures during bloom and results in flower clusters that collapse, turn brown to black are papery when dry, and eventually drop. Some scion-rootstock combinations (e.g., cv. Coral Champagne cherry on Mahaleb rootstock) are more susceptible than others. In addition to cultural practices, only copper products are currently widely available for the management for blossom blast. Copper, however, is not highly effective, is potentially damaging to blossoms and new growth, and resistance to copper occurs in many pathogen populations. For the evaluation of new bactericidal treatments that could be used in combination with fungicides during bloom, we developed a partial emasculation-inoculation method that proved highly effective for reproducing the disease in the field. New bactericidal treatments were also evaluated in natural incidence studies. Commercial formulations of kasugamycin, the biocontrol *Streptomyces lydicus*, and oxytetracycline proved highly effective; whereas copper had little or no effect. The registrants of kasugamycin and the biocontrol support registration of their products for the management of bacterial blast and these will be the first treatments ever registered in the United States for effective management of this disease.

Occurance of resistance to respiratory inhibitiors in *Corynespora cassiicola* isolates from Florida tomatoes

H. M. ADKISON (1), E. Margenthaler (1), V. Burlacu (1), R. Willis (1), G. E. Vallad (1)
(1) University of Florida Gulf Coast Research and Education Center, Wimauma, FL, U.S.A.
Phytopathology 102:S4.2

Target spot, caused by *Corynespora cassiicola*, is an important fungal disease of tomato in Florida. There are few published reports about pathogen diversity and fungicide resistance for *C. cassiicola*. Observations at grower sites and in field trials have raised concerns about the efficacy of certain fungicides for target spot management. Using an agar plug-based assay, 11 field isolates were tested for sensitivity to azoxystrobin, boscalid, fluopyram, and penthiopyrad. All isolates exhibited high levels of tolerance to azoxystrobin at 50 µg/ml, and two isolates were tolerant to boscalid at 50 µl/ml. To confirm our findings, tomato seedlings were treated with 0.5, 1.0, and 1.5 times the maximum labeled field rates of azoxystrobin, boscalid, chlorothalonil, fluopyram, mancozeb, and penthiopyrad, and inoculated with two boscalid-sensitive and -insensitive isolates in growth room trials. All four isolates showed an increase in disease severity greater than the control with treatment of azoxystrobin, regardless of the rate; whereas mancozeb performed better than chlorothalonil. All SDHI fungicides gave fairly consistent suppression of the two boscalid-sensitive isolates, while boscalid and penthiopyrad were ineffective against the two boscalid-insensitive isolates. Testing of additional isolates will continue to determine the frequency of fungicide resistance and to adjust fungicide recommendations for growers.

Biomass reduction potentials of a new leaf blight of *Miscanthus* x *giganteus* caused by *Pithomyces chartarum* and screening for effective fungicide control

M. O. AHONSI (1), K. A. Ames (1), M. E. Gray (1), C. A. Bradley (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.2

During a multistate survey of diseases of cellulosic ethanol grasses in the USA in 2009, a new leaf blight of *Miscanthus* x *giganteus* caused by *Pithomyces chartarum* was observed on 100% of the plants evaluated in research plots near Lexington, Kentucky. A greenhouse study was conducted to evaluate the effect of *P. chartarum* on *M.* x *giganteus* biomass, and to identify effective foliar fungicides against the disease. Eleven broad-spectrum fungicides were tested with rates typically used in agronomic field crops. Fungicides tested included active ingredients from four different chemical families, demethylation inhibitors (prothioconazole, tebuconazole, cyproconazole, propiconazole, tetraconazole, flutriafol, and metconazole), quinone outside inhibitors (pyraclostrobin), succinate dehydrogenase inhibitors (boscalid), methyl benzimidazole carbamates (thiophanate-methyl), and chloronitriles (chlorothalonil). The two controls were a no-fungicide – inoculated, and a no-fungicide – non-inoculated. The experiment was replicated eight times, repeated once over time, and data were pooled for analysis. Young plants were spray-inoculated with a *P. chartarum* spore suspension 24 h after fungicides were sprayed, incubated in growth chambers for seven days, and thereafter left in the greenhouse for 11 weeks. Infection from *P. chartarum* significantly lowered biomass accumulation. Application of cyproconazole, flutriafol, tebuconazole, or prothioconazole significantly reduced disease severity (up to 40% with cyproconazole or flutriafol). A significant negative correlation between disease severity and biomass was observed, but no fungicide treatment significantly mitigated biomass loss. More than one application or a higher fungicide dosage may be needed for adequate disease control that could result in significant biomass loss abatement.

Genetic diversity of *Apple stem grooving virus* and *Apple stem pitting virus* in North America

S. A. AKINBADE (1), D. V. Villamor (1), K. C. Eastwell (1)
(1) Washington State University, IAREC, Prosser, WA, U.S.A.
Phytopathology 102:S4.2

Apple stem grooving virus (ASGV) and *Apple stem pitting virus* (ASPV) occur in many apple orchards worldwide, and cause considerable yield loss. The genetic diversity of these viruses in North America were examined by comparing the coat protein (CP) sequences amplified from total RNA extracted from bark samples of apple trees. Restriction fragment length polymorphisms and sequences of cloned amplicons revealed a high degree of variability of ASPV but not of ASGV. Nucleotide identities of ASGV isolates from this study ranged from 91 to 100%, while at the amino acid level,

identities ranged from 98 to 100%. These sequences and those from Genbank formed a single phylogenic population. ASPV nucleotide identities ranged from 62 to 100% while predicted amino acids sequence identities ranged from 68 to 100%. ASPV sequences segregated into six distinct clades. We report for the first time the occurrence of several distinct North American isolates of ASPV that cluster into three of these groups. Our data clearly demonstrate that commercial apple trees in North America can be infected by a diverse population of ASPV isolates. Understanding the structure and genetic diversity of ASGV and ASPV populations in this geographic region facilitates detection and management of these agents.

Phenotypic reactions of 1050 barley accessions to a new spot blotch pathotype of *Cochliobolus sativus*

S. ALI (1), R. Wang (1), S. Zhong (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.3

Cochliobolus sativus is an important fungal pathogen, which causes spot blotch and common root rot in cereal crops in the northern Great Plains of the US. Previously, three pathotypes (0, 1, and 2) have been identified in the fungal isolates based on their differential virulence pattern on three barley genotypes (ND5883, ND B112, and Bowman). Barley genotypes with spot blotch resistance to these three pathotypes have also been identified and characterized. However, the recent emergence of a new pathotype represented by the isolate ND4008 makes currently available resistance sources ineffective, including ND B112, the most durable spot blotch resistance source in barley breeding programs. To identify effective resistance to this new pathotype, we screened 1050 barley accessions selected by the National Small Grains Collections from diverse genetic backgrounds. The seedlings were inoculated with isolate ND4008 at the second leaf stage and disease severity was rated 8 to 9 days after inoculation using a 1 to 9 rating scale, where 1 to 3 is considered as resistant; 4 to5 as intermediate; and 6 to 9 as susceptible. Of the 1050 accessions evaluated, only five exhibited resistant reaction to ND4008; 249 accessions showed intermediate reaction; and the rest of accessions were susceptible. The five resistant accessions identified will be utilized for developing spot blotch resistant cultivars in barley breeding programs.

Infection responses of diverse *Brachypodium distachyon* accessions to the cereal spot blotch pathogen *Cochliobolus sativus*

S. ALI (1), S. Zhong (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.3

Brachypodium distachyon (*Bd*) has emerged as a model system for studying temperate cereal species due to its small genome size, short growth cycle and close relationships to barley, wheat and many temperate grass species. To determine the host status of *Bd* to the spot blotch pathogen *Cochliobolus sativus* of barley and wheat, we inoculated 199 *Bd* accessions of diverse genetic background with a *C. sativus* isolate 07-47-1 recovered from wheat under greenhouse conditions. The *Bd* genotypes were grown in containers and inoculated at the heading stage. Disease reactions were rated 8 days post-inoculation using a 1 to 5 scale where 1 was the most resistant reaction and 5 was the most susceptible reaction. Of the 199 *Bd* accessions evaluated, 103 were grouped as resistant as they had a 1 to 2 rating score; 66 accessions were considered as intermediate with a rating score of 3. Only 30 accessions were considered susceptible as they had a rating score of 4 to 5. Our study demonstrated that *Bd* is a natural host of *C. sativus* and can serve as a model system for studying the *C. sativus*-cereals pathosystem. The results also indicated that *Bd* accessions varied in their reaction to spot blotch ranging from being very resistant to very susceptible. The resistant and susceptible accessions identified in this study will be utilized in unlocking the genetic mechanism of spot blotch resistance in cereals.

First detection of species *Stenotrophomonas maltipholia*, *S. rhizophila*, and *Alcaligenes faecalis* associated with citrus blast in Iran

M. ALIMI (1), M. Taghinasab Darzi (2)
(1) Department of Plant Pathology, Faculty of Agriculture, Gorgan Branch, Islamic Azad University, Gorgan, Iran; (2) Department of Plant Pathology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
Phytopathology 102:S4.3

The most important bacterial disease on citrus is bacteria blast in North of Iran that causes high level of loss. It is widespread in all citrus gardens in Northern provinces that are located in border of Caspian Sea. Therefore, suspected samples to bacterial blast of citrus were collected from different regions of Mazandaran and Golestan provinces. 185 isolates were gotten after segregation and purification. At first stage, ice nucleation activity of isolates was surveyed and then biochemical, physiological and morphological tests were done on ice nucleation positive isolates. In result, two species were diagnosed as agent of citrus blast, *Pseudomonas viridiflava* (*Pv*) and *P. syringae* pv. *syringae* (*Pss*). Some ice nucleation positive isolates were not identified by biochemical analysis; therefore PCR amplification was carried out on isolates by universal primers and PCR products were sequenced. Alignment of PCR products in comparison of sequences of Gene bank (NCBI) resulted in identification of three bacterial species. First, *Alcaligenes faecalis* that there was no report on isolation of this species from plants and it has been reported in this research for the first time. Next two ice nucleation positive species are *Stenotrophomonas maltipholia* and *S. rhizophila*. *S. rhizophila* has been reported only from water, soil and rhizosphere of plant. In this study it has been isolated from plant surface for the first time and *S. maltipholia* is reported for the first time from Iran.

Yield and net return on investment following an automatic fungicide application to field corn in Arkansas, Louisiana, and Mississippi

T. W. ALLEN (1), B. R. Golden (1), C. A. Hollier (2), G. Padgett (3), J. Kelley (4), D. Ingram (5), C. Coker (6), A. Henn (7), E. Larson (7), N. Buehring (8)
(1) Mississippi State University, Stoneville, MS, U.S.A.; (2) Louisiana State University AgCenter, Baton Rouge, LA, U.S.A.; (3) Louisiana State University AgCenter, Winnsboro, LA, U.S.A.; (4) University of Arkansas, Little Rock, AR, U.S.A.; (5) Mississippi State University, Raymond, MS, U.S.A.; (6) University of Arkansas, Monticello, AR, U.S.A.; (7) Mississippi State University, Starkville, MS, U.S.A.; (8) Mississippi State University, Verona, MS, U.S.A.
Phytopathology 102:S4.3

Since 2004 the use of strobilurin-based fungicides has dramatically increased in field corn production. Typically the fungicide application is timed for the tassel stage (VT) and made regardless of the presence of measureable foliar disease. Between 2005 and 2011, more than 80 locations in AR, LA, and MS received a timed fungicide application to assess the benefit of the application stragety for corn farmers in the Mid-south. In general, the majority of field trials were conducted at off-station locations. Plot size varied but generally ranged from small-plot trials conducted at experiment stations and off-station locations consisting of 4-row plots as well as large plot trials consisting of 8-row plots and larger. Application strategies differed by location but in general fungicides were applied by either air (< 30% of locations) or ground. While strobilurin-based fungicides were the crux of the trials, additional triazole-based programs were included. In all, 18 different fungicides were applied at several different rates and various growth stage timings (n=7) ranging from pre-tassel (V15) to the kernel dough stage (R4). In general, as a whole, fungicide application did not increase yield or net returns. In addition, disease pressure was considered to be low at greater than 85% of the trial locations. Even though yield was the important response variable, correlations between additional plot variables, fungicide, and fungicide rate were considered.

Genetic diversity among endogenous plant pararetroviral sequences from geographically diverse sources of dahlia (*Dahlia* spp.)

C. V. ALMEYDA (1), K. L. Druffel (1), S. G. Eid (2), H. R. Pappu (1)
(1) Washington State University, Pullman, WA, U.S.A.; (2) University of Idaho, Moscow, ID, U.S.A.
Phytopathology 102:S4.3

An endogenous plant pararetroviral sequence (DvEPRS), previously referred to as DMV-D10, was originally isolated in the US from the cultivated *Dahlia variabilis*, and has been found in New Zealand, Lithuania and Egypt as well as in wild dahlia species growing in their natural habitats in Mexico. Here we report the complete genome sequences of three new DvEPRS isolates from a Lithuanian cultivar (7159 nt), a New Zealander cultivar (7156 nt) and from the wild dahlia species *D. rupicola* (7133 nt). The three have the structure and organization typical of a caulimovirus species and showed identities between 71 and 97% at the nucleotide level (nt) among various open reading frames (ORFs) when compared to those of DvEPRS. A total of 7 full-length DvEPRS from cultivated and wild dahlia species were used for phylogenetic analyses, mutation frequencies, potential recombination events, selection and fitness as evolutionary evidences for genetic diversity. Phylogentic analyses showed one clade of all DvEPRS indicating a lack of clustering by geographical origin. When DvEPRS were grouped into two taxa, no difference was observed between those from cultivated and wild dahlia species. Strong negative selection for all ORFs was found, with the replicase region more variable than other ORFs. Identification of potential recombination events involving parents from different lineages provided strong evolutionary evidence for genetic diversity among various DvEPRS.

Genetic diversity of *Cercospora seminalis* causing false smut disease of buffalograss

B. S. AMARADASA (1), K. Amundsen (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, U.S.A.
Phytopathology 102:S4.4

Cercospora seminalis is an imperfect fungus causing disease in buffalograss caryopses. The disease is called false smut since the diseased caryopses resemble smut. The disease is spread by asexual spores and contaminated plant debris causing considerable loss of yield and reduced germination of buffalograss seed. No genetic diversity studies have been reported on this pathogen. The objective of this study was to obtain information on genetic diversity of fungal isolates collected from diseased buffalograss burs. A collection of 84 *C. seminalis* isolates were made from six sites in Nebraska. DNA fingerprinting analysis was performed by amplified fragment length polymorphism (AFLP) using four selective primer combinations. In total, 482 DNA markers were detected across all isolates. Out of that 478 markers were polymorphic while 4 were monomorphic. The unweighted pair-grouped method with arithmetic average (UPGMA) clustering resulted in six lineages (I to VI). AMOVA test supported the significance of these lineages at P = 0.05. However 14 isolates did not fall into any cluster indicating high heterogeneity of the fungus. Most of the isolates (62%) belonged to lineage I (36 isolates) and II (16 isolates). The geographic origin of the isolates and resulted clusters had no relationship. These results show high genetic diversity of *C. seminalis* isolates that are not site-specific. Analysis of more isolates from different geographic areas is needed to establish the population structure of the pathogen.

Characterization of *Sclerotinia sclerotiorum* sensitivity to metconazole in North Central United States

G. AMEEN (1), L. del Rio-Mendoza (1), B. D. Nelson (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.4

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic plant pathogenic fungus that affects canola, dry bean, soybean, sunflower, and many other crops of economic importance to states in North Central US. Farmers in the region depend largely on fungicide applications to control this pathogen. Fungicides registered for use against *S. sclerotiorum* include pyraclostrobin, thiophanate methyl, boscalid, and metconazole. Of these, the most recently introduced fungicide is metconazole. To establish baseline sensitivity to metconazole, 89 *S. sclerotiorum* isolates were collected from 13 states in North Central US. EC_{50} values were calculated by growing these isolates in potato dextrose medium amended with technical grade metconazole at different concentrations. Metconazole sensitivity ranged from 0.05 to 1.64 µg/ml. Isolates with EC_{50} > 1 µg/ml are considered resistant to DMI fungicides. Fitness of metconazole-resistant isolates will be determined in greenhouse trials.

Is the super-resistant fungus among us? Genesis and consequences of recent epidemic of *Botrytis cinerea* in strawberry fields in Florida

A. AMIRI (1), S. M. Heath (1), N. N. Peres (1)
(1) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.4

Strawberry growers in Central Florida experienced a severe epidemic of *Botrytis cinerea*, the causal agent of Botrytis fruit rot (BFR), in the last 10 days of February 2012. Economic consequences are being estimated but immediate investigations should be undertaken to understand the reasons behind such a sudden epidemic. Herein, we address the role of two major possible factors, the effect of weather conditions on the disease development and the prevalence of resistance to multiple fungicides. The disease cycle of *B. cinerea* can be completed in 15 to 20 days if favorable conditions persist for only a few hours (14) in the field. The Strawberry Advisory System (SAS) for monitoring the risk for *B. cinerea* and *Colletotrichum acutatum* indicated that between Feb 6 and 7 and Feb 17 and 18, leaf wetness duration and temperatures were highly conducive for BFR. These dates corresponded to peak bloom of early cultivars and beginning of bloom of mid-season and late cultivars, respectively. Severe symptoms of BFR were observed within the following 15 days. Furthermore, based on a recent fungicide resistance monitoring throughout Florida, a large number of *B. cinerea* isolates investigated were resistant to at least four of the most common fungicides used for BFR control. The widespread of *B. cinerea* populations with multiple fungicide-resistance has worsened the situation especially where appropriate sprays have not been applied at bloom. The implementation of programs warranting continuous monitoring of risks linked to disease and fungicide resistance development will be key components of future BFR management in strawberries.

Sensitivity of *Botrytis cinerea* field isolates to the novel succinate dehydrogenase inhibitors fluopyram, penthiopyrad, and fluxapyroxad

A. AMIRI (1), S. M. Heath (1), N. A. Peres (1)
(1) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.4

A recent monitoring of fungicide resistance in Florida revealed widespread resistance of *Botrytis cinerea*, the causal agent of Botrytis fruit rot (BFR) in strawberry, to almost all fungicides used for BFR control. If new alternatives are not implemented, growers may experience severe economic impacts. Fluopyram, penthiopyrad, and fluxapyroxad are three novel fungicides from the succinate dehydrogenase inhibitor (SdhI) group that are expected to be registered for BFR management. In this study, we determined the baseline sensitivity of 100 *B. cinerea* isolates to the three aforementioned fungicides and to the mixture of fluxapyroxad + pyraclostrobin (Merivon). EC_{50} values based upon conidial germination ranged from 0.08 to 2.8, 0.67 to 9.03, <0.05 to 10.7 and 0.09 to 12.9 µg/ml, for fluopyram, penthiopyrad, fluxapyroxad and Merivon, respectively. Respective EC_{50} values based on mycelial growth were 0.07 to 7.2, 0.09 to 6.8, 0.07 to 7.1 and 0.15 to 7.9 µg/ml. A positive cross-sensitivity was observed between the SdhI boscalid, to which resistance has been widely reported, and penthiopyrad (R^2 = 0.556) or fluxapyroxad (R^2 = 0.463) but not fluopyram (R^2 = 0.05). The three novel SdhIs sprayed preventively on strawberry fruits controlled all isolates regardless of their EC_{50} values including those characterized previously as highly resistant to boscalid. Our findings show great potential for BFR control with the new SdhIs. However, as with all single-site fungicides, wise spray programs should be recommended when using these new molecules to avoid a rapid buildup of resistant populations.

New insights into mechanisms of resistance to respiration inhibitor fungicides in *Botrytis cinerea*

A. AMIRI (1), S. M. Heath (1), N. N. Peres (1)
(1) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.4

Multiple single-site fungicides from succinate-dehydrogenase-inhibitor (SdhI) and quinone-outside-inhibitor (QoI) groups target complexes II and III of the mitochondrial respiration system also known as the succinate-ubiquinone oxydoreductase (SDH) and the cytochrome b (*cytb*), respectively. Herein, we report on the new findings on different mutations associated with resistance of *Botrytis cinerea* from strawberry fields in Florida to SdhIs and QoIs. In *B. cinerea* and in a few fungal species, the presence of an intron (*bi2*) after codon 143 of the *cytb* gene was thought to prevent mutation at this codon and thus prevent resistance development. We detected a restriction site (GC/AGC) 20 bp upstream the 3'end of *bi2* intron that was digested by *Sat*I enzyme. Such restrictions were only reported in QoI-resistant isolates and the restriction site found in the *bi2* intron might play a role in the later self-splicing. Regarding resistance to the SdhIs, in addition to the mutations previously reported at codon 272 of SdhB, we detected four linked simultaneous mutations in SdhC sub-unit of the complex II. These mutations were found in 95% of the *B. cinerea* isolates resistant to SdhIs. Preliminary investigation indicated a fitness penalty in isolates carrying mutations in the SdhC compared to the wild-type isolates. The role of these mutations is being characterized, however, we hypothesize an effect on the structure and the stability of the SDH complex or an impact on quinone and quinol interconversion.

Multiple fungicide resistance in *Botrytis cinerea* isolates from strawberry fields in Florida

A. AMIRI (1), S. M. Heath (1), N. A. Peres (1)
(1) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.4

Three hundred isolates of *Botrytis cinerea* collected between 2001 and 2012 from multiple strawberry fields in Florida were evaluated for their sensitivity to nine fungicides i.e. boscalid, pyraclostrobin, boscalid + pyraclostrobin (Pristine), fenhexamid, pyrimethanil, cyprodinil, fludioxonil and cyprodinil + fludioxonil (Switch). EC_{50} values were determined based upon conidial germination inhibition and mycelial growth inhibition for boscalid and for the other fungicides, respectively. Resistance frequencies were the highest for pyraclostrobin (91%) and boscalid (85%) followed by pyrimethanil (61%), cyprodinil (54%) and fenhexamid (45%). Respective resistance factors were 20866, 1612, 158, 100 and 223. A strong positive cross-resistance was observed between Pristine and boscalid (r =0.95), Pristine and pyraclostrobin (r = 0.89), and between the two anilino-pyrimidines, cyprodinil and pyrimethanil (r = 0.54). Overall, frequencies of isolates resistant to one, two, three or four fungicides were 7, 35, 44, and 14%, respectively. All fungicides sprayed preventively on detached strawberry fruits failed to control isolates with high levels of resistance to each fungicide with the exception of fludioxonil and Switch. Although an increase in EC_{50} values for fludioxonil

and Switch was observed, no actual resistance to them was noted. In light of widespread resistance to most common fungicides used for Botrytis fruit rot control in Florida strawberry fields, new fungicide application strategies have to be implemented to avoid a total control failure.

Stylet morphometrics and ultrastructure in relation to feeding behavior of nymphs and adults of the Asian citrus psyllid *Diaphorina citri*, vector of citrus huanglongbing bacterium

E. AMMAR (1), D. G. Hall (1)
(1) USDA-ARS, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.5

Newly developed methods were used to study the feeding behavior and stylet morphometrics in nymphs and adults of the Asian citrus psyllid (ACP), *Diaphorina citri* (Hemiptera, Psyllidae), vector of '*Candidatus* Liberibacter asiaticus' (Las) associated with citrus huanglongbing disease. The stylet length of first instar nymphs averaged 259 µm (80% of body length) whereas that of 5th instar nymphs was 614 µm (34% of body length). ACP nymphs feed only on young citrus leaves on secondary veins or on the sides (rather than the top) of the midrib, whereas adults can feed anywhere on the veins of young or old leaves. Cross sections in citrus leaves, showing ACP salivary/stylet sheaths branching inside the phloem tissue, indicated that the distance to the phloem is shorter from the sides of the midrib compared to that from the top, and is considerably shorter in young citrus leaves compared to that in mature leaves. Additionally, the thick-walled fibrous layer around the phloem is much more prominent in older than in younger leaves. These results at least partially explain the preference of nymphs to feed on the sides, rather than the top, of the midrib as well as their inability to feed on older leaves. Las bacterium is known to reside in the phloem of infected citrus plants. Transmission and scanning electron microscopy of the stylets in nymphs and adults are underway in order to determine if the maxillary food or salivary canals can act as Las transmission barriers at various stages of ACP development.

New and simple methods for studying the stylets of hemipteran nymphs and the salivary sheaths in host plants

E. AMMAR (1), D. G. Hall (1)
(1) USDA-ARS, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.5

Microscopic and behavioral studies on nymphal instars of hemipteran insects from four different families: Psyllidae, Aphididae, Aleyrodidae and Cicadellidae, showed that their molted skins (exuvia) normally had either fully or partially extended stylets in a feeding-like position. In most cases these stylets were still attached to the host plants after ecdysis, which indicates that hemipteran nymphs use their stylets to anchor themselves to their host plants during molting. This phenomenon can be used to study the length and other features of the stylets of hemipteran nymphs which is normally more difficult in nymphs than in adults because of the fragility and smaller size of nymphs. Additionally, a new simple method for studying the hemipteran salivary sheaths in host plants was developed. This method is based on fixation of hand sections of the plant material on which hemipteran insects have been feeding, staining of these sections with a fluorescent nuclear stain, then mounting and examination with fluorescence or confocal microscopy. No embedding or microtomy is needed for this method which makes it much faster and simpler than other methods. Furthermore, the hand sections allowed observation of the direction and branching of salivary sheaths in host tissues more clearly than in thinner microtome sections. This method was applied successfully with the salivary sheaths of aphids, psyllids, leafhoppers and whiteflies in their host plants.

Dynamics and environmental regulation of virulence gene expression in *Erwinia amylovora*

V. ANCONA (1), D. Wang (1), Y. Zhao (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.5

Erwinia amylovora is a bacterial plant pathogen that causes fire blight in apples and pears. A functional type III secretion system (T3SS) and production of the exopolysaccharide amylovoran are both required for *E. amylovora* to cause disease. In order to examine how these virulence factors interact with each other, we are studying the dynamics of T3SS and amylovoran biosynthesis gene expression. Quantitative RT-PCR approach and microarray were used to analyze gene expression profiles of virulence genes of *E. amylovora* strains both in vivo and in vitro. Our results showed that T3SS and amylovoran biosynthesis genes were rapidly induced both in vitro and in vivo; however, T3SS gene expression decreased very rapidly with time, whereas amylovoran biosynthetic genes remained at the same level for a prolonged time. On the other hand, environmental factors such as temperature, seems to play different roles in regulating their expression. While T3SS genes were induced to the same level at both 18°C and 28°C in vitro, amylovoran production decreased significantly at lower temperatures. This may explain why the disease is much severe at high temperature. These findings also indicate that, depending on the environmental conditions, T3SS and amylovoran production may play distinct roles in virulence and possibly at different stages of bacterial pathogenesis.

Influence of variable moisture patterns on the association between Fusarium head blight and deoxynivalenol contamination in wheat

K. F. ANDERSEN (1), K. T. Willyerd (1), L. V. Madden (1), P. A. Paul (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 102:S4.5

Visual symptoms of Fusarium head blight (FHB), caused by *Fusarium graminearum*, and grain contamination with deoxynivalenol (DON) are often positively correlated. However, this association breaks down under certain conditions, with DON being disproportionately lower or higher than expected based on visual symptoms. An experiment was conducted to evaluate the effects of different moisture patterns on the association between FHB index (IND) and DON in soft red winter wheat. Plots were planted on three dates (PD1, PD2 and PD3), and four 7-day misting treatments were assigned to separate plots (MIST1: mist every day; MIST2: 2 days of mist, 3 days off, 2 days of mist; MIST3: 2 days off, 3 days of mist, 2 days off; MIST4: mist every other day). In general, misting occurred before, during, and after anthesis for PD3, PD2, and PD1, respectively. Plots were either inoculated with *F. graminearum*-colonized corn kernels or naturally infected corn stubble, or left uninoculated. IND, DON, and IND:DON ratio were estimated for each planting date x misting treatment combination. Mean IND and DON were highest for PD1 and lowest for PD3, and highest in plots with corn stubble that were subjected to MIST1. DON contamination relative to IND varied with the timing and pattern of misting and the source of inoculum. For plots planted into cornstubble, IND:DON ratios were lowest for MIST2 (between 0.48 and 1.14), for all planting dates, and highest for MIST1 or MIST4 (between 1.60 and 1.95) for PD1 and PD3.

Development of mt*COI* primers for the rapid identification of three *Bemisia tabaci* biotypes and *Trialeurodes vaporariorum*

S. ANDREASON (1), J. K. Brown (2), J. Fletcher (3), F. M. Ochoa Corona (3), M. Arif (3), A. Wayadande (3)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) The University of Arizona, Tucson, AZ, U.S.A.; (3) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.5

The whitefly (Hemiptera; Aleyrodidae) species *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) are global agricultural pests and plant virus vectors. Rapid and reliable identification of these species commonly intercepted at U.S. ports of entry could be accomplished using molecular methods. Biotype-specific mtCOI endpoint PCR primers were designed for the B and Q biotypes, both 'high consequence' exotic *B. tabaci*, an A-like biotype consensus group, and *T. vaporariorum*. The PCR fragments were 613-, 116-, 329-, and 112-bp respectively. Genomic DNA was extracted from three adult *B. tabaci* representatives of each of the B, Q, and A biotypes, and *T. vaporariorum*. Endpoint PCRs using the four primer pairs were carried out for each sample. PCR products were separated using 1% agarose gel electrophoresis, and bands were eluted from the gel and sequenced to confirm primer specificity for each respective biotype. Helicase dependent amplification (HDA) primer sets were also designed for *B. tabaci* and *T. vaporariorum*. The IsoAmp III Universal tHDA Kit protocols were used per the manufacturer's instructions and followed by 1.5% agarose gel electrophoresis. These primer sets specifically amplified 87- and 99-bp mtCOI gene regions of *B. tabaci* and *T. vaporariorum*, respectively. The use of these primer sets in endpoint PCR or isothermic HDA reactions followed by gel electrophoresis allows for the specific and rapid identification of *B. tabaci* and *T. vaporariorum*.

The *Tomato bushy stunt virus* coat protein elicits a hypersensitive response in *Nicotiana* species

C. A. ANGEL (1), J. E. Schoelz (1)
(1) Division of Plant Sciences, University of Missouri, Columbia, MO, U.S.A.
Phytopathology 102:S4.5

Previous studies showed that the *Tomato bushy stunt virus* (TBSV) P22 protein elicits a hypersensitive response (HR) in *Nicotiana glutinosa* and *N. edwardsonii*, whereas the P19 protein elicits an HR in *N. tabacum* and *N. sylvestris*. In the present study we identified nine new *Nicotiana* species that responded to TBSV virion inoculation with HR. Of these, only *N. bonariensis* responded with HR to agroinfiltration of p19, whereas only an accession of *N.*

forgetiana responded to p22 with HR. By contrast, six species, all members of *Nicotiana* section Alatae, responded to agroinfiltration of the coat protein gene (p41) with HR. The only member of the Alatae section that was not resistant to TBSV was *N. plumbaginifolia*, which developed a systemic necrosis symptom upon inoculation of TBSV virions. To determine whether the TBSV coat protein or its nucleic acid sequence was responsible for HR elicitation in *Nicotiana* species, we created full length and truncated mutants of p41 in which increasingly larger portions of the 5' end sequence were deleted, and agroinfiltrated them into *N. langsdorfii* for HR analysis and into *N. benthamiana* for coat protein expression. Our results indicate that the coat protein of TBSV, rather than the viral RNA is responsible for eliciting HR in six species of *Nicotiana* section Alatae, and a sequence essential for eliciting HR is located between codons 80 and 211 of the p41 gene.

CHUP1, required for movement of chloroplasts on microfilaments, colocalizes with the P6 inclusion body protein of *Cauliflower mosaic virus*

C. A. ANGEL (1), L. Lutz (2), X. Yang (3), S. Leisner (2), R. S. Nelson (3), J. E. Schoelz (1)
(1) Division of Plant Sciences, University of Missouri, Columbia, MO, U.S.A.; (2) Department of Biological Sciences, University of Toledo, Toledo, OH, U.S.A.; (3) Plant Biology Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, OK, U.S.A.
Phytopathology 102:S4.6

The P6 protein of *Cauliflower mosaic virus* (CaMV) assembles in the cytoplasm into large, amorphous inclusion bodies (IBs) that localize to and require actin microfilaments for their intracellular movement. The CaMV IBs are considered virion factories, as they are the site for protein expression, genome amplification, and virion assembly. A yeast two-hybrid screen of an *Arabidopsis* cDNA library with CaMV P6 as the bait identified a protein whose function may explain the microfilament requirement for P6 IB movement. The *Arabidopsis* protein identified in the screen, CHUP1 (Chloroplast Unusual Positioning 1), is localized to the outer envelope of chloroplasts and is responsible for chloroplast movement on microfilaments. Transient co-expression of CHUP1 and P6 tagged with fluorescent proteins revealed that CHUP1 and P6 co-localize within the cell. Furthermore, expression of a truncated CHUP1 blocked the movement of P6 IBs. We evaluated the role of CHUP1 in CaMV infections through the use of an *A. thaliana chup1* T-DNA knockout line and through virus induced gene silencing in *Nicotiana edwardsonii*. In both hosts, the inactivation of CHUP1 measurably slowed the rate of the CaMV infection. However, viral infection was not abolished, indicating that additional proteins to CHUP1 may play a role on intracellular movement of CaMV.

Plant host effects on rhizosphere bacterial communities and pathogen suppression

B. E. ARENZ (1), J. M. Bradeen (1), L. K. Otto-Hansen (1), J. C. Anderson (1), L. L. Kinkel (1)
(1) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 102:S4.6

This study explored the influence of plant species on bacterial rhizosphere community composition, structure, and function (pathogen suppressive activity). Soil was collected from 6 agricultural and natural habitats with differing plant species histories, sieved, homogenized, and evaluated for edaphic characteristics. Subsequently, 2 grasses, *Andropogon gerardii* (Ag) and *Secale cereale* (Sc) were planted individually into each soil and maintained in a greenhouse for 14 weeks. DNA was extracted from both pre- and post-plant soils, amplified with bacterial rDNA-specific primers, and sequenced via 454 pyrosequencing. Suppressive activity against 3 plant pathogens was evaluated in vitro. Pre-plant samples from different locations supported distinctive bacterial communities, which remained distinct following planting. Both Ag and Sc significantly increased bacterial diversity compared to pre-plant soil communities. Groups that decreased in relative abundance after planting included the Flavobacteriaceae, Sphingobacteriales, Sphingomonodaceae, and Burkholderiales, indicating that the grass rhizosphere or the greenhouse environment is less favorable to these groups. In vitro tests of pre-plant communities were significantly different in *Streptomyces* spp., total bacterial, and inhibitor densities and inhibition zone size, (all; $p<0.0001$). Microbial community composition remained significantly different among distinct soils despite increases in diversity induced by Ag and Sc.

Application of primer and probe modifications in detection, biosecurity, and microbial forensics

M. ARIF (1), J. Fletcher (1), F. M. Ochoa Corona (1)
(1) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.6

Efficient PCR amplifications rely on harmonizing a precise design of oligonucleotide primers, chemistry and cycling conditions in the reaction. Despite software improvement and chemistry development, primer design still presents unresolved challenges. There is a need for increased sensitivity and higher DNA amplification efficiency for applications in microbial forensics, detection and diagnostic procedures in general. The aim of this research was to investigate primer modification to increase assay sensitivity and accuracy. Primers were designed from different target genes and modified at or close to the 5' terminus to achieve optimum thermodynamic performance during PCR and isothermal amplification. Other factors influencing the optimum primer design included the reaction format and condition; *i.e.* the type of PCR orisothermal amplification employed. Both PCR (multiplex, real time, and standard) and isothermal amplification assays, performed using primers modified in these ways, had higher DNA yield than those using traditionally-designed primers. Use of a multiprobe approach further increased the fluorescence level in qPCR reactions. Primer modifications enhance assay accuracy, and sensitivity, and will be beneficial for applications in detection, diagnostics, agricultural biosecurity, microbial forensics and forensic entomology, and have potential applications also for cases in which primers are designed from difficult sequences and there is little choice for primer selection.

Detection of *High plains virus* with loop-mediated isothermal amplification

M. ARIF (1), J. Daniels (1), C. Chalam (2), J. Fletcher (1), F. M. Ochoa Corona (1)
(1) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.; (2) Division of Plant Quarantine, National Bureau of Plant Genetic Resources, New Delhi, India
Phytopathology 102:S4.6

High plains virus (HPV), which infects wheat, corn, and other grasses in United States, occurs as a variety of strains. A field deployable, accurate, sensitive, and broad range detection method is needed for use by extension personnel and farmers to manage disease outbreaks and identify host reservoirs. Loop-mediated isothermal amplification (LAMP) is a novel technique that requires only one enzyme having strand displacement activity for amplification under isothermal conditions. LAMP has a higher specificity than PCR because its four primers recognize six distinct regions on the targeted genome. Specific LAMP primers HPV-F1, HPV-B3, HPV-FIP and HPV-BIP were designed using PrimerExplorer V4, targeting the RNA3 nucleoprotein of HPV. Primers HPV-FIP and HPV-BIP were used at 1.6 μM, while primers HPV-P3 and HPV-F3 were at 0.2 μM. The LAMP reactions were incubated at 65°C for 1 hour, and the amplified products were detected using Loopamp Florescent Detection Reagent and a UV lamp. All primers were validated for specificity *in silico* against published sequences and *in vivo* against infected plant samples. The described PCR assay is accurate, rapid, sensitive and useful for pathogen detection, disease diagnosis, and other applications in biosecurity, microbial forensics and forensic entomology. It also has potential applications in routine diagnostics laboratories.

Statistical parameters of spatial patterns of spread for leafroll disease (GLRaV-3)

K. L. ARNOLD (1), N. McRoberts (1), D. A. Golino (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.6

Data sets collected from the literature or available from previous observations were re-analyzed using quadrat-based approaches to characterize the spatial pattern of leafroll over time. Irrespective of the numerous differences among the studies, the analyses revealed a consistent spatio-temporal pattern across epidemics. In all cases vines with visual leafroll symptoms had a highly aggregated spatial pattern in which newly diseased plants tended to be nearest neighbors of previously infected plants. The degree of patchiness in the data was characterized by an effective sample size of $n = 3$ to $n = 3.5$ plants per quadrat. The smallest actual quadrats used in the analysis of the data were of size $n = 4$ and the quadrats used to analyze one vine block were $n = 30$. The analyses do not allow us to infer a specific mechanism of dispersal from the data, but they show a consistent mechanism dominated by short-range dispersal events. Information generated by these analyses will be used in a bio-economic toolkit to determine optimum disease management plans for individual vineyards and in the design of efficient sampling plans to detect early infection in vine blocks. We report consistent pattern dynamics among different studies and give the first estimates of statistical parameters for disease incidence distributions for leafroll disease.

Analysis of subjectivities about leafroll disease management among Napa grape growers and winemakers

K. L. ARNOLD (1), N. McRoberts (1), D. A. Golino (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.7

A Q-method approach was used to assess the opinions concerning GLRaV-3 management. Three workshops were held in the Napa Valley at which invited participants were asked to write down their views in response to a set of open-ended questions about leafroll, its impacts and the prospects for cooperative management of the disease. Responses were sorted into thematic groups (e.g. statements about financial issues, clean plant material, interpersonal trust, etc.). A small subset of response statements which encapsulated the groups of opinions was extracted. This resulted in a set of 47 statements. Invitations were issued via email and by personal contacts to a further group of participants drawn from the Napa Valley grower and winemaker communities generating a participant group of 37 individuals. These people were interviewed on a one-to-one basis and the participants ranked the statements by the degree to which each one accorded with their own views. The interviews were conducted during the fall and winter months of 2011. The resulting two-way table of data, in which each row gives the numerical rank assigned to each statement by one participant, was then subjected to a Principal Components analysis to extract information regarding the distribution of opinions over the group of participants and to identify meaningful classifications of the responses. The analysis revealed a wide diversity of opinion distributed among some broad categories of response.

Temperature functions as a repressor of ascocarp formation in strawberry powdery mildew *Podosphaera aphanis*

B. ASALF (1), A. Stensvand (2), D. M. Gadoury (3), L. Cadle-Davidson (4), R. C. Seem (3), N. A. Peres (5), A. Tronsmo (1)
(1) Norwegian University of Life Sciences, Aas, Norway; (2) Bioforsk, Aas, Norway; (3) Cornell University, Geneva, NY, U.S.A.; (4) USDA-ARS, Grape Genetics Research Unit, Geneva, NY, U.S.A.; (5) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.7

The asexual stage of *Podosphaera aphanis* occurs wherever strawberries are grown, but cleistothecia are reportedly rare in subtropical climates where the disease is nonetheless severe. We confirmed that the pathogen is heterothallic, and that both mating types are present in Florida, USA. Pairing compatible isolates under controlled temperatures indicated that ascocarp formation was suppressed above 15C. Abundant mycelial growth occurred at both 15C and 25C, thus lack of ascocarp production was not caused by lack of contact between mating types within the mildew colony. Furthermore, subsequent investigations of diurnal temperature fluctuation indicated that nightly exposure to cold (<13C) for 1 to 4 h was sufficient to stimulate ascocarp formation in the presence of higher (25C) daytime temperatures. Additional episodes of overnight cold resulted in more prolific ascocarp production despite higher daytime temperatures. Our results suggest that cleistothecia of *P. aphanis* may be suppressed in subtropical areas by high temperature during the warmest periods of the year, but may appear if strawberry plants in annual production systems are exposed to cooler temperatures late in the production cycle. The foregoing also suggests that ascocarp formation might be minimized or prevented in high tunnel production systems by avoiding temperatures below 13C. Geographical distribution of mating types as confirmed by specific markers will also be reported.

Early-season cryptic development of powdery mildew (*Podosphaera aphanis*) in June bearing strawberries

B. ASLAF (1), D. M. Gadoury (2), R. C. Seem (2), A. Tronsmo (3), A. Stensvand (3)
(1) Norwegian University of Life Sciences, Aas, Norway; (2) Cornell University, Geneva, NY, U.S.A.; (3) Bioforsk, Aas, Norway
Phytopathology 102:S4.7

Powdery mildew of strawberry (*Podosphaera aphanis*) is often described as a late-season disease, but ascospore discharge can occur as plants emerge from dormancy. Our hypothesis was that epidemics may enter a cryptic phase when environmental conditions do not favor sporulation, wherein the pathogen spreads but is not macroscopically visible. Shortly after snow-melt in 2010 and 2011, we inoculated emergent leaves with either ascospores or conidia, and assessed pathogen growth and sporulation starting one week after inoculation. Favorable periods for pathogen growth and sporulation, defined by both accumulated rain-free days and days with average temperatures > 10 °C, were significantly correlated with disease incidence ($p < 0.05$). In 2010, only 5 favorable days occurred before sporulation was detected 30 days after inoculation. In 2011, 11 favorable days occurred, and sporulation was detected on day 17 after inoculation. Macroscopically indistinct mycelial growth was confirmed 7 days after inoculation in both years. Spread of *P. aphanis* can precede macroscopic signs by several weeks. Two possible management options are suggested for further evaluation, and will be reported: (i) suppression of the cryptic phase from the earliest stages of crop growth, and (ii) initiation of suppression timed to precede forecasted sporulation, as contrasted with responding to macroscopically visible disease.

Detection of intrachromosomal recombination in *Sclerotinia sclerotiorum* populations

R. N. ATTANAYAKE (1), W. Chen (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.7

Genetic structure and reproductive mode of the homothallic fungal pathogen *Sclerotinia sclerotiorum* have been widely studied using linkage disequilibrium (LD) tests with putatively unlinked molecular markers. We previously observed random association between linked loci in *S. sclerotiorum* populations suggesting intrachromosomal recombination or high mutation rates at these loci. This study was aimed at testing intrachromosomal recombination using 12 microsatellite loci distributed over four chromosomes. Two hundred thirty isolates sampled from seven populations in the USA and China from a variety of crops were genotyped. Each isolate carried a single allele for each of the 12 loci suggesting the isolates were haploid and homokaryotic. Pairwise LD tests of all the intrachromosomal loci showed relationship ranged from linked to random association, and in many cases LD declined with increasing physical distance between loci. Thus the random associations of alleles cannot be simply attributed to random mutation. Majority of the isolates were mycelially incompatible, likely minimizing the possibility of heterokaryon formation and mitotic recombination. Thus the observed high intrachromosomal recombination is most likely due to meiotic recombination following outcross in these populations.

WITHDRAWN

Molecular detection and host adaptation of *Sclerotinia homoeocarpa*, the causal agent of dollar spot of turfgrass

B. A. AYNARDI (1), M. M. Jimenez-Gasco (1), W. Uddin (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.7

Dollar spot of turfgrass, caused by *Sclerotinia homoeocarpa*, affects cool and warm-season grasses throughout North America. The objective of this project was to develop a molecular method for the detection of pathogen DNA. Sequencing of the Internal Transcribed Spacer (ITS) region of the rDNA and comparisons of numerous dollar spot isolates collected throughout North America in the past three decades allowed for the design of species-specific primers that are able to amplify only DNA of the pathogen of interest. Specificity of the two newly designed primers was confirmed through testing its closest relatives in the Rutstroemiaceae and other turfgrass pathogens for amplification of DNA, which did not occur in either case. The implementation of these primers for the quantification of inoculum levels in the thatch/soil interface to predict the onset of disease will allow for proper fungicide appli-

cation timing in early spray programs. Additionally, phylogenetic analysis of the ITS region showed that two isolates concurrently collected from a bentgrass/*Poa* putting green and a bermudagrass fairway on the same golf course were genetically distinct. The maximum likelihood analysis indicated that the bermudagrass isolate grouped with other isolates from bermudagrass previously collected in Florida. This example provides direct evidence that genetically diverse populations of *S. homoeocarpa* host adapted for cool and warm-season grasses coexist in the same locality.

Occurrence of bacterial spot (*Xanthomonas cucurbitae*) in pumpkin fields in the Midwest

M. BABADOOST (1), A. Ravanlou (1), D. S. Egel (2), D. O'Brien (3)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) Purdue University, Vincennes, IN, U.S.A.; (3) Crop Production Services, Hudson, OH, U.S.A.
Phytopathology 102:S4.8

Bacterial spot, caused by *Xanthomonas cucurbitae*, was economically an insignificant disease in the Midwest until 2005. But, in the past four years, the disease occurred widely in pumpkin fields in the Midwest. Yield losses up to 90% forced some growers to abandon pumpkin production. A survey was conducted in 2011 to assess the occurrence of bacterial spot in pumpkin fields in Illinois, Indiana, Iowa, Kansas, Missouri, Nebraska, Ohio, and Wisconsin. During three weeks of pumpkin harvest, 111 pumpkin fields were visited and incidence and severity of bacterial spot on fruit were assessed. In each field, 60 fruit in 12 locations (five fruits per location) were examined in an M-shaped walking path. The incidence of *X. cucurbitae*-infected fruit were observed in 95 fields (86% of fields visited), with overall 26% of fruit infected. Severity of the disease on fruit (percent surface area of the fruit with bacterial lesions) ranged from 1 to 20%. Bacterial spot was observed in both jack-o-lantern and processing pumpkin fields. *X. cucurbitae* was isolated from infected fruit and the identity of the isolates was confirmed using biochemical tests and molecular methods. Koch's postulates for representative isolates were carried out on 'Howden' pumpkin in greenhouse trials. *X. cucurbitae* infection was also observed and confirmed on all winter squashes grown near pumpkin fields visited in 2011. In addition, greenhouse studies showed that all cucurbit crops are susceptible to *X. cucurbitae*.

Detection, seed transmission, and control of *Hyaloperonospora camelinae* on *Camelina sativa* (L.) in Washington State

E. M. BABIKER (1), S. H. Hulbert (2), T. Paulitz (3)
(1) Washington State University, Pullman, WA, U.S.A.; (2) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (3) USDA-ARS, Root Disease and Biological Control Research Unit/Washington State University, Pullman, WA, U.S.A.
Phytopathology 102:S4.8

Camelina (*Camelina sativa* [L.] Crantz) plants with symptoms of downy mildew were obtained from three different locations in Washington State. Based on PCR and sequencing of the ITS1-5.8S-ITS2 region, the causal pathogen was identified as *Hyaloperonospora camelinae*. The PCR primers consistently amplified 699 bp bands from the infected plants, but not from the healthy plants. A comparison of the sequences with those in GenBank revealed 100% sequence similarity to *H. camelinae*. Growth and development of the *H. camelinae* was observed in different tissues using light and scanning electron microscopy (SEM). Light microscopic observation revealed the presence of oospores in the infected leaves and SEM revealed the presence of conidia and conidiophores on the seed surface. To determine whether downy mildew is a seed-transmitted disease, seeds collected from infected plants were planted in potting mixes maintained in a growth chamber. Disease symptoms were observed in 96% of the seedlings compared to 3% of the seedlings grown from seed from healthy plants, which indicates that downy mildew of camelina is a seed-transmitted pathogen. Seeds treated with mefenoxam, a fungicide specific for Oomycetes, significantly reduced the incidence of the disease.

Effects of plant water stress on vector feeding behaviors that control acquisition and inoculation of *Xylella fastidiosa*

E. A. BACKUS (1), R. Krugner (1)
(1) USDA-ARS, Parlier, CA, U.S.A.
Phytopathology 102:S4.8

Xylella fastidiosa (Xf) is an economically important pathogen of grapevine (Pierce's disease), stone fruits, nursery trees, and ornamental plants (various scorch diseases) in California. The bacterium is transmitted by sharpshooter leafhopper vectors, such as the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar). Vector feeding behaviors directly control Xf acquisition and inoculation. The present study tested whether plant water stress affects vector performance of acquisition and/or inoculation behaviors. GWSS feeding behaviors on well-watered vs. water-stressed plants were recorded using electrical penetration graph (EPG); plants studied were almond, *Prunus dulcis*, and citrus, *Citrus sinensis* cv 'Navel'. EPG waveforms representing pathway phase (searching for xylem), X waves (xylem contact, likely to control Xf inoculation), and waveform C (ingestion of xylem fluid, Xf acquisition) were analyzed. Results showed that xylem-sap ingestion per insect was longer on well-watered than water-stressed plants. Numbers of X waves per insect also were higher when plants were well-watered. Thus, both acquisition and inoculation behaviors were decreased on water-stressed plants. These findings support other studies suggesting that diminished irrigation can impact Pierce's disease epidemiology by reducing bacterial acquisition and/or inoculation by the vector.

Thatch collapse: A new disease of golf course turfgrasses

A. M. BAETSEN (1), G. L. Miller (2), M. T. Kasson (1), D. D. Davis (1), J. E. Kaminski (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.; (2) University of Missouri, Columbia, MO, U.S.A.
Phytopathology 102:S4.8

In 2010, previously unreported disease symptoms were observed on golf course putting greens located in the United Kingdom and Pennsylvania. Since this initial discovery, similar symptoms have subsequently been reported in California, Michigan, South Dakota, Australia, and New Zealand on golf putting greens and fairways. Symptoms of this disease include circular patches of degraded organic matter (e.g. thatch) ranging from approximately 8 to 46 cm in diameter. Thatch degradation results in an indentation of the turf surface and disrupts playability. Commonly found in association with the thatch degradation is a basidiomycete not previously reported within stands of any turfgrass. Fungal signs included profuse, clamp connected mycelia within the upper 2.5 cm of the soil/thatch profile and peridium within the thatch and canopy of golf course putting greens. The fungus was isolated on oatmeal agar and DNA was extracted and the internal transcribed spacer region (ITS) was amplified with the primer set ITS4/ITS5. A 703 to 707-bp sequence was obtained and found to be 99% similar to an accession of *Sphaerobolus stellatus* in the NCBI database. *S. stellatus* is a basidiomycete commonly found within wood mulch. Based on these results, *S. stellatus* may be responsible for rapid lignocellulose degradation resulting in thatch collapse on turfgrass species. Ongoing studies are being conducted to better understand the biology of this fungus and to confirm *S. stellatus* as the cause of thatch collapse symptoms.

Seasonal dynamics of *Iris yellow spot virus* transmitters among *Thrips tabaci* populations from onion fields

S. BAG (1), S. I. Rondon (2), H. R. Pappu (1)
(1) Washington State University, Pullman, WA, U.S.A.; (2) Oregon State University, Hermiston, OR, U.S.A.
Phytopathology 102:S4.8

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a global pest in commercial onion (*Allium cepa* L.). *Thrips tabaci* can cause yield loss of >50%. Moreover, it is a vector of *Iris yellow spot virus* (IYSV, family *Bunyaviridae*, genus *Tospovirus*), a serious viral pathogen affecting both bulb and seed onion crops in the U.S. and many parts of the world. IYSV infection can lead to total crop loss. The role of *T. tabaci* in IYSV epidemiology is not completely understood. As part of an ongoing project to develop an IPM strategy for IYSV, the seasonal dynamics of *T. tabaci* that could serve as potential transmitters of IYSV was undertaken. Live adult *T. tabaci* were collected from two onion fields, one adjacent to an overwintering onion field and one far away from any onion field. Thrips were tested individually for the presence of IYSV by direct antigen coated–enzyme linked immunosorbent assay using antiserum specific to the non structural protein (NSs) of IYSV to differentiate between the transmitters from on-transmitters. Significantly more thrips populations were found in the middle of July and correlated with the highest percentage of potential transmitters during the same week during 2008 and 2009 seasons. The ELISA test facilitated rapid testing of a large number of field-collected thrips to determine the proportion of thrips that are potential virus transmitters. This information could help refine thrips management practices as part of an overall IPM strategy for reducing the impact of IYSV.

Molecular detection and characterization of phytoplasmas associated with blueberry stunt disease in New Jersey

P. G. Bagadia (1), J. Polashock (2), K. D. Bottner-Parker (3), Y. Zhao (3), R. E. Davis (3), I. LEE (3)
(1) University of Maryland, College Park, MD, U.S.A.; (2) USDA-ARS GIFVL, Chatsworth, NJ, U.S.A.; (3) USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD, U.S.A.
Phytopathology 102:S4.8

Blueberry, rich in antioxidants, is among the most important fruit crops in the world. Consumption and production of blueberries has increased in the last

decades and New Jersey is one of the top producers in the United States. Blueberries are susceptible to blueberry stunt disease, characterized by witches' broom, stunting, small and deformed leaves, cupping of leaves, and shortened internodes. This disease was attributed to a virus and only recently was the identity of causal pathogen clarified to be an unculturable mollicute, phytoplasma. It was widespread in eastern North America in mid 1900s, and has been largely controlled by the discovery of the primary insect vector (leafhoppers). Recently, however, the recurrence of blueberry stunt disease became evident in many old and new farms in New Jersey. The phytoplasmas attributed to the disease in New Jersey have never been characterized, prompting us to conduct a large scale field survey. In the present study, we conducted a state-wide survey of the causal pathogens of blueberry stunt disease present in all blueberry cultivars in the two major blueberry production counties in New Jersey. Sequence and phylogenetic analyses of 16S rRNA, ribosomal protein and secY genes indicated that phytoplasma strains, belonging to 16SrI group, subgroup 16SrI-E, were predominantly associated with infected blueberries in New Jersey, but a new phytoplasma belonging to group 16SrIX was also surprisingly detected in infected plants.

Virus diversity in Washington State Concord vineyards

B. W. BAHDER (1), N. A. Rayapati (1), D. B. Walsh (1)
(1) Washington State University, Prosser, WA, U.S.A.
Phytopathology 102:S4.9

Washington State is the largest producer of juice grapes, *Vitis labruscana* cv. 'Concord', and second largest producer of wine grapes in the United States. Grapevine leafroll disease (GLD) is the most widespread and economically significant viral disease of wine grapes in the world and in Washington State. Of the different species of this disease complex, *Grapevine leafroll associated virus-3* (GLRaV-3) is the predominant virus associated with GLD in wine grapes. Little is known about the occurrence of GLRaVs in juice grapes in Washington, with the first record of the GLRaV-3 in Concords occurring in 2006. The discovery of this virus in Concord vineyards highlighted the lack of knowledge of the occurrence of viruses in Concord grapes in Washington. Various Concord vineyards were surveyed in 2010 and 2011. Samples were taken randomly in each vineyard with a total of 400 samples taken in total (≈33 samples per vineyard). Each sample was tested for 16 different viruses that have been detected in wine grapes in Washington State. All denatured extract was tested for virus presence by RT-PCR and samples that tested positive were either sequenced directly or cloned and sequenced, depending on DNA quality. Thus far, six viruses have been detected; GLRaV-2, GLRaV-2rg, GLRaV-3, GLRaV-4, GLRaV-9, and grapevine virus A. The most common virus found was GLRaV-3 (50%). In 2012, more samples will be taken in new vineyards further away from the current study area. Mealybug and scale insects will also be tested to help assess their role in vectoring of viruses.

Population change of *Phytophthora* spp. in two streams over a one-year period

Y. BALCI (1), N. Hoang (1), N. Mirjafary (1)
(1) University of Maryland, College Park, MD, U.S.A.
Phytopathology 102:S4.9

The population fluctuation of *Phytophthora* was quantified for a one-year period in two streams. The Paint Branch creek (PB) and Comcast Center creek (CC), part of the Anacostia watershed in Maryland, were sampled. Streams differed in both their size and the amount of water available. The CC creek was considerably smaller and is a tributary of PB. Every week for two days (Monday and Wednesday) 1 L water sample was collected twice at 8 AM and 2 PM. Of the 1 L water sample, two or three 200 ml subsamples were filtered through a 3 µm pore size 9 cm diameter Millipore membrane filter. Filters were placed on clarified V8 juice based PARPNH growth media selective for isolation of *Phytophthora* spp. After two days of incubationin darkness at room temperature, colonies were counted. Streams differed significantly in *Phytophthora* colony numbers. The smaller CC stream had almost twice as many colonies compared to PB stream. However, there was no significant difference between the samples that were collected during the morning (8am) or afternoon (2pm). A significant decrease of population of *Phytophthora* was found with increasing temperatures in both streams. The month samples were collected also played a significant role in *Phytophthora* colony numbers. In both streams June, October and November had the greatest number of colony counts.

Detached leaf assay adapted to tomato pericarp sections for modeling contamination of tomato fruit by *Salmonella* Typhimurium

J. BARTZ (1), M. Mahovic (2), D. Spiceland (3), M. Teplitski (4)
(1) Plant Pathology Department, University of Florida, Gainesville, FL, U.S.A.; (2) FDA, CFSAN, Office of Food Safety, College Park, MD, U.S.A.; (3) University of Florida, Gainesville, FL, U.S.A.; (4) Soil and Water Science Department, University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.9

Sections of pericarp were excised with a cork borer from tomato fruit. Section diameter ranged from 11.5 to 19.5 mm depending on the test. Section thickness ranged from 5 to 8 mm. Methods for inoculation and storage of sections evolved to one where a wound that was 3.4 mm at the base, 1.2 mm at the tip with a depth of 2 mm was made in the cuticle of the pericarp. The wound held 10 µL of suspension or solution without spillage. Proliferation of *Salmonella* Typhimurium in the wound was similar to that measured when the inner surface of a pericarp was inoculated. Sections were incubated on porcelain depression plates resting on wet paper towels in a plastic snap-lid container. Spoilage was usually not observed during an up to 96 h of incubation. Weight loss was minimal. Inocula (*Erwinia carotovora* or *S.* Typhimurium) migrated through the sections within 120 min, based on movement of a water-soluble dye and colony development after inoculated sections were removed from CVP or XLT media, respectively. In most tests on proliferation of *S.* Typhimurium, section weight was not a significant co-variable. However, somewhat greater proliferation per wound was detected with section diameter 19.5 mm as compared with 11.5 mm. Incubation of pericarp sections at 35 versus 22°C consistently enhanced proliferation of *Salmonella* by ca. 2.5-fold in 24 h and more than 10-fold within 48 h. Populations ranged from Log 6.7 cfu/wd after 24 h at 22°C to Log 8.8 cfu/wd after 48 h at 35°C.

Extraction of *Pratylenchus* sp. and *Hoplolaimus* sp. from corn roots using two methods and two extraction solutions

M. BATISTA DA SILVA (1), G. L. Tylka (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.9

Plant-parasitic nematodes occur in most fields where corn is grown in Iowa, although most are not thought to be damaging to the crop at low population densities. The types and numbers of nematodes present must be known to assess if yield loss is occurring and management is necessary. Some of these nematodes exist within roots as well as in soil and must be extracted from the roots to identify and count. The recovery of root-lesion (*Pratylenchus* sp.) and lance (*Hoplolaimus* sp.) nematodes from corn root tissue using two extraction methods and two solutions were compared in laboratory experiments. Corn plants were grown in soil infested with both nematodes. After 60 days, roots were washed of soil and cut into 1-cm-long fragments. A 3-g root sample from each plant was assigned to an extraction solution and method combination. Treatment combinations were replicated six times, and the experiment was conducted twice with consistent results. More root-lesion nematodes were extracted from roots after seven days on a platform shaker than in a Baermann funnel. Also, more were extracted from roots in water than in a dihydrostreptomycin sulfate-mercuric chloride (strep-HgCl) solution with the platform shaker, but not with Baermann funnels. More lance nematodes were recovered using the platform shaker than the Baermann funnel, and more were recovered using water than the strep-HgCl solution. There was no interaction between extraction method and solution for lance nematode.

The first report of *Columnea latent viroid* (CLVd) in tomato in West Africa

O. BATUMAN (1), R. L. Gilbertson (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.9

Virus-like symptoms, including stunted growth, epinasty and chlorosis of leaves, and necrosis of leaf veins and stems were observed in 1-5% of tomato plants (*Solanum lycopersicum*) in field surveys conducted in Ghana and Mali in 2011. Representative leaf samples were tested with PCR or RT-PCR for known tomato-infecting DNA and RNA viruses, phytoplasma and '*Candidatus* Liberibacter'. Results of these tests were negative, but a putative virus-like agent was transmitted to tomato seedlings following rub-inoculation with sap prepared from a sample from Niono, Mali. Furthermore, tomato seedlings rub-inoculated with total RNA extracts from leaves of symptomatic tomato plants developed similar symptoms, indicating that the casual agent might be a viroid. RT-PCR tests with RNA extracts from symptomatic tomato leaves and universal- and specific-pospiviroid primer sets were negative. However, a PCR fragment of the expected size (~370 bp) was amplified by RT-PCR with the pCLV4/pCLVR4 primer pair, specific for *Columnea latent viroid* (CLVd). The sequence of this PCR fragment (GenBank accession no. JQ362419) was 99% identical with that of an isolate of CLVd from the Netherlands (accession nos. AY373446 and AY372396). In host range studies, the African CLVd isolate only induced symptoms in tomato, although symptomless infection was detected in pepper (*Capsicum annuum*), *Nicotiana*

benthamiana and *N. tabacum* plants by RT-PCR. This is the first report of CLVd in West Africa, and it may have been introduced in association with tomato seed.

Identification of tree-crop rootstocks with resistance to Armillaria root disease

K. BAUMGARTNER (1), P. Fujiyoshi (1), D. Kluepfel (1), G. Browne (1), C. Leslie (2)
(1) USDA-ARS, Davis, CA, U.S.A.; (2) Department of Plant Sciences, University of California, Davis, CA, U.S.A.
Phytopathology 102:S4.10

Armillaria root disease attacks a broad range of tree crops in California. Instead of re-tooling ineffective conventional controls, namely soil fumigation, we focused on identification of *Armillaria*-resistant *Juglans* rootstocks. Our work is part of a collaborative project to identify rootstocks with resistance to several major root diseases (e.g., crown gall). Plants were grown in a standard tissue-culture medium, which supports both the plant and the pathogen, *A. mellea*. This approach overcame the obstacles of greenhouse inoculations by eliminating escapes and facilitating repeatable mortality starting at 3 weeks post-inoculation. Rootstocks were challenged with three *A. mellea* strains in three replicate experiments. We inoculated paradox hybrids (*J. regia* x black walnut; AX1, PX1, RX1, RR4-11A, Vlach, and VX211), Northern California black walnut (*J. hindsii*) 'W17', English walnut (*J. regia*) 'Chandler', and relative *Pterocarya stenoptera* 'WNxW'. At 2 months post-inoculation, AX1, PX1, and RX1 exhibited the lowest percent mortality for all strains of *A. mellea* in all three experiments (10, 31, and 34%, respectively; n = 99 observations). The most susceptible to *A. mellea* infection were VX211, W17, and WNxW (70, 86, and 94% mortality, respectively). Applications of these findings to the field are difficult to evaluate, due to inconsistent observations and poor design of past field trials. As such, we are establishing additional inoculations in both the lab and field to help validate our results.

Developing an integrated approach to cucumber downy mildew management

F. BAYSAL-GUREL (1), J. Mera (1), S. A. Miller (1)
(1) The Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.10

Downy mildew (DM) is a highly destructive disease of cucumber in the eastern US. The efficacy of fungicide treatments against DM and risks and benefits of using extended spray intervals (10-or 14-days vs. 7-days) with 'Tasty Green' (moderately resistant) and 'Intimidator' (susceptible) varieties were assessed in 2010 and 2011. The effects of treatment and variety on DM severity were significant. Across all treatments DM severity was higher and progressed more rapidly on 'Intimidator' than on 'Tasty Green'. With the exception of Bravo WeatherStik 6SC applied to 'Tasty Green' on a 10-or 14-day spray schedule, all treatments significantly reduced DM compared to the non-treated control of the same cultivar. Three additional applications of Bravo WeatherStik 6SC significantly reduced DM severity on 'Tasty Green'. There was no differences in DM severity on 'Intimidator' between the 7-and 14-day Bravo Weatherstick 6SC application schedules in 2010 and no difference on "Tasty Green" between the 7-and 10-day application schedules of Presidio 4SC+BravoWeatherStik 6SC alternated with Ranman 400SC+Bravo WeatherStik 6SC or the 7-day application schedule of Bravo WeatherStik 6SC in DM severity in 2011. DM severity throughout the season was higher in 'Intimidator' treated with BravoWeatherstick 6SC alone than Presidio 4SC+Bravo WeatherStik 6SC alternated with Ranman 400SC+Bravo WeatherStik 6SC. There was a significant effect of treatment and variety on marketable yield in 2011.

Assessing organic vegetable growers' beliefs regarding soilborne disease management

F. BAYSAL-GUREL (1), K. Parajuli (2), B. McSpadden Gardener (1), G. Norton (2), S. A. Miller (1)
(1) The Ohio State University, Wooster, OH, U.S.A.; (2) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 102:S4.10

Soil-borne diseases can be economic threats to both conventional and organic production systems. It is important to understand the needs, knowledge, perceptions, and challenges of organic vegetable growers regarding their management. Major factors influencing organic growers' perspectives and practices, and potential gaps in knowledge were identified from qualitative companion models and a survey-based confirmatory assessment of organic growers in OH, MD, MI, NY, PA, VI, IN,IL, WV and KY. The survey consisted of four sections: organic farming activities, grower perspectives, response and communication and grower information. Descriptive data from 93 growers were analyzed. The majority indicated that foliar (86.9%) and soil-borne diseases (68.5%) were a problem on their farms. 40.8% and 35.5% of the participants, respectively, indicated they strongly agreed or somewhat agreed that disease management was a challenge or obstacle. Just overhalf of the participants indicated that they were somewhat familiar with soil-borne disease management practices (51.6%) and 5.4% of the growers indicated that they were very familiar. The most cited sources for assistance on soil-borne diseases were other organic growers, organic farming organizations and university extension. The majority of the growers (94.6%) indicated that they need more information about soil-borne disease management in organic vegetable production.

An expert perspective on the organic vegetable grower decision-making process related to soilborne disease management

F. BAYSAL-GUREL (1), B. McSpadden Gardener (1), S. A. Miller (1)
(1) The Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.10

Organic production is one of the fastest growing segments of U.S. agriculture. Soil-borne diseases are a major limitation to vegetable production. It is important to understand the characteristics, management strategies, information sources, perceptions, opinions, and challenges of organic vegetable growers regarding soil-borne diseases. The research findings reported here are the first of five stages proposed to improve the effectiveness of on-organic farm decision making related to soil-borne disease management. Meetings with experts were conducted to develop a summary of expert knowledge. The resulting comprehensive, technical model consists of six major content areas, 56 embedded subcontent areas, 41 embedded sub-subcontent areas and 19 embedded individual concepts. This expert model was used as the analytical framework for subsequent companion surveys of organic vegetable growers to describe and visualize how organic vegetable grower concepts map onto the framework to identify specific opportunities for future research, education, and extension efforts.

The development of a mobile app for the diagnosis and management of ornamental plant health problems

J. BECKERMAN (1), C. Sadof (1), S. Koenig (1), A. Witte (1)
(1) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 102:S4.10

The iPod/iPad technology is a cultural phenomenon that has tremendous potential to assist users in the diagnosis and management of plant health problems, and in the education of practitioners that require these skills. Unlike much of the current educational work (i.e., podcasting), this project focused on developing an interactive and integrated field guide to assist learners in the diagnosis and management of ornamental plant health problems, and includes a database of 225 pests, diseases and disorders, and over 1000 images. Unlike a static field guide, the developed app was designed to better accommodate diverse learning styles and engage users' visual and tactile senses. Thus, we adopted a 'differentiated instruction and learning' approach and provided three different search menus (host, pathogen, or by name) to access information. This results in an app that is presented more like a website that allows users to explore, as opposed to a downloaded book or pdf that forces learners into a linearity they may not desire. These multiple entry points into the content can be especially valuable in formal educational settings, in that it offers greater accommodation to the many learning styles of a diverse group of students. Ultimately, the goal is to turn users into "citizen-scientists" that can provide data to monitor plant health problems across the urban landscape nationwide.

Zoospore lysis occurs in sporangial suspensions made from petunia late blight lesions

M. C. BECKTELL (1)
(1) Mesa State College, Grand Junction, CO, U.S.A.
Phytopathology 102:S4.10

Zoospores are an important stage in the pathogenicity of *Phytophthora infestans*, the causal agent of late blight of potatoes, tomatoes, and petunias. Of these hosts, petunias are known to be the least susceptible to the disease. During investigations of tomato and petunia late blight, we observed lysis of zoospores released into leachate from petunia late blight lesions. If sporangia produced from petunia lesions were washed before being incubated in conditions favorable to zoospore release, the zoospores did not lyse. Zoospores released into leachate from tomato late blight lesions did not lyse. Addition of petunia late blight lesion leachate to sporangia produced from tomato late blight lesions caused the subsequently released zoospores to lyse. Lysis typically occurred within a few minutes of exposure. Germination rates and infection efficiency of washed and unwashed sporangia obtained from petunia lesions were compared to sporangia obtained from tomato lesions. Sporangia produced on tomato late blight lesions had a higher total

germination rate (78%) than did sporangia produced from petunia late blight lesions, whether they were washed (61%) or unwashed (41%). Washed sporangia from petunia lesions had a higher infection rate compared to unwashed sporangia from petunia and tomato lesions. The identity of the factor(s) responsible for lysis and suppressed germination is currently under investigation.

Characterizing *Xylella fastidiosa* subsp. *multiplex* in symptomatic northeastern and mid-Atlantic oak trees

G. BEHRINGER (1), A. B. Gould (1), D. Kobayashi (1)
(1) Rutgers University, New Brunswick, NJ, U.S.A.
Phytopathology 102:S4.11

Xylella fastidosa is the causal agent of bacterial leaf scorch of oak. Although its pathogenic mechanisms are not fully understood, the bacterium is thought to produce symptoms via vascular establishment, occlusion, and repeated reestablishment through episodic planktonic migration. Although some strains of the pathogen have been well described, strains infecting native hardwoods have not. Data from 2002-2011 studies concentrated in New Jersey as well as in the mid-Atlantic and Northeastern regions of the United States have confirmed that disease severity has increased within affected populations of susceptible oaks and has spread to previously asymptomatic individuals. Since this vectored generalist appears to have an ever expanding host range, these pathogen reservoirs can no longer be ignored. In an attempt to further define geographically distinct *Xylella fastidiosa* subsp. *multiplex* strains isolated from oak, pathogenicity-focused multilocus sequence analysis was considered in addition to whole genomic sequencing. Comparison to existing strains and large sample clade establishment may help to predict future hosts and environmental and commercial impact.

Transmission and population frequency of viruses in the soybean cyst nematode

S. BEKAL (1), J. P. Bond (1), K. N. Lambert (2), A. M. Fakhoury (1)
(1) Southern Illinois University, Carbondale, IL, U.S.A.; (2) University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.
Phytopathology 102:S4.11

Viruses have not been exploited for phytoparasitic nematode control because few nematode infecting viruses have been identified. Recently, four viruses were discovered that infect *Heterodera glycines*, the soybean cyst nematode (SCN). The viruses must be efficiently transmissible to nematodes to be effective biological control agents. Our objectives were to identify mechanisms of nematode virus transmission and to assess virus frequency in natural and laboratory SCN populations. Viruses were detected in SCN populations using a relative quantitative reverse transcription PCR assay. All developmental stages of SCN, including eggs, juvenile stages, adult females and males were tested and found to contain viruses. This suggests that viruses are transmitted vertically (transovarial), but could also be transmitted via the male during mating. The application of a mixture of viruses to uninfected SCN showed that two viruses had the ability to infect SCN. This indicates a possible route of horizontal transmission of the viruses. While laboratory cultures of SCN showed high levels of viral infection, only 10% of wild SCN populations harbored the viruses. Our studies show that laboratory SCN populations can have more than one thousand times the viral load found in natural populations. Laboratory SCN populations were also suppressed in their reproduction, suggesting that some of the SCN viruses may be exploited as biological control agents.

Plant health benefits of strobilurin fungicide applications incorporated within a programmatic fungicide approach on creeping bentgrass

J. J. BENELLI (1), B. J. Horvath (1), J. T. Brosnan (1), D. A. Kopsell (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 102:S4.11

Pyraclostrobin, a member of the strobilurin class of fungicides, has recently been labeled for plant health benefits in addition to its fungistatic properties. A fungicide that controls disease causing organisms while potentially reducing the impact of abiotic disorders could enhance the quality of turf during stressful conditions. An experiment was conducted on a creeping bentgrass (*Agrostis stolonifera* L.) putting green located at Ruggles Ferry Golf Club in east Tennessee to better understand the plant health benefits associated with strobilurin fungicides when integrated within a summer fungicide program. The four strobilurins labeled for turfgrass applications, pyraclostrobin, azoxystrobin, fluoxastrobin, and trifloxystrobin, were applied at their highest label rates of 0.55, 0.61, 0.55, and 0.38 kg ai ha^{-1}, respectively on 15 June and 27 July during 2011. Normalized Difference Vegetation Index (NDVI), Digital Image Analysis (DIA), and visual turfgrass quality were evaluated 14 days post strobilurin application. Significant differences were not detected for NDVI, DIA, or visual turfgrass quality between any of the strobilurin containing fungicide programs and the untreated control. Our results suggest that strobilurin fungicides may not provide a field observable promotion of plant health, beyond that of disease control, in certain varieties of creeping bentgrass.

Genome sequencing of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 strain II5

A. S. BERG (1), M. Dita (2), T. Nan (1), T. Shea (3), S. Zhou (4), W. Jonkers (5), Q. Zeng (3), S. Young (3), M. E. Beleza Yamagishi (6), P. Giachetto (6), R. Herai (7), M. Souza (8), C. Waalwijk (8), G. Haatje Jan Kema (8), H. Kistler (5), L. Ma (1)
(1) University of Massachusetts at Amherst, Amherst, MA, U.S.A.; (2) Bioversity International, Turrialba, Costa Rica; (3) Broad Institute, Cambridge, MA, U.S.A.; (4) University of Wisconsin-Madison, Madison, WI, U.S.A.; (5) University of Minnesota, St. Paul, MN, U.S.A.; (6) Embrapa Bioinformatics, Campinas, Brazil; (7) University of Campinas, Campinas, Brazil; (8) Plant Research International, Wageningen, Netherlands
Phytopathology 102:S4.11

Fusarium oxysporum f. sp. *cubense* (*Foc*) is the causal agent of Fusarium wilt of banana, also known as Panama disease. Tropical race 4 (TR4) of *Foc* emerged in the early 1990s and is spreading throughout Asia and northern Australia. There are no effective treatments or viable export cultivars resistant to *Foc* TR4. A genome assembly of *Foc* TR4 strain II5 has been generated using Illumina sequencing technology. A combination of paired-end reads from 180 bp fragments and 5kb insert libraries delivered a high quality assembly with a total of 46.5M assembled bases in 418 scaffolds of 1.13 N50 scaffold. Based on kmer estimation, the II5 genome size is approximately 51Mb, including about 4.3Mb lineage specific (LS) sequences that are highly repetitive, a characteristic described in other LS sequences within *F. oxysporum* f. sp. *lycopersici*. An initial genome annotation was conducted, incorporating RNA-seq data generated with 454 sequencing technology. We will report the preliminary analysis of the genome and the annotation results, with a focus on detection of potential virulence factors, such as effectors, to further elucidate the methods of pathogenesis of TR4. Additionally, we will validate the completeness of the current assembly by comparing independently generated transcripts. Further understanding of the profile of the effectors secreted between the four races of *Foc* may speed development of TR4 resistant banana cultivars as well as integrated management strategies.

Identification and characterization of genes conferring resistance to the photoactivated *Cercospora* toxin cercosporin

A. BESELI (1), S. Herrero (1), M. E. Daub (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.11

Cercospora species produce the photoactivated, active-oxygen-producing toxin cercosporin. Because cercosporin has broad-spectrum toxicity, we are interested in understanding toxin resistance mechanisms in *Cercospora* species. A suppressive subtractive hybridization library was recovered of genes differentially regulated between the cercosporin-resistant wild-type and a toxin-sensitive mutant deficient for a transcription factor required for resistance. We hypothesize that some of the 185 genes recovered are involved in cercosporin resistance. Up-regulation of these genes under conditions of cercosporin toxicity was tested by quantitative RT-PCR analysis in a cercosporin-sensitive *C. nicotianae* transporter mutant exposed to cercosporin. Of 33 genes tested, six were found to be significantly upregulated. These included genes for two transporters, two hypothetical proteins, hydroxynicotine oxidase and cyanide hydratase. Gene disruption mutants for the cyanide hydratase were not altered in cercosporin resistance as compared to wild type. Further, transformation of the cyanide hydratase gene into the cercosporin-sensitive fungus, *Neurospora crassa* confirmed no increase in cercosporin resistance. By contrast, transformation of *N. crassa* with one of the transporter genes significantly increased cercosporin resistance. Efforts are continuing to characterize the putative resistance genes by gene disruption in *C. nicotianae* and transformation in *N. crassa*.

First report of *Phomopsis amygdali* causing dieback of *Pieris japonica* in the United States

J. C. BIENAPFL (1), Y. Balci (1)
(1) University of Maryland, College Park, MD, U.S.A.
Phytopathology 102:S4.11

Japanese andromeda (*Pieris japonica*) is an economically valuable floricultural plant used in landscapes across the United States. In 2010 and 2011, P. japonica cultivar Mountain Fire plants growing in a Maryland nursery were observed with symptoms of dieback. All plants were shipped from a West Coast nursery. Necrosis was evident on shoot tips and often advanced into lateral shoots, as well as down to the crowns. Symptomatic shoots were

surface disinfested and plated on potato dextroxe agar amended with streptomycin sulfate. The resulting isolates had morphological characteristics that were consistent with those for *Phomopsis* species. Six representative isolates were grown in potoato dextrose broth and mycelium was used to extract genomic DNA. The internal transcribed spacer and translation elongation factor 1-alpha loci were sequenced for each isolate and subjected to BLAST searches. The isolates shared 100 and 99.6% identity with known sequences of *Phomopsis amygdali* for each locus, respectively. Inoculation experiments are underway to demonstrate Koch's postulates and results from pathogenicity experiments will be presented. This is the first association of this pathogen with an ornamental plant species and its impact remains to be determined.

Development of diagnostic assays for detection of *Verticillium* in alfalfa and flax and detection of blackleg (*Leptosphaeria maculans*) in canola using real-time PCR

G. J. BILODEAU (1), R. Tropiano (1), S. C. Briere (1)
(1) Canadian Food Inspection Agency, Ottawa, ON, Canada
Phytopathology 102:S4.12

Canada is an significant producer of grains and oilseeds. For phytosanitary export certification, methods to identify plant pathogens associated with grain and seeds is important. Wilt of alfalfa caused by the *Verticillium albo-atrum* (Vaa) is one of the most damaging diseases of alfalfa. Recent work shows Vaa forming 3 separate groups with one infecting alfalfa. Some countries require that alfalfa hay and seed must be exempt of Vaa. In order to meet these requirements, a qPCR assay for Vaa of alfalfa using the rDNA intergenic region was developed. In addition a *Verticillium dahliae* (Vd) assay for detection from flax grain was also used. Specific primers with a specific probe for Vaa alfalfa and Vd in flax were designed and tested for specificity and sensitivity with multiple *Verticillium* species from different hosts. The Blackleg of brassicas caused by *Leptosphaeria maculans* and *L. biglobosa* (less virulent), is a disease of canola found in Canada. On seeds, no symptoms are visible, showing the importance of having molecular method for detection. To improve sensitivity and high volume processing of samples for blackleg in canola, a real-time PCR adapted from conventional PCR already in use, using ITS region was developed using SYBRGreen and Taqman probe. The sensitivity of the assay was improved by several orders of magnitude and which allowed the reduction of sub samples from 4 to 2. A panel of naturally and artificially infested seeds was tested for specificity and a sensitivity of 3 fg was calculated.

Characterization of *CbCyp51* from field isolates of *Cercospora beticola*

K. BIRLA (1), M. Bolton (2), V. Rivera (1), K. Rudolph (1), G. Secor (1)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS, Fargo, ND, U.S.A.
Phytopathology 102:S4.12

The hemibiotrophic fungus *Cercospora beticola* causes leaf spot of sugar beet. Demethylation inhibitors (DMIs) are an important group of fungicides used to manage leaf spot. These fungicides act by inhibiting cytochrome P450-dependent sterol 14α-demethylase (CYP51), which is required for biosynthesis of the cell membrane component ergosterol in fungi. Resistance to DMIs have been reported in many fungal pathogens, including *C. betocola.* The purpose of this study was to determine the molecular basis of DMI resistance in *C. beticola.* The study was initiated by cloning *CbCyp51*, which is a single copy 1,632-bp intron-free gene with homology to other fungal *Cyp51* genes. Because resistance to DMIs can be related to polymorphism in promoter or coding sequences, >2,440 nucleotides were sequenced encompassing *CbCyp51* coding and flanking regions from isolates with varying EC_{50} values (effective concentration to reduce growth by 50%) to DMI fungicides. However, no mutations or haplotypes were associated with DMI resistance or sensitivity. No evidence of alternative splicing of *CbCyp51* was found that might explain reduced sensitivity to DMIs. However, *CbCyp51* was overexpressed in isolates with high EC_{50} values compared to isolates with low EC_{50} values. After exposure to tetraconazole, isolates with high EC_{50} values responded with further induction of *CbCyp51*, with a positive correlation of *CbCyp51* expression and tetraconazole concentration up to 2.5 $\mu g\ ml^{-1}$.

Fusarium oxysporum* produces volatile organic compounds that affect the growth and disease defense of *Arabidopsis thaliana

V. BITAS (1), S. Kang (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.12

Fusarium oxysporum is a cosmopolitan soil-borne plant pathogen well-known for causing wilt symptoms in a large number of plant species. However, not all isolates are plant pathogenic, and some of the non-pathogenic isolates even function as biocontrol agents protecting plants from other pathogens through various mechanisms. Our research indicates that certain *F. oxysporum* isolates produce volatile organic compounds (VOC) that promote shoot and root growth, as well as alter morphological and physiological properties of the model plant *Arabidopsis thaliana*. In order to analyze and comprehend the mode of action by which those volatiles affect plant growth we have employed an array of *A. thaliana* mutants, defective in hormonal regulatory pathways controlling plant growth. We are also trying to isolate and identify those compounds through the use of gas chromatography and mass spectrometry. Finally, certain growth-promoting isolates also seem to enhance plant resistance against the bacterial pathogen *Pseudomonas syringae*. Identification of the fungal VOC underpinning these plant alterations and the elucidation of their nature and mode of action will help us better understand volatile-mediated plant-microbe interactions in the complex soil environment and enhance our understanding of the nature and mechanisms of this poorly studied area of soil-ecology and plant-microbe interactions.

Effect of simultaneous application of selected fungal endophytes and *Coniothyrium minitans* against sclerotia of *Sclerotinia sclerotiorum*

N. BITSADZE (1), J. Strauss (2), H. R. Dillard (2)
(1) Agricultural University of Georgia, Tibilisi, Georgia; (2) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.12

Sclerotinia sclerotiorum is a geographically cosmopolitan, soilborne, non-specific, plant pathogenic fungus that can attack a wide range of plants, including many economically important crops. *Coniothyrium minitans* is a naturally occurring fungal mycoparasite of sclerotia formed by *Sclerotinia* spp. and has been shown to be an effective biological control agent in several crops. *Beauveria bassiana*, *Metarhizium brunneum* and *Isaria fumosorosea* are well known as entomopathogenic fungi, with worldwide distribution. These fungi have been recovered as endophytic colonists from several plant species and have been shown to protect plants against plant pathogens. A bioassay was conducted to observe the biocontrol potential and interaction of the fungal endophytes (*M. brunneum* Strain F 52, *B. bassiana* Strain GHA, *I. fumosorosea* Strain 97) and *C. minitans* against sclerotia of *S. sclerotiorum.* Two to three weeks after treatment, *C. minitans* and *M. brunneum* exhibited additive interaction at concentrations $10^2 – 10^6$ sp/ml but at the concentration 10^7 sp/ml interaction were antagonistic. *C. minitans* and *B. bassiana* at low concentrations $10^2 – 10^3$ sp/ml interaction were additive but at concentrations $10^4 – 10^7$ sp/ml they were antagonistic. At concentrations $10^2 – 10^3$ sp/ml, the interaction between *C. minitans* and *I. fumosorosea* was synergistic and at concentrations $10^4 – 10^7$ sp/ml the interactions were additive.

Effect of temperature on resistance to bacterial wilt, caused by *Ralstonia solanacearum*, in tobacco cultivars

R. BITTNER (1), A. Mila (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.12

Resistance to *Ralstonia solanacearum* (Rs) was investigated in six flue-cured tobacco cultivars: K346 and Speight168 (highly), NC71 (moderate), and K326, RJR15, and RJR75 (low) resistant, at 10, 15, 20, 25, 30, and 35°C. Inoculations were performed with four strains of Rs collected in North Carolina in 2007 from stems of diseased tobacco plants. The highest disease incidence, measured as area under the disease progress curve (AUDPC), was observed in all cultivars at 30 and 35°C 18 days after inoculation; no symptoms were observed at 10 and 15°C with any strains on any cultivars. Temperature, cultivar, and strain were all significant to AUDPC values. The mechanism of resistance to Rs was examined in histological studies conducted at 10, 20, and 30°C. Strain AW1-gfp38, expressing green fluorescent protein, was used for these experiments. We observed faster bacterial colonization in cultivars incubated at 30 than at 20 or 10°C. At 30°C, the only difference among cultivars was the rate of colonization in the stem. At 20°C, infections occurred, but colonization was limited to the site of infection in all cultivars. At 10°C, the pathogen infected epidermal cells but rarely progressed to the xylem vessels of the roots in all cultivars. Based on our results, we suggest that the mechanism of resistance to Rs in flue-cured tobacco cultivars is associated with the ability to limit colonization of stem tissues.

Identifying resistance to Sclerotinia stalk and root rot in perennial sunflower germplasm

C. BLOCK (1), L. F. Marek (2), T. J. Gulya (3)
(1) USDA-ARS, Ames, IA, U.S.A.; (2) Iowa State University, Ames, IA, U.S.A.; (3) USDA-ARS, Fargo, ND, U.S.A.
Phytopathology 102:S4.12

The objective of the research was to identify resistance to Sclerotinia stalk and root rot in perennial sunflower species from the USDA germplasm collection. Two diploid species, *Helianthus grosseserratus* and *H. salicifolius*, and four

hexaploid species, *H. californicus*, *H. pauciflorus*, *H. resinosus*, and *H. tuberosus* were evaluated in greenhouse trials. Screening was conducted by placing *Sclerotinia*-infested millet into individual cells of plastic flats and transferring one seedling with 6 to 8 leaves into each cell. Plants were monitored for days to permanent wilt and accessions were ranked on the basis of plant survival after 21 days. All of the perennial species showed remarkable resistance. From the 144 accessions tested across six species, 106 accessions had 90% or more surviving plants. By comparison, the most resistant hybrid check, Croplan 305, ranged from 40-55% plant survival and the susceptible check ranged from 0-5% survival. Forty-four accessions had 100% plant survival including 7 of 10 accessions from *H. californicus*, 8 of 14 from *H. resinosus*, 7 of 14 from *H. salicifolius*, 9 of 38 from *H. tuberosus*, and 13 of 37 from *H. grosseserratus*. Five of 31 *H. pauciflorus* accessions had 95% or better plant survival, with the highest at 98%. The results demonstrate that perennial sunflowers may be a potentially valuable source of *Sclerotinia* resistance genes for improving cultivated sunflower.

Ground application provides adequate control of pecan scab on tall pecan trees in moderate to low epidemic risk years

C. H. Bock (1), B. W. Wood (1), M. H. HOTCHKISS (1), T. E. Cottrell (1)
(1) USDA-ARS-SEFTNRL, Byron, GA, U.S.A.
Phytopathology 102:S4.13

Fungicides are used to manage pecan scab (*Fusicladium effusum*), which can develop in the upper canopies of tall trees, beyond the reach of air-blast sprayers, thus justifying aerial application. One experiment in 2010 and two in 2011 (cvs. Desirable and Wichita) investigated the distribution of scab in fungicide-treated pecan trees (>12.5 m tall). In 2010, propiconazole (Orbit, 4 applications @ 0.59 L/ha) was followed by triphenylytin hydroxide (TPTH, Supertin 4L, 2 applications @ 0.54 L/ha) and in 2011 only TPTH was applied (7 applications). Leaf and fruit samples from 4 replicate trees at 0-5, 5.0-7.5, 7.5-10, 10-12.5 and >12.5 m were assessed for disease. A Cerium (Ce) tracer was used to monitor spray coverage on two treated trees in 2010. GC-MS quantification showed approx 40% of the total Ce collected below 5 m reached 7.5-10 m, and <11% reached >12.5 m. General linear modeling showed no difference in disease severity at any height (F=0.31-2.2, P=0.07-0.9), except for Wichita in 2011 when there more severe disease in the lower canopy (F=2.41, P=0.05). There was no linear relationship between disease severity and height. The disease epidemic in 2010 was moderate, but light during the dry 2011 season. Thus, in average or low-inoculum pressure years, there does not appear to be greater scab severity in the upper canopy of fungicide-treated trees compared to the lower canopy suggesting that aerial application to manage disease would not be warranted.

Predisposition of citrus foliage to infection with *Xanthomonas citri* subsp. *citri*

C. H. BOCK (1), J. H. Graham (2), A. Z. Cook (3), P. E. Parker (3), T. R. Gottwald (4)
(1) USDA-ARS SEFTNRL, Byron, GA, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.; (3) USDA-APHIS, Edinburg, TX, U.S.A.; (4) USDA-ARS, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.13

Citrus canker (caused by *Xanthomonas citri* subsp. *citri*, *Xcc*) is a serious disease of susceptible citrus in Florida and other citrus-growing areas of the world. The specific effects of predisposing factors for bacterial penetration of leaves are poorly characterized. Experiments were designed to investigate the effects of wind and rain (to simulate a storm), high humidity (>90%) and mild abrasion with sand (to simulate wind blow sand and debris) on infection of citrus foliage with *Xcc*. Exposure of leaves of Swingle citrumelo seedlings to wind (16 m sec^{-1}) and rain (235 mm h^{-1}) for 15 or 30 min caused significant injury and leaves developed up to two fold as high an incidence and 10-fold as high severity compared to leaves on seedlings of non-treated control plants. The points of attachment of the lamina to the petioles were particularly susceptible to wind-induced infection with up to 25% showing disease symptoms compared to 0% for the non-treated control. Over 80% of injured leaves had lesions associated with the site of injury. There was little or no effect of humidity >90% for 1.5 or 2.5 h on disease incidence or severity compared to the non-treated control. Mild leaf abrasion of grapefruit seedling leaves with sand increased incidence and severity of disease two-fold. Minimizing wind speed in orchards via windbreaks will reduce foliar injury and decrease canker incidence and severity.

The effect of height on severity of pecan scab in nontreated mature pecan trees

C. H. BOCK (1), B. W. Wood (1), T. E. Cottrell (1)
(1) USDA-ARS SEFTNRL, Byron, GA, U.S.A.
Phytopathology 102:S4.13

Pecan scab (*Fusicladium effusum*) is a destructive disease of pecan. There is concern that disease in the upper canopy of tall trees is difficult to control using ground-based sprayers. To establish a basic understanding of the distribution of scab in a non-treated pecan canopy, the vertical distribution of disease was assessed in three separate experiments in 2010 and 2011 (moderate and light scab years, respectively) on cvs. Desirable and Wichita. Samples of 5 or 10 leaves or fruit were collected from 4 replicate trees at heights of 0-5, 5.0^{+}-7.5, 7.5^{+}-10, 10^{+}-12.5 and 12.5^{+} m and assessed for disease. General linear modeling demonstrated disease was greatest <5m above ground, and least at heights >12.5 m (F=2.55-12.3, P=<0.0001-0.04). Only on foliage of cv. Desirable in June 2011 was there no significant effect (F=0.86, P=0.9). However, disease severity was variable in the mid-section of trees. There was a slight but significant negative linear relationship between sample height and disease severity on fruit on cvs. Wichita and Desirable in August 2011 (P=0.04, R^2=0.22 and P=0.0006, R^2=0.49) and on Desirable in October 2011 (P=0.05, R^2=0.20), but not on foliage on Desirable in 2010, or Wichita or Desirable in June 2011 or on fruit in October 2010. Disease severity is often greatest at the base of the canopy, and least at the apex of the tree, but the precise distribution likely depends on sources of inoculum, canopy architecture and the environment.

Resistance of sugarcane varieties to *Puccinia kuehnii* in Brazil

J. BOMBECINI (1), C. Gonçalves (2), I. Ascencio (3), A. Urashima (1)
(1) Universidade Federal de Sao Carlos, Araras, Brazil; (2) Centro Tecnologia Canavieira, Piracicaba, Brazil; (3) ESALQ, Universidade Sao Paulo, Piracicaba, Brazil
Phytopathology 102:S4.13

Sugarcane covers 8.3 million hectares in Brazil with production of 571,471 million ton. The availability of more than 20 different varieties has been a great asset to cope with many challenges the crop have faced. The most recent threat to sugarcane industry in Brazil was the outbreak of orange rust in 2009. Field observations have identified varieties with high susceptibility and they are not planted any longer. Nevertheless, sporadic reports of orange rust still exist in some regions. Therefore, the present work aimed to assess orange rust resistance of 10 varieties/lineages on a scientific basis. Inoculation was made by spraying uredioniospore suspension (3 x 105 spores/ml, germination 40%) on plants of 4 weeks until run off and keeping them in water saturated atmosphere for 20h at 25 °C. Varieties were evaluated for their reaction to rust 14 days after inoculation based on their severity (number and length of pustules). Data showed RB72454 as susceptible and RB975242, RB975157, RB966928, RB855453, RB867515 and RB935744 as resistant. Intermediate varieties included: RB92579, RB855156, and RB975201. Those intermediate varieties should be monitored closely since occasional epidemics might happen under favorable conditions.

Effects of dew-period temperature changes on initiation of infection in soybean by *Phakopsora pachyrhizi*

M. R. BONDE (1), S. E. Nester (1), D. K. Berner (1)
(1) USDA-ARS, Frederick, MD, U.S.A.
Phytopathology 102:S4.13

Our previous research suggested night-time and early morning dew-period temperatures in much of the U.S. are highly conducive for soybean rust. During these studies, dew-period temperatures were held constant. However, recognizing that dew-period temperatures are rarely, if ever, constant in nature, we decided to determine what effects temperature changes during the dew period might have on urediniospore germination and infection. Williams 82 plants were inoculated with isolate Alabama 04-1 at $2\text{-}3X10^4$ urediniospores/ml 0.01% Tween 20, then incubated in dew 3 or 6 h at selected temperatures above the optimum (20°C) dew-period temperature for infection, or conversely 3 or 6 h below the optimum. Plants then were transferred to a 20°C dew chamber for the remainder of the 24-h dew period. Plants at 20°C the entire dew period served as controls. Water agar dishes seeded with urediniospores accompanied each plant. At the end of the dew periods, dishes were examined for germination and plants placed in the greenhouse. By 2 weeks, in each of four experiments, twice as many lesions had developed on plants that had begun the dew period at 29 or 32°C and then were transferred after 3 h to 20°C than on plants at 20°C the entire dew period. No or few urediniospores germinated at a constant 29 or 32°C. The study showed infection was more efficient with a declining dew-period temperature, and supported the original conclusion.

Evaluation of the effect of butternut canker on the genetic diversity of regenerating butternut in New England

A. BORAKS (1), K. Broders (1)
(1) University of New Hampshire, Durham, NH, U.S.A.
Phytopathology 102:S4.13

Comprehensive knowledge of the genetic diversity for an endangered species is necessary for proper management and long-term survival. Genetic uniformity in trees can increase vulnerability to disease and in turn, reduces the stability of entire ecosystems. Butternut (*Juglans cinerea*) is currently being extirpated from its natural range in North America by the introduced fungal pathogen *Ophiognomonia clavigignenti-juglandacearum*. Whether sufficient genetic variability remains in butternut populations to adapt to environmental changes and resist disease is still unknown. The objective of this study is to compare the population structure of mature butternut, to that of regenerating butternut saplings. Age class, diameter at breast height (DBH), health, habitat and DNA were sampled and recorded from 158 butternut trees in 10 locations across New Hampshire and Vermont. Initial results indicate low levels of structure comparing juvenile (<20cm DBH) and adult (>20cm DBH) populations, with an F_{st} value of 0.022. Using a rare faction analysis, juvenile trees were estimated to have a 12% reduction in allelic richness, indicative of a potential genetic bottleneck. Sufficient sampling of regenerating butternuts, post infection, may provide insight of a selection towards genetic resistance. The molecular identification and genetic diversity of putatively resistant butternut trees will be used as the basis for future recovery projects.

WITHDRAWN

Optimization of copper resistance testing methods for foliar bacterial pathogens of tomato

S. BOST (1), J. Mixon (1), B. Ownley (2), K. Gwinn (2), C. Sams (2)
(1) University of Tennessee, Nashville, TN, U.S.A.; (2) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 102:S4.14

Copper is a primary control product for bacterial spot (*Xanthomonas* spp.), bacterial speck (*Pseudomonas syringae* pv. *tomato*) and bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) of tomato. There is a need for a technique that would accurately measure the level of resistance to copper in foliar bacterial pathogens of tomato in a protocol that would be practical for processing large numbers of samples. A protocol was developed that included colony growth measurement on solid medium amended with $CuSO_4*5H_2O$ (200 µg/ml), expressed as a percent of that on a non-amended control. Parameters were modified to produce copper sensitivity reactions that approximated *in-vivo* disease-control levels. Factors causing greatest variability in cell resistance to copper were medium composition, medium pH, and age of prepared medium. Freshly-prepared sucrose peptone agar adjusted to pH 6.8 (*Xanthomonas* spp. and *C. michiganensis* subsp. *michiganensis*) or 6.2 (*P. syringae* pv. *tomato*), provided copper sensitivity ratings that corresponded well with bioassay ratings. Because of its known low-complexing activity, 2-(*N*-morpholino) ethanesulfonic acid (20 mM) was used to counteract the depressing effect of copper on medium pH.

Investigation of the etiology of black choke and tangle top disease on perennial ornamental grasses

L. BOSTIC (1), L. Lacey (2), M. Benson (1), K. Ivors (2)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (2) Department of Plant Pathology, North Carolina State University, Mills River, NC, U.S.A.
Phytopathology 102:S4.14

Ephelis spp. infect a wide range of warm and cool season grasses, causing a disease known as 'black choke'. This disease results in white streaks of mycelium on the leaf blades and dense hyphal growth surrounding the panicle causing black, hardened spikes rather than the feather-like plume which gives ornamental grasses their desirability in the landscape. *Myriogenospora* spp. infect many of the same grass species as *Ephelis* spp., resulting in a tangled arrangement of the leaf blades and inflorescences known as 'tangle-top disease'. Both diseases reduce plant marketability and are becoming increasingly problematic for the ornamental grass industry. Due to the similar host range of these two fungi, we hypothesized that symptoms were caused by different stages (teleomorph vs. anamorph) of the same fungus. ITS sequences of isolates collected from infected tissues suggest that these fungi are closely related, but distinct. These fungi are often described as endophytes; however other researchers have found that *Ephelis* is essentially an epiphyte. Using a modified staining and clearing technique on host plant tissues, we determined that *Ephelis* hyphae were mostly restricted to epiphytic growth on the leaf blade. On occasion, hyphae did grow across and into leaf stomata, indicating that some endophytic growth is possible but infrequent. The biological interaction of these fungi and their host plants has yet to be resolved.

WITHDRAWN

Impact of fungicides on rust intensity and biomass yield of switchgrass

K. L. BOWEN (1), A. K. Hagan (1), J. Akridge (2)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Auburn University, Brewton, AL, U.S.A.
Phytopathology 102:S4.14

Switchgrass cv 'Dallas Blues' was transplanted in 2010 into a Benndale sandy loam soil (≤ 1% OM) at the Brewton Agricultural Research Unit. Plants were watered with a drip system as needed. An application of 400 lb/A of 5-10-15 fertilizer was made in March. A randomized complete block design with six single-plant replications was used. Fungicide treatments were applied with a tractor mounted sprayer from June to September at 2-wk intervals in 2010 and 2011. Rust intensity was visually rated monthly during the study period using a modified Florida 1 to 10 peanut leaf spot rating scale. In December 2011, tops of all plants were cut and weighed. To calculate dry biomass yield, shoot samples were weighed wet, oven dried, and weighed dry. In both study years, Eagle 40W and Heritage 50WDG provided superior protection from rust. Symptoms were limited to light to moderate pustule formation on lower leaves and leaf sheaths. While rust ratings were often lower compared with the non-treated control, severe rusting was noted in both years on switchgrass treated with 3336 4.5F, Banner MAXX, Daconil Ultrex, Concert II, Palladium, and Medallion 50W. Biomass yields for the Eagle 40W and Heritage 50WDG-treated switchgrass were higher compared with the other fungicides and the non-treated control, which all had similarly low yields. Dry biomass yield for the non-treated control was 37 and 40% lower when compared with Heritage 50WDG and Eagle 40W, respectively.

Weather variables for predicting aflatoxin occurrence in peanuts

K. L. BOWEN (1), H. L. Campbell (1), A. K. Hagan (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.14

Aflatoxin contamination of peanuts can cause substantial economic losses. Reliable prediction of aflatoxin occurrence in peanuts is needed that can be used by growers and peanut buyers. The importance of high temperatures and dry conditions during the last 4-6 weeks of peanut growth on aflatoxin accumulation has been proven in controlled-environment plots. However, field conditions during peanut growth are more variable than had occurred in

those plots. We monitored aflatoxin concentrations in peanuts over 5 years; in each year, 4 planting dates subjected the crop to different temperatures and rainfall amounts. Daily maximum temperatures averaged over the final 42 days prior to inversion and the number of 3-day intervals with less than 0.25 cm rainfall ('dry periods') for 28 days prior to inversion were found to be better related to aflatoxin concentrations than were many other variables including dry periods over shorter or longer intervals, other temperature variables, and rain days or rain amounts. Further, we found that maximum temperature averages defined risk groups for aflatoxin concentrations, while dry intervals provided predictive precision within each risk group. When higher daily maximum temperatures prevailed (>31.1°C), 15 dry periods could result in unacceptable aflatoxin concentrations (> 15 ppb) while with cooler maximum temperatures, more than 20 dry periods were needed during the 28 days prior to inversion.

Evaluation and adaptation of the Lincoln Nucleic-acid kit (LiNK) technology for rapid extraction of plant pathogen DNA

H. BOWMAN (1), K. Rappaport (1), L. Parameswaran (2), C. R. Cabrera (2), F. Nargi (2), L. Levy (3), Z. Liu (1)
(1) USDA APHIS PPQ CPHST, Beltsville, MD, U.S.A.; (2) MIT Lincoln Laboratory, Lexington, MA, U.S.A.; (3) USDA APHIS PPQ CPHST, Riverdale, MD, U.S.A.
Phytopathology 102:S4.15

The Lincoln Nucleic-acid Kit (LiNK) cartridge was developed by the Massachusetts Institute of Technology's Lincoln Laboratory, to provide fast (~ 6 minutes), field deployable, simple isolation of PCR-ready DNA. The LiNK cartridge can be used to collect surface-wipe and liquid samples, and provides advantages over a widely used commercially-available spin column DNA extraction kit that is more time and labor intensive (~ 2 hours) and not field deployable, requiring centrifugation, heating and cooling steps. In this study, the LiNK cartridge was evaluated against the commercial kit using five economically significant plant pathogens and two sample types. The purity and quantity of DNA extracted with either the LiNK or the commercial kit were measured, and the extracted DNA was subsequently subjected to PCR for detection and identification of the pathogens. DNA extracted using the LiNK was of higher quantity but lower purity than DNA extracted using the commercial kit. Samples processed with the commercial kit produced positive PCR identifications for all 3 replicates of each pathogen, whereas the LiNK-processed samples produced positive PCR results for at least 2 of 3 replicates of each pathogen-sample type combination. The LiNK system is very promising and will be further optimized and adapted for use in plant pathogen diagnostics.

Evaluation and adaptation of CANARY technology for rapid detection of *Phytophthora*

H. BOWMAN (1), K. Rappaport (1), Z. Abad (1), L. Levy (1), Z. Liu (1)
(1) USDA APHIS PPQ CPHST, Beltsville, MD, U.S.A.
Phytopathology 102:S4.15

CANARY is a rapid and sensitive immunological B cell based assay. We evaluated nine *Phytophthora* specific CANARY B cell lines using *P. ramorum* pure cultures, selected the most sensitive line, and determined its shelf life. The B cell line 3812 clone 37-4 had the highest sensitivity to *P. ramorum* and a shelf life of 14 days. Capture protocols using monoclonal antibody-coated magnetic beads are included in the *Phytophthora* sample preparation to enhance the CANARY assay performance. Two different monoclonal antibody coated magnetic beads were compared for capturing the *Phytophthora* antigen. The optimum incubation period and temperature for the best performing monoclonal antibody coated beads was determined. Four sample preparation platforms were examined using *P. ramorum* inoculated Rhododendron leaves to optimize sample preparation. Two preparation platforms were selected, one field deployable and one for a central laboratory. The optimized *Phytophthora* CANARY system was used to test field collected and laboratory inoculated *Phytophthora* samples. All field collected and laboratory inoculated subsamples tested positive for *Phytophthora* as expected. The *Phytophthora* CANARY assay is very promising and will be further optimized and adapted for use in *Phytophthora* diagnostics.

Response of wild and cultivated blackberry (*Rubus* spp.) species to infection of *Peronospora sparsa* under controlled conditions

J. Boyzo-Marín (1), A. REBOLLAR-ALVITER (2), S. D. Segura-Ledesma (1), H. V. Silva-Rojas (3), N. Avila-Alistac (1)
(1) Centro Regional Morelia/Universidad Autónoma Chapingo, Morelia, Michoacan, Mexico; (2) Universidad Autónoma Chapingo, Morelia, Michoacan, Mexico; (3) Colegio de Postgraduados, Produccion de Semillas, Campus Montecillo, Texcoco, Mexico
Phytopathology 102:S4.15

Downy mildew of blackberry (dryberry) is one of the most important diseases in Mexico. Up to 100% of losses have been recorded. Although several species of wild blackberry have been reported in Mexico, their susceptibility to this pathogen has not been tested. The objective of this research was to evaluate some resistant components of 4 wild and 2 cultivated blackberry species under controlled conditions. An isolate from disease fruits was obtained, and inoculated on blackberry cv Kiowa for its ability to sporulate abundantly. Fifteen days later sporangia were washed off from sporulated leaves and a 10 5 sporangia/ ml suspension was prepared. 1 ml of sporangia suspension was sprayed to the underside of disinfested blackberry leaves of blackberry and placed in Petry dishes containing water-agar media (0.5%) sealed with parafilm. Plates were incubated in a growth chamber at 16 C and 12:12 L:D. After 3 days, number of spongiophores/cm ^{2} was recorded every three days on each leave. Latent period was determined and disease severity recorded every 3 days up to day 21 after inoculation. Statistical analysis showed there were significant differences ($P<0.001$) in sporulation among species. *Rubus cymosus* (wild) showed the lowest level of sporulation, followed by tupy (cultivated), Kiowa, *R. adenotrichus*, *R. pringley*, *R. sapidus*. *Rubus pringley* showed the shortest latent period (7 days) and *R. cymosus* the longest (20 days). Final disease severity was higher in *Rubus sapidus* with an average of 70%. The lowest severity was found in *R. cymosus* with average of 3%.

Our expanding SolaR80 system: Toward comprehensive survey of the solanaceae R-gene space

J. BRADEEN (1), E. Quirin (1), H. Mann (1), A. Traini (2), M. L. Chiusano (2), D. Carputo (2)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) University of Naples Federico II, Portici, Italy
Phytopathology 102:S4.15

Most plant disease resistance (R) genes encode a conserved nucleotide binding site (NBS) and a variable leucine rich repeat (LRR) domain. NBS R gene fragments (Resistance Gene Analogs; RGAs) have been amplified from the genomes of many plant species via PCR. We amplified 97 RGAs from the wild potato *Solanum bulbocastanum* and combined these, RGAs from eight other *Solanum* species and sequences of previously cloned Solanaceae R genes into a single meta-analysis. Based on an 80% DNA sequence homology threshold, nearly 800 NBS sequences were assigned to 56 diversity bins that we call "SolaR80" (SOLAnaceae R gene) groups. We demonstrated that SolaR80 groups reflect both DNA homology and evolutionary relationships among the R gene sequences. Distribution of SolaR80 groups across *Solanum* species was arrayed adjacent to a neighbor joining tree illustrating relationships among the SolaR80 groups. The result is the SolaR80 System. This system provides a simple visual means of exploring cross-species distribution of R gene families, reveals patterns of allelic diversification, and facilitates comparative genomics approaches by establishing a common terminology. Importantly, this system is also amendable and expandable. Current research efforts include genome-wide analysis of tomato and potato genome sequences to identify NBS-LRR genes for integration into the SolaR80 System and generation of RGA resources for strategic *Solanum* species using next generation sequencing.

Evidence that organ-specific modulation of R gene function is achieved through transcriptional regulation

J. M. BRADEEN (1), B. P. Millett (1), L. Gao (1), M. Iorizzo (2), D. Carputo (2)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) University of Naples Federico II, Portici, Italy
Phytopathology 102:S4.15

Different plant organs face divergent pathogen pressures. Aboveground organs frequently encounter biotrophs, against which the R gene-triggered hypersensitive response (HR) is effective. In contrast, necrotrophs are more common in the soil and belowground organs predominantly deploy non-HR defenses. Surprisingly, how plants modulate R gene function in different organs is poorly understood. *Phytophthora infestans* causes late blight disease of potato both in aboveground foliage and belowground tubers. We examined the function of foliar late blight R gene *RB* in tubers of 11 transgenic potato lines. In nine lines, *RB* failed to function in the tuber despite being transcribed in the tuber and fully functional in the foliage. However, two other lines showed both enhanced tuber blight resistance and extremely high *RB* transcription. Resistance in these lines was age dependent; older tubers became phenotypically equivalent to susceptible controls. Importantly, as these tubers aged, *RB* transcript levels also declined, reaching levels comparable to susceptible lines by six weeks after harvest. Our data indicate that organ-specific modulation of *RB* function is achieved through direct transcriptional regulation of the R gene itself. In the foliage, *RB* triggers an HR in response to *P. infestans*. The identification of tuber blight resistant *RB*

lines enables study of tuber response pathways activated in the presence of *P. infestans*, an effort we are pursuing.

Seed and in-furrow fungicides with and without postemergence azoxystrobin for control of *Rhizoctonia solani* on sugar beet
J. R. BRANTNER (1), C. E. Windels (1), J. D. Nielsen (1)
(1) University of Minnesota, Northwest Research and Outreach Center, Crookston, MN, U.S.A.
Phytopathology 102:S4.16

Damping-off and crown and root rot (RCRR) caused by *Rhizoctonia solani* AG 2-2 is the most common root disease on sugar beet in Minnesota and North Dakota. Azoxystrobin typically is applied postemergence (PE), but disease control is inconsistent because of uncertain optimal timing for application. We evaluated efficacy of seed and in-furrow (I-F) fungicides, with and without PE application of azoxystrobin, for disease control and enhancement of sugar beet yields in two field trials infested with *R. solani*. Treatments included: azoxystrobin, penthiopyrad and pyraclostrobin on seed and I-F; sedaxane on seed; and an untreated control. PE azoxystrobin was applied 4 wk after planting. For both sites, there was no interaction between at-planting and PE treatments. At site 1, stand loss began 3 wk after planting and disease was severe. The three I-F treatments and penthiopyrad seed treatment reduced damping-off and RCRR and increased sugar yield by 44% compared to the untreated control. Azoxystrobin applied PE did not affect RCRR or sugar yield. At site 2, damping-off did not occur and RCRR was mild. Seed treatments did not significantly affect RCRR or sugar yield. All I-F fungicides and the PE application of azoxystrobin, however, reduced RCRR and increased sugar yield. Overall, I-F application of fungicides provided excellent disease control under both severe and mild disease pressure without the addition of a PE application of azoxystrobin.

Tanslocation path of *Banana bunchy top virus* (*Nanoviridae*) in the aphid vector *Pentalonia nigronervosa* as revealed by real-time PCR and immunofluorescence assays
A. BRESSAN (1), S. Watanabe (1)
(1) University of Hawaii, Honolulu, HI, U.S.A.
Phytopathology 102:S4.16

Banana bunchy top virus (BBTV) (family *Nanoviridae*, genus *Babuvirus*) is transmitted by the aphid vector, *Pentalonia nigronervosa* in a circulative persistent manner. There is little information on the process of BBTV internalization and transport through the aphid vector. In this study, we performed time-course experiments coupled with real time PCR and immunofluorescence localization assays to examine the tropism and concentration of BBTV in dissected guts, hemolymph and salivary glands. Our results indicate that BBTV translocates rapidly through the aphid vector. It internalizes through the anterior midgut where it accumulates and get retained at a very high concentrations. BBTV antigens were localized through immunofluorescence assays on the basal surface of cells forming the anterior midgut and principal salivary glands, suggesting an ongoing process of virus escaping and internalization, respectively. Interestingly, those organs can get in direct contact into the aphid, thus suggesting a possible direct translocation of virions. This study reveals a pattern of virus transport through the vector that has more in common with viruses of the family *Geminiviridae* (transmitted by leafhoppers, treehoppers and whiteflies) rather than aphid-transmitted *Luteoviridae*.

Development of microsatellite markers for assessing diversity of *Didymella bryoniae* in the southeastern United States
M. T. BREWER (1), M. Rath (1), A. N. Turner (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 102:S4.16

To best manage plant diseases it is essential to identify sources of inoculum, as well as to understand the underlying population structure of the pathogens. The major source of inoculum for gummy stem blight of watermelon, caused by *Didymella bryoniae*, is not clear; however, seed, wild cucurbit hosts, seedling transplants, and plant debris in the field are all potential sources. In order to assess the underlying diversity within and among fields and to compare the genetic similarity of samples from epidemics with those from potential inoculum sources we developed 20 microsatellite markers using a next-generation sequencing approach. A library was developed from genomic DNA by enriching for microsatellite repeat motifs and sequencing fragments using Roche 454. Reads were assembled into contigs and regions containing microsatellite motifs were identified. Primers were designed for PCR amplification of fragments containing microsatellite motifs. We tested putative markers on a panel of diverse isolates until 20 polymorphic markers were identified. Analysis of 20 isolates from diverse cucurbit hosts across the southeastern USA show high levels of diversity. Three of the isolates, which were collected from the same host and field location, had the same multilocus genotype, however all other isolates had unique genotypes. Even after removing identical genotypes, multilocus linkage disequilibrium was significant, which may result from this fungus being homothallic. Principal components analysis of genetic similarity of isolates showed no clustering of genotypes by host species or geographic origin. The utility of these markers on isolates from a worldwide collection of *D. bryoniae* will be determined.

Growth of new rootstocks for *Prunus* spp. in fumigated and non-fumigated replant soil
G. T. BROWNE (1), L. S. Schmidt (1), R. G. Bhat (2), J. Gartung (3), D. Wang (3), D. A. Kluepfel (1)
(1) USDA-ARS CPGRU, Davis, CA, U.S.A.; (2) University of California, Davis, CA, U.S.A.; (3) USDA-ARS WMRL, Parlier, CA, U.S.A.
Phytopathology 102:S4.16

Growth and productivity of replanted almond and stone fruit orchards often are suppressed by Prunus replant disease (PRD), an ill-defined soilborne complex that is distinct from parasitic nematode damage. Pre-plant soil fumigation can prevent PRD, but mandated fumigation restrictions are motivating development of improved rootstocks as an alternative. We tested resistance to PRD in five widely planted and 17 new rootstocks near Parlier, CA. The field site was cleared from almonds on Nemaguard rootstock in summer 2010. In Oct 2010, plots were shank fumigated with Telone C35 (605 kg ha^{-1}) or shanked without fumigant (control). Rootstocks were planted in Apr 2012. Resistance was assessed in winter 2012 by determining growth proportions (GPs), calculated by dividing increases in growth (stem diameter, shoot weight) in non-fumigated plots by the corresponding increases in fumigated plots. Rootstocks with peach parentage (Empyrean #1; Harrow Blood x Okinawa clones 1, 10, 28, 32, and 50; Lovell; and Nemaguard) were relatively susceptible (mean stem diameter GPs of 0.28 to 0.52); while those with peach and almond parentage (Bright Hybrid clones 5 and 106; Garnem; Hansen 536; Rootpac 20; and Tempropac) did better (0.56 to 0.76), and rootstocks with some plum parentage (Controller 5; Krymsk clones 1, 2, 9, and 86; Marianna 2624; Myrobalan; and Replantpac) were variable (0.37 to 0.96). Judicious development and use of rootstocks for *Prunus* spp. have potential to manage PRD.

***Pilidium concavum* on *Fallopia japonica* in the United States**
W. L. BRUCKART (1), F. M. Eskandari (1), E. M. Coombs (2), A. Y. Rossman (3), M. E. Palm (4)
(1) USDA-ARS FDWSRU, Fort Detrick, MD, U.S.A.; (2) Oregon Department of Agriculture, Salem, OR, U.S.A.; (3) USDA-ARS SMML, Beltsville, MD, U.S.A.; (4) USDA, Animal and Plant Health Inspection Service, Riverdale, MD, U.S.A.
Phytopathology 102:S4.16

Fallopia japonica (Houtt.) Ronse Decr. (= *Polygonum cuspidatum* Siebold & Zucc.; Japanese knotweed) (Polygonaceae) is a perennial subshrub or herb that is invasive in many parts of the world. Large, brown, necrotic spots were observed on plants at the Oregon Department of Agriculture, Salem (44.9299 N -122.9936 W). Spots were 1 – 3 cm diameter and spreading, occupying up to 30% of the leaf surface. Conidiomata in necrotic areas of leaves were of two types, hemispherical or discoid, both exuding a brown spore mass. Both types of fruiting structures were produced on potato dextrose agar, and they exuded similar spore masses. Discoid conidiomata had dark, pedicellate bases, and the hemispherical conidiomata were black, circular, and somewhat flattened. Conidia were unicellular, cylindrical to fusiform, hyaline, and 4.5 - 7.2 x 0.9 - 1.8 µm. Morphology of this fungus is comparable to that of *Pilidium concavum* (Desm.) Höhn. A sequence of the ITS1-5.8S-ITS2 region of this isolate was identical to that of a *P. concavum* isolate, BPI 1107275 (GenBank Accession No. AY487094), based upon NCBI BLAST comparison. Koch's postulates were satisfied by inoculations of *F. japonica* from Oregon. A specimen, BPI 883546, has been submitted to the USDA National Fungus Collection.

Temperature shifts compromise resistance to yellow rust in wheat
R. Bryant (1), C. Uauy (1), S. Dorling (2), L. A. Boyd (1), C. J. RIDOUT (1)
(1) John Innes Centre, Norwich, United Kingdom; (2) University of East Anglia, Norwich, United Kingdom
Phytopathology 102:S4.16

Disease resistance of wheat can vary from year to year due to the environment, causing concern to growers and breeders alike. We are studying temperature sensitive resistance responses in the interaction between hexaploid wheat and the yellow (stripe) rust pathogen *Puccinia striiformis* f. sp *tritici* (*Pst*) in controlled environment chambers. With variety UC1041 there was no significant difference in leaf pustule cover between plants kept at day temperatures of 18°C and 25°C respectively, both with a night tem-

perature of 12°C. However when plants were shifted following infection with *Pst* from 25°C to 18°C, resistance was notably compromised with percent infection increasing up to 3 fold. In contrast, plants shifted from the 18°C to 25°C day temperatures were virtually resistant. Alpowa responds in a similar manner to UC1041 under the same temperature treatments, but Jupateco does not. We hypothesise that temperature fluctuations in the field may compromise resistance in some wheat varieties but not others. Further experiments are being performed to establish how the magnitude of the shift affects resistance and whether temperature-compromised resistance occurs in other wheat varieties on the recommended list. We are also analysing climate projections to estimate temperature fluctuations that will occur in the future. Our results will inform breeding practices and help develop wheat varieties with improved resistance performance in the future.

The role of fungal stress reponse in *Colletotrichum graminicola* pathogenicity
E. A. BUIATE (1), M. F. Torres (1), S. Amyotte (2), R. O'Connel (3), L. Vaillancourt (1)
(1) University of Kentucky, Lexington, KY, U.S.A.; (2) University of Ottawa, Ottawa, ON, Canada; (3) Max Planck Institute, Cologne, Germany
Phytopathology 102:S4.17

Maize anthracnose, caused by *Colletotrichum graminicola*, causes annual losses in the U.S. of around 1 billion dollars. A *C. graminicola* random insertional mutant is almost normal in culture, but completely non-pathogenic *in planta*, although it germinates, penetrates, and colonizes the first host cell apparently normally. The mutation is located in the 3'UTR of the *Cpr1* gene, predicted to encode a non-catalytic component of the ER signal peptidase homologous to yeast Spc3. It was reported that a *Cpr1* homologue in *Aspergillus niger* was significantly up-regulated during chemically-induced secretion stress, and another study demonstrated that the *Medicago truncatula* homologue was essential for nodule establishment. The *Cpr1* mutant is more sensitive to stress, including oxidative and secretion stress, *in vitro*. An *in planta* transcriptome study showed that genes predicted to be important in fungal stress response were differentially regulated during pathogenic development. There were minor differences in expression of these genes in the mutant strain. Fluorescent reporter gene constructs were used to provide additional evidence that the fungi were experiencing and responding to stress *in planta*, and that the mutant response differed from the WT. Northern blot analyses revealed differences between the mutant and WT in the expression of stress response genes *in vitro*. Our hypothesis is that the mutant is nonpathogenic due to an inability to respond normally to stress experienced during growth and development *in planta*.

Regulation of the oxidative stress response in *Pantoea stewartii* subsp. *stewartii*, an important aspect of Stewart's wilt development in sweet corn
L. BURBANK (1), M. Roper (1)
(1) University of California-Riverside, Riverside, CA, U.S.A.
Phytopathology 102:S4.17

Pantoea stewartii subsp. *stewartii*, the etiological agent of Stewart's wilt, is a serious bacterial pathogen of sweet corn. An important aspect of bacterial plant colonization is the ability to withstand exposure to reactive oxygen species (ROS) arising from the host defense response or normal plant developmental processes. The two transcriptional regulators, OxyR and SoxR modulate the bacterial oxidative stress response through regulation of ROS-detoxifying enzymes, such as catalase and superoxide dismutase. *P. stewartii* mutants in one or both of these oxidative stress sensors were more sensitive to exogenous ROS. Interestingly, the OxyR and SoxR regulons also include genes involved in biofilm formation, another important bacterial survival mechanism and a mutation in *oxyR* causes a marked decrease in the production of stewartan exopolysaccharide, a key component of the mature biofilm matrix. Both the Δ*soxR* and Δ*oxyR* mutants were also less virulent *in planta* during either the apoplastic or xylem colonization phases of Stewart's wilt development. Moreover, Δ*oxyR* showed a striking increase in expression of *soxS*, which encodes a sensor protein that works in conjunction with SoxR. This suggests a partial overlap of the OxyR and SoxRS regulons and that both of these systems play important regulatory roles at different stages of the disease process.

Population genetics of the fungal pathogen *Monilinia vaccinii-corymbosi* in blueberry fields throughout the United States
K. M. BURCHHARDT (1), M. A. Cubeta (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.17

Monilinia vaccinii-corymbosi (Mvc) is an economically important pathogen of blueberry (*Vaccinium* spp.) that causes mummy berry disease, resulting in yield loss by mummification of infected fruit ("mummies"). The fungus overwinters inside mummies and in early spring produces sexual spores that infect blueberry shoots and cause blighting. Blighted shoots form asexual spores that are disseminated by bees to the flower and infect the ovary through the gynoecial pathway. Long-term disease management strategies require information about the spread and evolution of pathogen populations, however little is known about the population dynamics of Mvc. The primary objective of this study was to utilize microsatellite-based genetic markers to examine intraspecific genetic diversity, population structure, and gene flow among populations of Mvc throughout blueberry growing regions of the United States. In this study, 438 isolates of Mvc sampled from 15 blueberry fields in New York, New Jersey, Massachusetts, Georgia, Mississippi, Oregon, Washington, Michigan, and North Carolina were screened with 10 polymorphic microsatellite markers. Results based on analyzing a geographically diverse subsample of 55 isolates from six fields indicate high intraspecific genetic diversity. Forty-eight private alleles were detected across the 10 loci, providing evidence for population differentiation. Results from population genetic analyses of a more comprehensive dataset of 438 isolates will be presented.

A potential vector of *Blueberry necrotic ring blotch virus* and symptoms on various host genotypes
C. BURKLE (1), J. W. Olmstead (1), P. F. Harmon (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.17

Blueberry necrotic ring blotch has recently been associated with the presence of the newly described *Blueberry necrotic ring blotch virus* (BNRBV). A range of symptoms has been observed on a variety of plant accessions in the blueberry breeding program at the University of Florida. A scale to quantify disease incidence and severity on blueberry plants affected by BNRBV was developed and used to assess disease symptoms on a variety of genotypes in the University of Florida blueberry breeding program. Some plant accessions were more severely affected than others, which may have relevance to breeding efforts for blueberry resistance to BNRBV. An isolate of BNRBV was maintained in a greenhouse facility where transmission of the virus to healthy plants was observed. An eriophyid mite was associated with the symptomatic plants, and the same mite was observed in commercial fields and research plots in Alachua and Marion County, Florida where disease was prevalent. Adult mites were on average 118 µm in length and 47 µm in width. Based on morphological characteristics, the mite is a potential new species in the genus *Calacarus* and is a vagrant foliar feeder. Additional research is underway to describe this potential new species and to establish whether this mite vectors BNRBV.

Evaluation of epidemiology and prediction tools of gray mold, anthracnose fruit rot, and powdery mildew in field and high-tunnel-grown day-neutral strawberries
R. R. BURLAKOTI (1), J. Zandstra (2), K. Jackson (1)
(1) Weather INnovations Incorporated, Chatham, ON, Canada; (2) University of Guelph-Ridgetown Campus, Ridgetown, ON, Canada
Phytopathology 102:S4.17

Epidemics of anthracnose fruit rot (AFR), Botrytis gray mold (BGM) and powdery mildew (PM) in two day-neutral strawberries were compared in outdoor and high-tunnel plots at Cedar Springs, Ontario during summer and fall seasons in 2009, 2010, and 2011. Model-based fungicide sprays and calendar sprays were also evaluated. Fungicide treatments were either single applications or rotations of: Captan (Maestro 80 DF), Cabrio EG, Nova 40W, and Pristine WG and Switch 62.5 WG. Compared to outdoor fields, BGM and AFR pressure was very low in high-tunnels in all three years, suggesting fungicide spray is not needed to control BGM and AFR in high-tunnels. PM was more problematic, however, in high-tunnels compared to outdoor plots. PM incidences on fruits were observed around mid-August and increased thereafter. The cultivar Seascape was highly susceptible to PM. Sulfur spray; alternate sprays of Switch 62.5 WG, Nova 40 W and Quintec; and alternate sprays of Phostrol and Regalia Max were effective to control PM. In outdoor plots, AFR epidemic periods were variable among years and were greatly influenced by rainfall, wetness period and temperature. BGM incidence was relatively lower in all three years compared to AFR incidence. Model-based fungicide sprays were as effective as calendar sprays to reduce AFR (up to 79% reduction in cumulative incidence) and increase the fruit yield (up to 100%) compared to unsprayed plots. Rotating Pristine WG, Cabrio EG, and SWITCHTM 62.5WG were more effective to reduce both AFR and BGM than calendar sprays of Captan.

Epidemiology and management of *Septoria tritici* leaf blotch in winter wheat in Ontario
R. BURLAKOTI (1), K. Jackson (1)
(1) Weather INnovations Incorporated, Chatham, ON, Canada
Phytopathology 102:S4.17

Septoria tritici leaf blotch (SLB) is an economically important leaf disease of winter wheat in Ontario. To understand the disease epidemics in commonly grown winter wheat cultivars in Ontario and identify the suitable types and timing of fungicides, six cultivars (Ava, CM614, E0028W, Emmit, Keldin, and 25R51) were evaluated in 2011. Three fungicide treatments were evaluated, along with unsprayed plots, arranged in factorial randomized plots with 3 replicates. The fungicide treatments were: (i) single spray of Folicur (100% amount) at booting stage; (ii) two Folicur (Tebuconazole) sprays: 50% amount at booting and 100% amount at flowering; (iii) two sprays: 50% amount of Folicur at booting and 100% amount of Prosaro (Prothioconazole + Tebuconazole) at flowering. SLB was monitored periodically from tillering (ZS 20) to soft dough stage (ZS 85). SLB symptoms appeared first in the lowermost leaves in the 2nd to third week of May at stem elongation stage (ZS 31 to 33) and progressed to the uppermost two leaves during flowering to early milking stages (GS 65 to 73). Analysis of variance (ANOVA) showed significant difference ($P<0.01$ to <0.001) among fungicide treatments and cultivars on SLB severity and yield. SLB incidence and progress was higher in cultivars Keldin, 25R51 and E0028W compared to other cultivars. Fungicide sprays significantly reduced SLB severity (up to 69% reduction in two sprays and 52% reduction in single spray) compared to unsprayed plots. Yields in fungicide-sprayed plots (3.74 to 4.47 t/ha) were significantly higher ($P<0.01$) than in unsprayed plots (3.45 t/ha).

Development of infectious clones for *Maize chlorotic mottle virus* (Tombusviridae) using long RT-PCR

D. R. CABANAS (1), A. Bressan (1)
(1) University of Hawaii, Honolulu, HI, U.S.A.
Phytopathology 102:S4.18

Maize chlorotic mottle virus (MCMV) (*Tombusviridae*: *Machlomovirus*) has become one of the most widespread viruses of corn in the Hawaiian Islands, where the virus is spread by the corn thrips, *Frakliniella williamsi*. Infectious clones have been previously developed to examine the expression strategies and pathogenicity of MCMV. To begin studying the molecular determinants in MCMV-vector transmission, we developed infectious clones of MCMV from Hawaii (MCMV-HI) using long RT-PCR assays. MCMV viral particles were purified from laboratory-infected corn plants and the genomic RNA, of approximately 4.4 kb, was purified and sequenced. Viral RNA was used to synthesize cDNA using a primer specific to the 3' end of the MCMV-HI genome. The cDNA was then amplified in long PCR assays with a primer pair that hybridized to the beginning and end of the MCMV genome. After agarose gelelectrophoresis, a DNA fragment of approximately 4.4 kb was purified and cloned into the pCR 4-TOPO vector. Transformed plasmids were used as template in long PCR to produce DNA having the T7 promoter sequence at their 5' end. In vitro synthesized infectious RNA was used to challenge healthy corn seedlings. Two weeks following infection, challenged plants displayed the typical MCMV symptoms of chlorotic mottling and stunting. MCMV infection was confirmed by ELISA and RT-PCR. The infectious clones will be applied for future studies on vector transmission.

VitisGenPM: A precision phenotyping center for powdery mildew resistance breeding in grapevine

L. CADLE-DAVIDSON (1), A. Nowogrodzki (2), M. Schaub (2), P. Barba (2), B. I. Reisch (2), R. C. Seem (2), D. M. Gadoury (2)
(1) USDA-ARS, Grape Genetics Research Unit, Geneva, NY, U.S.A.; (2) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.18

Analyzing resistance to powdery mildew (*Erysiphe necator*) can be complicated by race specificity, environment, phenology, and other factors. Of necessity, breeding programs often rely upon natural infection in greenhouses, nurseries, and/or vineyards and use categorical ratings to assess resistance once or several times per year. While directly applicable to breeding and selection, natural infection results in multiple, uncontrolled sources of variation, which can hinder genetic characterization of resistance and development of predictive molecular markers. As part of a USDA-SCRI project on grapevine breeding (http://www.vitisgen.org), we established a phenotyping center (VitisGenPM) for detailed evaluation of resistance to powdery mildew. The center employs genetically diverse isolates from an array of wild hosts to provide quantitative phenotypic analysis of race-specificity and mechanisms of resistance. By phenotyping detached leaves received from breeding programs, the center provides highly controlled, replicated analysis of resistance segregation among progeny for association analysis with molecular markers on high-density genetic linkage maps. In addition to providing markers for pyramiding multiple resistance genes in individual breeding progeny, this approach enables breeders to compare the relative strength, race-specificity, and resistance mechanism of breeding lines and to make informed decisions in the selection of parents.

Occurrence and phenotypes of pyrimethanil resistance in *Penicillium expansum* from apple

R. CAIAZZO (1), Y. K. Kim (1), C. Xiao (2)
(1) Washington State University, TFREC, Wenatchee, WA, U.S.A.; (2) USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA, U.S.A.
Phytopathology 102:S4.18

Penicillium expansum is the primary cause of blue mold of apple. Pyrimethanil is a recently registered postharvest fungicide for decay control. Resistance to pyrimethanil has emerged in *P. expansum*. To monitor and characterize pyrimethanil resistance, blue mold-decayed fruit were collected in 2010 and 2011 from 5 packinghouses and the causal agents were identified to species. Isolates of *P. expansum* were tested for resistance and further classified as low resistance if conidia germinated at 0.5 µg/ml but not 10 µg/ml, moderate resistance if germinated at 10 µg/ml but not 40 µg/ml, and high resistance if germinated at 40 µg/ml. In 2010, 85% and 7% of the isolates were resistant in packinghouse A and B, respectively, where pyrimethanil had been used for 4-5 years. In 2011, either pyrimethanil or fludioxonil was used in packinghouse A, and 96% of the isolates from the fruit treated with pyrimethanil were resistant but only 4% of the isolates from the fruit treated with fludioxonil were resistant to pyrimethanil, suggesting fungicide rotation helped reduce resistance frequency. In packinghouse B, resistance frequency was reduced to 1% when fludioxonil was used instead of pyrimethnail. No resistant isolates were detected in 2010 in other 3 packinghouses where the fungicide was just recently used at a small scale, but 1% of the isolates from one of the three packinghouses in 2011 were resistant to pyrimethanil. Of the resistant isolates tested, 37-52%, 4-5% and 44-58% were phenotyped as low, moderate, and high resistance, respectively.

Morphological characterization of *Colletotrichum* species isolated from mango and tree tomato in Cundinamarca and Tolima, Colombia

C. CALDERÓN (1), M. Cárdenas (1), S. Restrepo (1), P. Jiménez (2)
(1) Universidad de Los Andes, Bogotá, Colombia; (2) Universidad Militar Nueva Granada, Bogotá, Colombia
Phytopathology 102:S4.18

Colletotrichum species are important pathogens in commercially important fruits like mango and tree tomato. This pathogen is responsible for anthracnose and necrotic lesion in fruit, leaves, and stems of these plants. Fruit and leaf samples, from tree tomato and mango, were colleted in 17 locations between Cundinamarca and Tolima. A total of 88 strains were isolated, and their morphology was characterized by growing them on different culture media as PDA (Potato Dextrose Agar), malt agar and B media. As result it was determined that most common isolates from tree tomato were *Colletotrichum acutatum*, and *Colletotrichum gloesporioides* from both mango and tree tomato. As expected, the morphological traits of colonies such as colony pigments on different media, conidia size, sporulation rate and other physiological traits, differed between species and we discussed how these physiological and morphological traits contribute to the polyphasic identification of the species in the genus *Colletotrichum*.

Molecular characterization of *Colletotrichum* species isolated from mango and tree tomato in Cundinamarca and Tolima, Colombia

C. CALDERÓN (1), J. Tabima (1), S. Restrepo (1), P. Jiménez (2)
(1) Universidad de Los Andes, Bogotá, Colombia; (2) Universidad Militar Nueva Granada, Bogotá, Colombia
Phytopathology 102:S4.18

Colletotrichum gloeosporioides is the most common anthracnose causal agent in two different hosts (mango and tree tomato) while *Colletotrichum acutatum* attacks only tree tomato. However, *Colletotrichum* species are difficult to separate when morphological and molecular features are considered separately. Many studies have shown the importance of combining different genes in polyphasical approaches. Weevaluated 4 genic regions ITS, Beta-tubulin, GDPH (glycerol-3-phosphate dehydrogenase) and AOX (alternative oxidase) in 88 strains collected from different hosts in order to characterize the anthracnose causal agents in Colombia. These results are consistent with morphological parameters evaluated for identification (see abstract by Calderon et al. in this issue). In this work we show that the combined loci clearly separate the different species of *Colletotrichum*. Moreover, Aox is a new genic region assayed in this fungus, and it proved to be an interesting alternative as a barcode as it clearly separated different species of *Colletotrichum*.

Corynespora leaf spot: A new disease in Alabama cotton

H. L. CAMPBELL (1), A. K. Hagan (1), K. L. Bowen (1), S. P. Nightengale (2)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Plant Breeding Unit, Tallassee, AL, U.S.A.
Phytopathology 102:S4.18

Corynespora leaf spot caused by the fungus *Corynespora cassiicola*, has damaged cotton in southwest Georgia for the last five years. Prior to 2011, Corynespora leaf spot had not been previously reported in Alabama but noticeable outbreaks occurred in on irrigated cotton in Baldwin, Talladega, and Lawrence Counties. Also, leaf spotting and defoliation were observed in variety trials in Henry and Elmore County. Heaviest leaf spotting was observed in irrigated cotton, however Corynespora leaf spot was also observed in dry-land cotton that received late summer rains. This disease was also more likely to occur in fields where cotton follows cotton particularly under strip or no-till. Disease ratings were taken from two cotton variety trials located at the Wiregrass Research and Extension Center in Headland, AL and the Plant Breeding Unit in Tallassee, AL using the Florida 1-10 leaf spot scoring scale. In both trials, the several Phytogen varieties had the highest leaf spot rating and the lowest rating was with Stoneville 5288 at WREC and DP1050 at PBU, respectively. Future studies will look at the impact of cotton cropping frequency, tillage, and variety selection as well as fungicides on Corynespora leaf spot intensity and yield.

Development and application of a degree-day model to predict thrips growth and development of *Tomato spotted wilt virus* in California tomato fields

A. CAMPBELL (1), O. Batuman (1), L. Chen (1), L. B. Coop (2), R. L. Gilbertson (1), N. McRoberts (1)
(1) University of California-Davis, Davis, CA, U.S.A.; (2) Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 102:S4.19

Tomato spotted wilt disease, caused by the RNA virus *Tomato spotted wilt virus* (TSWV), represents a major constraint to the processing tomato crop in the Central Valley of California. The insect vectored virus is transmitted primarily by thrips, particularly the Western Flower Thrips, *Frankliniella occidentalis.* Thrips acquire TSWV as larvae and stay infective for life. In an integrated pest management approach to controlling the disease, an understanding of thrips development and life-cycle is therefore critical. Because the development of thrips is temperature dependent we created a degree day accumulation phenology model, driven by data collected from five meteorological stations in key Central Valley locations to predict timing of thrips development. The model uses current and predicted weather to indicate when the key stages for thrips acquiring and transmitting TSWV will occur during a growing season. This allows growers to make a decision regarding control measures, and to know when the optimal times for disease control tend to be. Through the use of the phenology model, we anticipate heightened awareness and more efficient methods for managing Tomato spotted wilt disease in the Central Valley of California.

Reduced azole sensitivity in the oilseed rape pathogen *Pyrenopeziza brassicae*

H. CARTER (1), H. Cools (1), J. West (1), M. Shaw (2), A. Mehl (3), B. Fraaije (1)
(1) Rothamsted Research, Harpenden, United Kingdom; (2) University of Reading, Reading, United Kingdom; (3) Bayer CropScience, Monheim, Germany
Phytopathology 102:S4.19

Light leaf spot (*Pyrenopeziza brassicae*) is one of the most economically important diseases of oilseed rape in Northern Europe. Azole fungicides are used to control *P. brassicae*. The emergence of azole resistance could therefore compromise disease control. The aims of this research are to determine whether *P. brassicae* is evolving reduced azole sensitivity and to characterise the underlying resistance mechanisms. The sensitivities of *P. brassicae* strains to different azoles have been determined *in vitro* using high-throughput microtitre plate methods. Some strains showed an up to 35-fold reduction in azole sensitivity compared to sensitive strains. Sequencing the azole target encoding gene, sterol 14alpha-demethylase (*CYP51*), identified two mutations associated with azole insensitivity. In other strains, azole insensitivity correlated with DNA inserts of 150 or 232 bp upstream of the predicted start sequence of *CYP51*. Strains with these promoter inserts constitutively overexpressed *CYP51* up to 4-fold in comparison to wild-type strains. Therefore, both target-site mutations and increased *CYP51* expression may be important azole resistance mechanisms in *P. brassicae*. We are currently developing pyrosequencing assays in order to rapidly detect and quantify these azole resistance markers in leaf and aerosol populations. These tools have a great potential to assess the value of anti-resistance strategies designed to prolong the lifetime of fungicides.

Further studies on upright dieback in cranberry

F. L. CARUSO (1)
(1) University of Massachusetts, East Wareham, MA, U.S.A.
Phytopathology 102:S4.19

Upright dieback caused by *Phomopsis vaccinii* occurs in all areas where cranberries are grown. Although the worst case scenario in some beds involves 20% symptomatic uprights, cranberry yields did not appear to be impacted. A three year study examined the ratio of vegetative to fruiting uprights affected by the disease and its relationship to yield in that bed. Several transects were walked in each bed in order to get 20 representative samples of uprights in a unit area. The ratio of vegetative to fruiting uprights varied from 1:1 to 1000:1, and the ratio within a single bed varied significantly within the three year period. Just prior to harvest, similar transects were walked in order to determine yields. Yields were largely unimpacted by the disease, even when the ratio of uprights was 1:1. Both vegetative and fruiting uprights were sampled, stripped of their leaves and 1-cm pieces were surface sterilized and plated on ACMA. Fungi were identified at three weeks. *Phomopsis* incidence was high in both types of uprights, but generally higher in the fruiting uprights. was also cultured from both types of uprights at significantly lower levels, but usually equivalent for each upright type. When uprights were sampled the following spring, *Fusicoccum* was isolated much more frequently than *Phomopsis*. Because both of these fungi also cause cranberry fruit rot, fungicide applications for control are still warranted.

Regulation of effector protein translocation by type III secretion chaperones and HrpN in *Erwinia amylovora*

L. F. CASTIBLANCO (1), L. R. Triplett (2), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI, U.S.A.; (2) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.19

Translocation of effector proteins into the host cytoplasm through the Type III secretion system (TTSS) is a major pathogenicity determinant of Gram-negative bacterial pathogens. Moreover, the successful delivery of many effector proteins depends on the association with type III secretion (TTS) chaperones and other regulatory proteins in the bacterial cytoplasm prior to their export to the plant cell. *Erwinia amylovora*, the causal agent of fire blight disease of rosaceous plants, secretes at least four effector proteins: DspE, Eop1, Eop3 and Eop4. DspE specifically interacts with the TTS chaperone protein DspF, which stabilizes the effector protein in the cytoplasm and promotes its efficient translocation through the TTSS. In addition, the harpin protein HrpN has been demonstrated to be required for efficient translocation of DspE. The effector gene *eop1* is located adjacent to a TTS chaperone gene, named *esc1* which interacts not only with Eop1 but also with DspE in yeast, suggesting that TTS chaperones in *E. amylovora* may be involved in the translocation of non-partner effectors. In the present study we identified functional interactions between effector proteins DspE, Eop1, and Eop3 with the TTS chaperones DspF, Esc1 and Esc3 and the harpin protein HrpN. Additionally, using site-directed mutagenesis and translocation assays, we determined how these proteins play a functional role in regulating effector translocation dynamics.

WITHDRAWN

Identification and characterization of soft-rot pathogens isolated from *Vanda*, *Phalaenopsis*, *Oncidium*, and *Tolumnia* orchids in Florida

R. A. CATING (1), M. A. Hoy (2), E. R. Dickstein (2), A. J. Palmateer (3)
(1) Twyford International, Apopka, FL, U.S.A.; (2) University of Florida, Gainesville, FL, U.S.A.; (3) University of Florida, Homestead, FL, U.S.A.
Phytopathology 102:S4.19

Very little is known about bacteria that cause soft-rot in orchids. Although soft-rot pathogens typically have wide host ranges, recent isolates from orchids have not fallen into previously described taxa. In this study, 18 bacterial strains causing soft-rot were recovered from four orchid genera: *Vanda*, *Phalaenopsis*, *Oncidium*, and *Tolumnia*. Strains were characterized using standard biochemical tests, fatty acid analysis, 16S rDNA and the pectate lyase coding gene cluster (*pel*) sequences. Phylogenetic analysis using obtained sequence data was also performed. The results suggest that there are two distinct orchid clades of *Dickeya* in Florida, one isolated from *Vanda* and another from *Tolumnia* orchids. Carbohydrate utilization tests indicated that these two orchid isolates could be differentiated from the *Dickeya* taxa that were described by Samson et al. (2005). Isolates from *Phalaenopsis* and *Oncidium* did not form distinct clades in either the 16S or pel analyses, and were most similar to *D. dadantii*. Thus, there are at least three clades of *Dickeya* attacking orchids in Florida.

Expression of the cloned *IS53* transposase promoter from *Pseudomonas savastanoi* under heat-shock

T. R. CERVONE (1), S. D. Soby (1)
(1) Midwestern University, Biomedical Sciences, Glendale, AZ, U.S.A.
Phytopathology 102:S4.20

Pseudomonas savastanoi causes tumors on olive and oleander trees, in part by the synthesis of indole-3-acetic acid (IAA) and cytokinins (CK). Oleander isolates carry the IAA and CK genes on virulence plasmids which also contain insertion sequence (IS) elements that have been associated with loss of virulence due to deletion of the IAA genes. The role of IS elements in bacterial mutation is well-established, but it is not known if IS transpositional mutation rates change in response to environmental stresses. The transposase (*tnp*) gene of *P. savastanoi IS53* is homologous to the consensus *sigma-32* heat-shock promoter of *E. coli*. The *tnp* gene may be up-regulated by heat-shock, providing a mechanism for transduction of environmental stress. To test this hypothesis, the *IS53 tnp* promoter (p*tnp*) was cloned into a reporter gene vector upstream of a promoterless *lacZ* gene. Twenty minutes of heat-shock at 50°C resulted in increased *lacZ* expression in *E. coli* for up to two hours. Expression of the putative *ptnp* was maximized under highly aerobic conditions at mid-log phase. If heat-stress induces the *tnp* gene in *P. savastanoi*, then *IS53* transpose activity should also increase. The purpose of this research is (1) to define *ptnp* activity under stress conditions in *E. coli via* transcriptional fusions, and (2) to investigate the extent of *tnp* activity of *IS53* by RFLP and Southern blotting.

Detection of *Bean pod mottle virus* using RT-PCR, RT-qPCR, and isothermal amplification

C. Chalam (1), M. ARIF (2), J. Fletcher (2), F. M. Ochoa Corona (2)
(1) Division of Plant Quarantine, National Bureau of Plant Genetic Resources, New Delhi, India; (2) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.20

Seed-transmitted viruses pose an important threat to several crops, and are easily introduced into new areas when infected seed is planted. *Bean pod mottle virus* (BPMV) occurs in both the USA and India. In the US, BPMV is spreading widely in major soybean growing areas in the south and south-east. In India, BPMV has high quarantine significance. A reliable, rapid and sensitive detection method is needed by plant health officials for inspection of products from quarantined locations, and by extension specialists for improving safe-trading and monitoring. Specific primers targeting the capsid polyprotein gene in BPMV was designed using Web-interface softwares Primer3, mFOLD, and BLASTn. The primers were modified at the 5' position for efficient helicase dependent amplification (HDA). Primer set BPMV-CpF and BPMV-CpR amplified a 61 bp PCR product, which was validated *in silico* and *in vivo* against an infected plant positive control. The specificity of the primers was confirmed using an exclusivity panel composed of non-target but closely related pathogens. The described assays are sensitive, detecting as little as 100 fg with both qPCR and endpoint PCR, and 10 fg using HDA. The described PCR and isothermal amplification assays are accurate, rapid, sensitive and useful for pathogen detection and disease diagnosis, microbial quantification, and applications in biosecurity and microbial forensics.

Discrimination among *Cherry leafroll virus*, *Grapevine fanleaf virus*, and *Tomato ringspot virus* using multiplex RT-PCR

C. Chalam (1), M. ARIF (2), D. R. Caasi (2), J. Fletcher (2), F. M. Ochoa Corona (2)
(1) Division of Plant Quarantine, National Bureau of Plant Genetic Resources, Stillwater, OK, U.S.A.; (2) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.20

Cherry leafroll virus (CLRV), *Grapevine fanleaf virus* (GFLV) and *Tomato ringspot virus* (ToRSV) are seed-transmitted nepoviruses that cause economic yield losses. Fanleaf degeneration/decline disease is caused by several viruses including CLRV, GFLV, and ToRSV but GFLV is the best characterized and the most widespread of the three. All three viruses have been reported in USA, but CLRV and ToRSV have not been reported in India, and GFLV is known to be present there on grapevine but not on legume crops, hence CLRV, GFLV and ToRSV all are of quarantine significance. We developed a method for simultaneous detection and discrimination of these three viruses. Specific primer pairs for each virus were taken from the literature and their compatibility for multiplex PCR was enhanced by modifying the 5' terminus. All primer pairs were tested in silico and in vivo against infected plant positive controls. The specificity of the primers was confirmed against an exclusivity panel composed of closely related pathogens. The assay is sensitive and able to detect as little as 10 pg of ToRSV, and 100 fg of CLRV and GFLV in multiplex PCR. The described multiplex PCR assay is accurate, sensitive and useful for pathogen detection and disease diagnosis, enabling microbial forensic management through quarantine, and can be used also in virus-free trade, germplasm exchange, and certification programs.

Polar auxin transport is mandatory for gall formation by *Pantoea agglomerans* on gypsophila

L. Chalupowicz (1), D. Weinthal (1), V. Gaba (1), G. Sessa (2), I. BARASH (2), S. Manulis-Sasson (1)
(1) ARO The Volcani Center, Bet Dagan, Israel; (2) Tel Aviv University, Tel Aviv, Israel
Phytopathology 102:S4.20

Virulence of *Pantoea agglomerans* pv. *gypsophilae* (*Pag*) on gypsophila is dependent on the pPATH plasmid containing a functional *hrp/hrc* gene cluster, genes encoding for type III effectors and a cluster of genes for biosynthesis of indole-3-acetic acid (IAA) and cytokinin (CK). Mutation in the *hrp*/*hrc* gene cluster abolished gall formation, whereas simultaneous inactivation of the pathways for IAA and CK biosynthesis reduced gall size by only 50%. The hypothesis that plant-produced IAA is involved in gall development was tested by disruption of polar auxin transport with the specific inhibitors 2,3,5-tri-iodobenzoic acid (TIBA) or N-1-naphthylphthalamic acid (NPA) applied as a lanolin ring around gypsophila stems. *Pag* inoculation was performed below and above the lanolin rings containing TIBA or NPA. Galls were developed above rings containing either TIBA or NPA, but not below. Colonization of *Pag* was reduced by 2 orders of magnitude after 96h on inoculated gypsophila stems below the lanolin-TIBA ring. Transcription of the *hrp* regulatory gene *hrpL* and the quorum sensing-regulatory gene *pagR* were significantly reduced in *Pag* inoculated-stem below the TIBA treatment. Expression of a putative auxin efflux carrier PIN2 was significantly affected in *Pag*-inoculated gypsophila at initial 24h. Results presented demonstrate that plant-produced auxin is crucial for gall formation as opposed to the significant but secondary role of pathogen-produced IAA.

Selection of candidate genes involved in the defense mechanisms of *Phytophthora infestans* against fungicides by EST analysis

E. R. Champaco (1), R. P. LARKIN (2), B. G. de los Reyes (3)
(1) University of Maine, Department of Molecular and Biomedical Sciences, Orono, ME, U.S.A.; (2) USDA-ARS, New England Plant, Soil, and Water Laboratory, Orono, ME, U.S.A.; (3) University of Maine, School of Biology and Ecology, Orono, ME, U.S.A.
Phytopathology 102:S4.20

Preliminary research using a functional genomics approach was conducted to gain insights on how *P. infestans* responds to fungicides and the possible implications of these responses on its ability to adapt to such selection pressure. Two isolates with subtle differences in mefenoxam resistance were exposed to low and moderate concentrations of mancozeb or mefenoxam. A high-titer composite primary library consisting of 1.3×10^7 near full-length cDNA clones was constructed to ensure broad representation of genes expressed by both strains under fungicide treatments. Initial low-depth sequencing of 500 cDNA clones revealed that a large proportion of transcripts represent genes with binding (47%) and catalytic activities (34%). A smaller proportion represent genes with structural (10%), transport (4%), protein serine/threonine kinase (3%) and efflux, plant pathogenesis and defense (2%) related functions. This latter category is represented by multidrug resistance, elicitin-like and crinkler family proteins known to play important roles in programmed cell death and defense. These trends suggest a potentially interesting parallel between the mechanisms of plant responses to pathogens and pathogen responses to xenobiotic chemicals, perhaps through specialized functions of evolutionarily conserved proteins. Our initial results underscore the need to investigate the potential of these candidate genes to confer a selective advantage in pathogen-fungicide interactions.

Baseline sensitivity of *Exserohilum turcicum* to the quinone outside inhibitor pyraclostrobin

V. CHAPARA (1), D. K. Pedersen (1), P. Balint-Kurti (2), P. D. Esker (3), A. E. Robertson (4), P. A. Paul (5), C. A. Bradley (1)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.; (3) University of Wisconsin-Madison, Madison, WI, U.S.A.; (4) Iowa State University, Ames, IA, U.S.A.; (5) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.21

Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum* can cause severe yield reductions in corn (*Zea mays*) in the United States. Among the practices used to manage this disease, spraying foliar fungicides is becoming increasingly popular. Quinone outside inhibitor (QoI) fungicides, such as pyraclostrobin, are extensively used to manage NCLB. Baseline isolates of *E. turcicum* were obtained from NCLB affected corn leaf samples collected from fields across 10 states in the United States in years prior to the registration of QoI fungicides on corn. In total, 43 *E. turcicum* isolates were tested in vitro using pyraclostrobin amended potato dextrose broth in multi-well plates. Based on the chemical properties and mode of action, varying concentrations (0, 0.001, 0.01, 0.1, and 1 µg/ml) of pyraclostrobin were used to determine their effects on *E. turcicum* conidial germination after 48 hours. Salicylhydroxamic acid (SHAM) was added to the liquid media to prevent an alternative oxidative pathway. Baseline EC_{50} values of pyraclostrobin ranged from 0.01 to 0.15 µg/ml with mean and median values of 0.037 and 0.033 µg/ml, respectively. The determination of the baseline sensitivity of *E. turcicum* to QoI fungicides will help in monitoring the development of resistance of this pathogen to QoI fungicides.

Incidence and diversity of fungal endophytes in *Elymus* species

N. CHARLTON (1), C. Young (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.
Phytopathology 102:S4.21

Elymus species are cool season bunch grasses that are native to much of North America. Many *Elymus* species, including *El. canadensis* (Canada wildrye), *El. hystrix*, *El. villosus*, and *El. virginicus* (Virginia wildrye), are known to harbor epichloid endophytes. Predominantly, the sexual species, *Epichloë elymi* have been described within *Elymus*. However, an asexual non-hybrid, *E. amarillans* was found in Virginia wildrye and we have recently described an asexual hybrid, *E. canadensis* (*E. elymi* × *E. amarillans*), from Canada wildrye. The objectives of this study were to 1) investigate the occurrence of endophytic fungi in *Elymus* species especially within Canada and Virginia wildryes, 2) determine the potential alkaloid production of each population through PCR of key alkaloid genes, and 3) determine whether the endophyte is of hybrid or non-hybrid origin using microsatellite markers. Seeds of *Elymus* spp. were collected from 100 sites ranging from Kansas to South Texas. Total DNA isolated from 12-24 individual seeds/location was examined for endophyte infection using a PCR based approach that indicated infection frequencies ranging from 0-100%. We identified ten-alkaloid gene profiles based on analysis of key pathway genes. Microsatellite markers will be used to distinguish hybrids (*E. canadensis*) from non-hybrids (*E. elymi* or *E. amarillans*). We will evaluate whether alkaloid diversity translates into differences in fitness and persistence of the host.

Evaluation of commercial soybean cultivars for pathogen and pest resistance

S. CHAWLA (1), C. R. Bowen (2), H. A. Hobbs (1), G. L. Hartman (3)
(1) University of Illinois, National Soybean Research Center, Department of Crop Sciences, Urbana, IL, U.S.A.; (2) USDA-ARS, National Soybean Research Center, Urbana, IL, U.S.A.; (3) USDA-ARS, National Soybean Research Center, University of Illinois, Department of Crop Sciences, Urbana, IL, U.S.A.
Phytopathology 102:S4.21

From 2009-2011, 1776 cultivars were evaluated under controlled greenhouse conditions for soybean pathogen and pest resistance. In 2009, 65% of 633 cultivars, in 2010, 67% of 395 cultivars, and in 2011, 65% of 375 cultivars evaluated with *Phytophthora sojae* race 17 were resistant. Additionally, 82% of 357 cultivars evaluated with *P. sojae* race 7 were resistant in 2011. For Sclerotinia stem rot evaluations 49% of 179, 0.5% of 183, and 41% of 167 cultivars were considered as partially resistant in 2009, 2010, and 2011, respectively. In the evaluation with soybean aphid 9% of 44, 1% of 154, and 3% of 154 cultivars were resistant in the three years, respectively. For *Soybean mosaic virus* evaluations 0% of 569 cultivars in 2009, 2% of 394 cultivars in 2010, and 5% of 331 cultivars in 2011, were resistant. In the evaluation for sudden death syndrome 3% of 656, 0.7% of 599, and 4% of 521 cultivars were considered partially resistant in 2009, 2010, and 2011, respectively.

Dual fungicide resistance in *Monilinia fructicola* and fungicide-mediated transposition of genetic elements

F. CHEN (1), X. Liu (2), G. Schnabel (1)
(1) Clemson University, Clemson, SC, U.S.A.; (2) China Agricultural University, Beijing, Peoples Republic of China
Phytopathology 102:S4.21

Monilinia fructicola (G. Wint.) Honey is a causal agent of brown rot, a serious disease of stone fruits. The most commonly used fungicides for brown rot control belong to the demethylase inhibitors (DMIs) and respiration inhibitors (RIs) including quinone outside inhibitors and succinate dehydrogenase inhibitors (SDHIs). Although resistance to DMI and RI fungicides were reported in *M. fructicola* recently, resistance to both in the same isolates has not been reported in this or any other *Monilinia* species. Single-spore isolates from a peach orchard with severe brown rot damage despite DMI and RI applications revealed dual resistance to DMI (growth rate more than 80% on PDA amended with 0.3 µg/ml propiconazole) and SDHI (EC_{50} values greater than 2.1 µg/ml for boscalid assessed on minimum medium) fungicides. The resistance determinant 'Mona' was present in these isolates but no point mutations in the SDH subunits A, B, C, or D were associated with SDHI resistance. An investigation was launched as to whether exposure to site-specific fungicides would prompt the transposition of mobile genetic elements such as 'Mona'. DMI-sensitive isolates lacking Mona upstream the *cyp*51 gene were exposed to azoxystrobin in 12 weekly transfers on PDA. A transposon around 1500 bp in size was translocated into the upstream region of the *cyp*51 gene at generation six on azoxystrobin-amended medium but not on unamended medium. The transposon remained at this position until the 12th generation. We report for the first time dual resistance to DMI and SDHI fungicides in a *Monilinia* species and transposition of mobile genetic elements in a fungal plant pathogen induced by a site-specific fungicide.

Dynamics of growth regulators during infection of apple leaves by *Alternaria alternata* apple pathotype

Y. CHEN (1), P. Cong (2), C. Zhang (2)
(1) Cornell University, Geneva, NY, U.S.A.; (2) Institute of Pomology, Chinese Academy of Agricultural Sciences, Xing Cheng, Liaoning Province, Peoples Republic of China
Phytopathology 102:S4.21

Apple Alternaria blotch caused by *Alternaria alternata* apple pathotype has a severe negative effect on apple production. It can cause tissue necrosis on leaves, young shoots and fruit. Recent studies on this pathogen have mostly focused on phytotoxicity and pathogenicity. There are few reports on the roles of signaling and metabolism in the process of infection. A filial generation with substantial differences in resistance between individuals with similar genetic background was used as host, and an aggressive strain of *A. alternata* that can complete the infection process in 72 hr after inoculation served as the pathogen. A reproducible and reliable *in vitro* inoculation system for plant growth regulator determination was established to overcome the difficulties of inoculation of attached leaves. Alterations in growth regulator concentrations were detected, including indole-3-acetic acid (IAA), zeatin riboside (ZR), gibberellin A_3 (GA_3), abscissic acid (ABA) and thepolyamines, putrescine (Put), spermidine (Spd) and spermine (Spm). Results indicated the plant growth regulators interacted with each other to modulate signaling and metabolic networks. A biotrophic-like phase was inferred to exist before necrosis developed. GA_3 and ABA appeared to be involved in the phase transformation from the biotrophic-like stage to the necrotrophic stage. CK, Put and Spd appeared to be related to disease resistance. This study advances our knowledge of the pathological mechanisms of apple Alternaria blotch and provides useful resources for disease control and prevention.

Biofilm formation of *Bacillus subtilis* on tomato roots enhances biocontrol efficacy against tomato bacterial wilt disease caused by *Ralstonia solanacearum*

Y. CHEN (1), F. Yan (1), H. Liu (1), Y. Chai (2), J. Guo (1)
(1) Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Nanjing, Peoples Republic of China; (2) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, U.S.A.
Phytopathology 102:S4.21

The soil-living bacterium *Bacillus subtilis* is able to form architecturally complex cell communities, known as biofilms. *B. subtilis* has long been used in the field of agriculture as a biocontrol reagent to protect plants against soil-borne plant pathogens. The exact mechanisms for plant biocontrol have not been clearly addressed. In this study, we established *B. subtilis*-tomato interaction system to explore the biocontrol mechanisms. We isolated many *B. subtilis* strains form various natural environments in China, and obtained 6 wild stains demonstrated above 50% biocontrol efficacy against the *R.*

solanacearum under greenhouse conditions. They also showed robust biofilm formation both in biofilm-inducing media and on tomato plant roots, and exhibited strong antagonistic activities again various plant pathogens *in vitro*. We knocked out 7 key genes related to *B. subtilis* biofilm formation by transformation and evaluated the biocontrol efficacy of these mutants. Results indicated the biofilm formation ability of *B. subtilis* is positively correlated to biocontrol efficiency against tomato bacterail wilt disease. Meanwhile, the antimicrobial capability is also necessary to inhibit *R. solanacearum*, especially surfactin. The surfactin mutant strains seriously decreased biocontrol efficacy. Finally, we suggested *B. subtilis* biofilm formation and production of anti-microbial agents may act synergistically to enhance biocontrol efficacy during the plant diseases management.

Molecular determinants of resistance activation and suppression by *Phytophthora infestans* effector IPI-O

Y. Chen (1), Z. Liu (1), D. HALTERMAN (2)
(1) University of Wisconsin-Madison, Plant Pathology, Madison, WI, U.S.A.; (2) USDA-ARS, Madison, WI, U.S.A.
Phytopathology 102:S4.22

The potato late blight pathogen, *Phytophthora infestans*, is able to rapidly evolve to overcome resistance genes. The pathogen accomplishes this by secreting an arsenal of proteins, termed effectors, that function to modify host cells. Although hundreds of candidate effectors have been identified in *P. infestans*, their roles in pathogenicity or virulence remains basically unknown. The potato *RB* gene, derived from the wild species *Solanum bulbocastanum*, confers resistance to most *P. infestans* strains through recognition of members of the pathogen effector family IPI-O. While the majority of IPI-O proteins are recognized by RB to elicit resistance (e.g. IPI-O1, IPI-O2), some family members are able to elude detection (e.g. IPI-O4). Our results showed that IPI-O4 functions to turn off resistance mediated by the potato gene *RB*. This effector accomplishes this by directly interacting with the RB protein, which likely modifies its ability to turn on host resistance. Further molecular analysis identified two amino acids within the effector that determine interaction, which can assist in developing appropriate disease control strategies.

tofM* Encoding a *rsaM* homolog is required for the quorum sensing-independent biosynthesis of toxoflavin in *Burkholderia glumae

R. CHEN (1), I. K. Barphagha (1), J. Ham (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.22

Burkholderia glumae is the major causal agent of bacterial panicle blight of rice. The phytotoxin, toxoflavin, as well as flagella and lipase are important virulence factors of *B. glumae*. These virulence factors are regulated by the *N*-acyl homoserine lactone(AHL)-mediated quorum-sensing (QS) system of *B. glumae*, which consists of *tofI* encoding an AHL synthase and *tofR* encoding an AHL receptor. *B. glumae* mutants in which both *tofI* and *tofR* were deleted were still able to produce toxoflavin on solid media despite their deficiency in AHL production, indicating the presence of an unknown pathway for toxoflavin production that is independent of the AHL-mediated QS system conferred by *tofI* and *tofR*. Interestingly, additional deletion of the 453 bp ORF present in the intergenic region between *tofI* and *tofR* from the Δ*tofI*/*tofR* mutant abolished this *tofI*/*tofR*-independent toxoflavin production. This ORF is homologous (21.9% identity) to *rsaM* of another rice-pathogenic bacterium, *Pseudomonas fuscovaginae. rsaM* is also located between an AHL synthase gene (*pfsI*) and an AHL receptor gene (*pfsR*) and divergent from *pfsR* like its counterpart of *B. glumae*. Thus, the *rsaM* homolog of *B. glumae* was named *tofM* after *rsaM*. *rsaM* was recently reported to function as a built-in QS homeostasis regulator and to be required for the full virulence of *P. fuscovaginae* in rice. Our observations in this study strongly suggest that TofM is another regulator of virulence in *B. glumae* and may modulate the TofI/TofR QS system.

Detection of small RNAs in *Xylella fastidiosa*

J. CHEN (1), H. Huang (2)
(1) USDA-ARS PWA, Parlier, CA, U.S.A.; (2) University of South Florida, Tampa, FL, U.S.A.
Phytopathology 102:S4.22

Non-coding small RNAs (sRNAs) are regarded as ubiquitous regulatory elements in bacteria. For *Xylella fastidiosa*, a plant pathogen causing many economically important crop diseases, research attention to sRNAs has been limited. With the availability of whole genome sequences and increasing bioinformatic knowledge, putative sRNA genes can be identified based on computational analyses. In the genome of *X. fastidiosa* strain M23, 49 sRNA genes were predicted. The goal of this study was to experimentally verify the presence of sRNAs in the bacterium. Due to the low expression levels of sRNAs, compounded by the nutritional fastidiousness of *X. fastidiosa*, implementation of the commonly used techniques such as Northern-blotting has been problematic. An alternative method was developed that took advantage of the sensitivity of PCR technology. Primers were designed within (internal) and outside (external) the putative sRNA genes. sRNAs in bacterial cultures were verified by real-time quantitative reverse- transcriptase PCR (qRT-PCR) when internal primer sets yielded positive amplifications and external primer sets yielded weak or no products. At least nine sRNAs have been detected in *X. fastidiosa* strain M23 so far. Profiles of sRNA types varied depending on culture media. Results from this study provide the first experimental proof of sRNAs in *X. fastidiosa*. The developed technique also has a potential to be implemented in sRNA detection of other microbes including fastidious prokaryotes.

Canada-wide spore-trapping network provides effective monitoring of microbial phytopathogens in air and rain samples

W. CHEN (1), C. T. Lewis (1), J. T. Chapados (1), S. Hambleton (1), K. A. Seifert (1), K. Temple (1), A. Biernacka-Larocque (1), Z. Robleh Djama (1), C. A. Lévesque (1)
(1) Agriculture and Agri-Food Canada, Ottawa, ON, Canada
Phytopathology 102:S4.22

Shifts of the aero-microbial populations can affect the health of the agro-ecosystem. To determine the baseline profile of microbial biodiversity and to search for potential bioindicators related to climate change, we established nine spore-trapping sites in five Canadian provinces. Air and rain samples were collected by three types of collectors through the summer from 2009 to 2011. Amplicons of the bacteria16S rRNA gene and fungal internal transcribed spacer (ITS) region were subjected to 454 pyrosequencing. To date, 90 million sequences have been generated from over 700 samples. Data analysis pipelines were developed to classify sequences down to the species level using public data and in-house reference data. Approximately 11 million ITS1 and 12 million ITS2 sequences were extracted from the full ITS sequences for downstream fungal diversity analysis, including 76,000 ITS1 and 48,000 ITS2 assigned to the rust fungus order *Pucciniales*, which are obligate parasites of vascular plants. The combined rust community recovered using the selected primers represented 11 families and 24 genera, with over 85% of the sequences assigned to *Puccinia* and *Melampsora*. Geographic and temporal comparisons are being performed among community profiles of rust and other phytopathogens. Fluctuations of the microbial profile against the baseline, among locations and time points, can detect agriculturally significant species in field.

A reporter gene-transactivation system to study early events in the curtovirus infection process

L. CHEN (1), R. L. Gilbertson (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.22

Curtoviruses are a genus of circular, single-stranded DNA viruses in the family *Geminviridae* that are phloem-limited and transmitted by the beet leafhopper (*Circulifer tenellus*). Little is known of the early events in the viral infection process. To assess whether curtoviruses initially infect cells in inoculated leaves or move, long distance, to sink tissues for initial replication and infection, *Beet severe curly top virus* was inoculated into leaves of shepherd's purse plants by the beet leafhopper. Tissues of inoculated and newly emerged leaves and roots were collected at 1, 2 and 7 days post leafhopper transmission (dpt). Southern blot hybridization analysis revealed that viral DNA was primarily in newly emerged leaves and roots at 7 dpt, and not in the inoculated leaves. These results suggest that curtovirus virions move long distance in the phloem following inoculation by leafhoppers, and that replication and infection occur in sink tissues. To investigate when and where the virus initiates replication in these tissues, an inducible green fluorescent protein (GFP) expression system was developed. Here, the GFP gene is in upstream of the 35S promoter and this expression cassette is flanked by two viral intergenic regions. Upon virus infection, the expression cassette is re-leased by rolling circle replication, activating GFP expression. This inducible transactivation system should be a useful tool to study the mechanism of curtovirus movement and replication.

Virulence and SSR markers revealed only asexual reproduction in the *Puccinia striiformis* f. sp. *tritici* population of the U.S. Pacific Northwest

P. Cheng (1), X. CHEN (2), D. R. See (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.22

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), occurs every year and caused frequent epidemics in the US Pacific Northwest (PNW). Races of Pst change rapidly and barberry plants, which could be alter-

nate hosts of the fungus, are found in the region. However, whether sexual reproduction occurs in the Pst population is not clear. To determine the reproduction mode of Pst, a systemic collection of single-stripe leaf samples of Pst was made in 26 fields in the PNW in 2010. A total of 270 isolates obtained from the PNW collection, together with 66 isolates from other 20 states collected in the same year, were characterized by virulence tests and simple sequence repeat (SSR) markers. A total of 21 races and 66 genotypes were detected, of which 15 races and 32 genotypes were found in the PNW. Cluster analysis with the SSR marker data revealed two genetic groups, the PNW group with more homozygous isolates and the rest US group with more heterozygous isolates. The molecular genetic groups generally corresponded to the two virulence groups. The analyses of multi-locus association, parsimony tree length permutation, and Hard-Weinberg equilibrium ruled out the possibility of sexual reproduction, indicating that Pst reproduces asexually and that barberry may not have an effect on stripe rust in the PNW.

***Plasmopara viticola* isolates from the Lake Erie region with the G143A mutation that confers resistance to strobilurin-class fungicide**

S. L. Chestnut (1), C. T. GEE (1)
(1) Penn State University, Erie, PA, U.S.A.
Phytopathology 102:S4.23

Downy mildew (*Plasmopara viticola*) is a significant problem in grape vineyards throughout the growing season. Control of downy mildew is carried out with a combination of host tolerance and chemical applications. Even in vineyards planted with very tolerant varieties (*e.g.* Concord), increased chemical control is important in years where conditions for pathogen development are ideal. For fungicides with a single-site mode of action, the potential for resistance is very high, especially when host cultivar sensitivity makes the use of a multi-site control unavailable. This potential for resistance has been observed in the strobilurin class of fungicides. This project aims to ascertain the levels of strobilurin resistance in downy mildew colonies on diseased leaves using ARMS-SYBR Green real-time PCR and CAPS-PCR to quantify the glycine to alanine mutation (G143A) known to confer a qualitative level of resistance in fungal pathogens. Our data shows a small percentage of the collected samples contain G143A, suggesting an overall low level of strobilurin resistance in the sampled vineyards. The low prevalence of the resistant SNP suggests that strobilurins should remain a viable control mechanism in Lake Erie vineyards. Additionally, it appears that a viticultural region where tolerant hosts predominant, and strobilurin use is minimal, resistance buildup in the pathogen population will remain quite low.

A new recombinant *Potato virus Y* isolate classified as belonging to PVY^{Z} strain group may help to define viral determinant responsible for tuber necrosis in potato

M. CHIKH ALI (1), T. Maoka (2), T. Natsuaki (3), A. V. Karasev (1)
(1) University of Idaho, Moscow, ID, U.S.A.; (2) National Research Center for Hokkaido Region, Sapporo, Japan; (3) Utsunomiya University, Utsunomiya, Japan
Phytopathology 102:S4.23

A *Potato virus Y* (PVY) isolate from Syria, SYR-III-L4, overcame the resistance genes Ny_{tbr} and *Nc* but induced a hypersensitive reaction (HR) in a potato cultivar carrying the gene *Nz* typical of PVY^{Z} strain group, reported previously for a recombinant isolate PVY-L26 from the U.S. Like PVY-L26, the genome of SYR-III-L4 was found recombinant with two recombination junctions (RJs), RJ2 and RJ3, identical in L26 and other Eu-PVY^{NTN} genomes, and located in the HC-Pro/P3 and VPg regions. However, unlike PVY-L26, SYR-III-L4 had one additional RJ, RJ1, in the P1 region, at nucleotide position 686. Another RJ, RJ4, the closest to the 3'-end of the SYR-III-L4 genome, was shifted relative to a similar RJ in PVY-L26 and other PVY^{NTN} genomes, to the position 8,432 in the NIb/CP region. This shift in the RJ4 position in the SYR-III-L4 genome led to an O-serotype of this isolate, as opposed to an N-serotype in other PVY^{NTN} isolates. SYR-III-L4 was not able to induce PTNRD in any potato cultivar tested including a susceptible cultivar, Nishiyutaka. Comparison of the SYR-III-L4 genome to genomes of several other recombinant PVY isolates from Syria with similar genome structures, and PVY^{NTN} genomes from the GenBank database, all of them inducing PTNRD, allowed to suggest possible genome area involved in the PTNRD induction. Genome analysis of SYR-III-L4 suggests that genetic determinants of PTNRD and HR in the genetic background of the *Nz* gene are not linked.

An outbreak of the *Potato virus* Y^{NTN} (PVY^{NTN}) strain in foundation seed potatoes in Japan

M. CHIKH ALI (1), A. Karasev (1), N. Furutani (2), M. Taniguchi (3), Y. Kano (3), M. Sato (2), T. Natsuaki (4), T. Maoka (5)
(1) University of Idaho, Moscow, ID, U.S.A.; (2) National Center for Seed and Seedlings, Tsukuba, Japan; (3) National Center for Seed and Seedlings, Sapporo, Japan; (4) Utsunomiya University, Utsunomiya, Japan; (5) National Research Center for Hokkaido Region, Sapporo, Japan
Phytopathology 102:S4.23

Due to the isolated geographic location of Japan and restrictions on seed potato import, the composition of *Potato virus Y* (PVY) strains is stable and included so far PVY^{O} and PVY^{NA-N}. In 2008 and 2009, a sudden increase in PVY incidence was noticed in foundation seed potatoes in Hokkaido, northern Japan. Molecular typing revealed that besides PVY^{O} and PVY^{NA-N}, the Eu-PVY^{NTN} was the most common strain with over 60% of all PVY positives. The Eu-PVY^{NTN} isolates from Japan were recombinants with three recombinant junctions typical of the Eu-PVY^{NTN}. The occurrence of Eu-PVY^{NTN} was not restricted to Hokkaido Island since they were also detected in samples collected from seed potatoes in Nagasaki prefecture, southern Japan. Eu-PVY^{NTN} isolates from Japan were closely related to Eu-PVY^{NTN} isolates from Idaho, USA, reported during a PVY outbreak in 2007-2008. Although the Eu-PVY^{NTN} is the main cause of the potato tuber necrotic ringspot disease (PTNRD) in the world, no tuber necrosis was noticed at the three seed producing stations. An isolate of Eu-PVY^{NTN} from Japan, Eu-12Jp, did not induce PTNRD in 65 potato cultivars tested in both primarily and secondarily infected plants. Two cultivars with the resistance gene Ry_{chc} were immune to the infection with Eu-PVY^{NTN}, hence presenting good sources for resistant cultivar breeding. The outbreak of Eu-PVY^{NTN} in Japan may lead to an increased risk of PTNRD.

Fungal gene expression patterns during infection of canola by *S. sclerotiorum*

K. CHITTEM (1), W. Yajima (1), L. E. del Rio-Mendoza (1), R. S. Goswami (2)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) DuPont Crop Protection, Newark, DE, U.S.A.
Phytopathology 102:S4.23

Sclerotinia Stem Rot (SSR) is an economically important disease of canola (*Brassica napus*) caused by necrotrophic plant pathogen *Sclerotinia sclerotiorum*. This study focuses on identifying *S. sclerotiorum* genes associated with disease development on canola. The petiole inoculation method was used to inoculate a double haploid canola line. cDNA libraries were created from RNA collected 8, 16, 24 and 48 hours post inoculation (hpi) and samples from two consecutive time points were pooled. cDNA libraries were also prepared from *S. sclerotiorum* growing in pure culture. A total of 61,730,444, 76-bp reads were generated from inoculated libraries of susceptible line NEP 32 and 18.27% of the reads were aligned to the *Sclerotinia* genome. Compared to the *in vitro* library, 244 *S. sclerotiorum* genes were differentially expressed *in planta* within 16 hpi , with 56 of them being up-regulated. At 24 and 48 hpi, 1,371 genes were differentially expressed with 486 of them being up-regulated by at least two-fold. Preliminary findings indicate that most of the up-regulated genes at the different time points were involved either in cell wall degradation or detoxification. Further analyses of the genes expressed during this interaction will be presented.

Modeling flush-to-flush transmission of huanglongbing in a citrus tree and effects of control strategies on disease dynamics

C. Chiyaka (1), B. H. Singer (1), S. E. Halbert (2), J. G. Morris (1), A. H. VAN BRUGGEN (3)
(1) Emerging Pathogens Institute, University of Florida, Gainesville, FL, U.S.A.; (2) FDACS Division of Plant Industry, Gainesville, FL, U.S.A.; (3) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.23

Since the discovery of Huanglongbing (HLB) in Florida in 2005, the disease has spread through most of the state. To understand the factors that influence disease development, a mathematical model of the transmission of HLB between its psyllid vector and citrus host has been developed. The model describes the spread of the pathogen from flush to flush within a tree via the vector and through the tree. Dynamics of vector and host populations are simulated realistically as the flush population approaches complete infection. Model analysis indicates that vector activity is essential for initial infection but is not necessary for continued infection since infection can occur through internal movement in the tree. Flush production, within-tree spread and latent period are the most important parameters influencing HLB development. The model shows that the effect of spraying of psyllids depends on time of initial spraying, frequency and efficacy of insecticides. Effects of removal of symptomatic flush depend on the frequency of removal and time of initiation of this practice since the start of the epidemic. Within-tree resistance to spread, possibly affected by inherent or induced resistance, is a major factor affecting epidemic development, supporting the notion that alternate routes of transmission besides that by the vector can be important for epidemic development.

Functional characterization of four transcription factors regulating pathogenesis in the plant-pathogenic fungus *Alternaria brassicicola*
Y. CHO (1)
(1) University of Hawaii at Manoa, Honolulu, HI, U.S.A.
Phytopathology 102:S4.24

Alternaria brassicicola is a successful saprophyte and necrotrophic plant pathogen. It is relatively well understood that the fungus undergoes a few steps of morphological changes during early pathogenesis within 24 hours post inoculation. In comparison, understanding of molecular basis of the process is scrawny. Through a functional genomics approach, we have identified and characterized the functions of four transcription factor coding genes. One gene is a determinant of the fungal life cycle between the saprophytic to parasitic. Another gene is associated with detoxification of phytoalexins, which is important for early plant colonization. The other two genes regulate mainly hydrolytic enzyme genes that encoded putative cell wall-degrading enzymes. The molecular mechanisms of pathogenesis and their regulation in necrotrophic fungi are in an early stage of research. This study sets the stage for discovery of downstream genes associated with pathogenesis and the characterization of their functions. It promises an identification of novel proteins associated with the elusive early events of pathogenesis. Currently available data from this study indicate the importance of transcription factors as regulators of pathogenesis and as future targets for disease management.

WITHDRAWN

Disruption of poly(A) RNA polymerase gene alters morphology of *Phoma medicaginis*
K. CHOI (1), C. A. Smith (1), M. R. Dhulipala (1), J. N. Enis (1), S. M. Marek (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.24

Phoma medicaginis causes spring black stem and leaf spot of alfalfa (*Medicago sativa*) and annual medics worldwide, including the model legume *M. truncatula*. *P. medicaginis* is genetically tractable using *Agrobacterium* mediated transformation (AMT), with which T-DNA-tagged mutants altered in pycnidial morphology were generated. One mutant (P265) displayed fewer and smaller pycnidia and more aerial hyphae than the wild type. A single T-DNA disrupted a putative poly(A) RNA polymerase gene, Pm*CID13*, which in yeasts interacts with ribonucleotide reductase (RNR). As in yeast mutants, P265 showed sensitivity to hydroxyurea (HU), a RNR inhibitor. To functionally characterize Pm*CID13*, targeted Pm*Δcid13* mutants were created using a hygromycin selectable marker flanked by 1 kbp regions of Pm*CID13*. Pm*Δcid13* mutants possess similar morphological features to those of P265. The complementation vector pCAM-NAT (nourseothricin selection) was constructed and used to introduce full-length Pm*CID13* into mutants. Complemented mutants recovered wild type morphologies and often lost the original T-DNA due to homologous integration. To our knowledge, this is the first *CID13* ortholog to be examined in a filamentous fungus.

Characterization of *Phoma medicaginis* mutant forming hyaline pycnidia
K. CHOI (1), C. A. Smith (1), M. R. Dhulipala (1), J. N. Enis (1), J. M. Stacey (1), S. M. Marek (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.24

Phoma medicaginis causes spring black stem and leaf spot of alfalfa and the model legume *Medicago truncatula*. *P. medicaginis* produces uninucleate conidia in melanized pycnidia and is genetically tractable through *Agrobacterium* mediated transformation (AMT), which can result in insertional mutants. One T-DNA-tagged mutant produced conidia in non-melanized (hyaline) pycnidia. Pycnidial melanization recovered if the mutant was supplemented with phenoloxidase substrates or allowed to age. DNA sequences flanking the insertion did not predict any disrupted open reading frames (ORF) unless a Coccidioides prediction algorithm was used. The Coccidioides predicted ORF (CPO) was expressed in the wild type, but not the mutant (cpo), and has not been annotated in any genomes, to date. Expression of two conserved genes flanking the T-DNA-disrupted cpo was unchanged from the wild type. Complementation of cpo mutants with wild type CPO partially recovered pycnidial melanization. CPO appears to be a novel regulator of pycnidium specific melanization.

Production of monoclonal antibodies to the expressed coat protein of cytoplasmic *Citrus leprosis virus* and its application in immunodiagnosis
N. CHOUDHARY (1), A. Roy (1), G. A. Leon (2), D. D. Picton (3), G. Wei (3), M. K. Nakhla (3), L. Levy (4), R. H. Brlansky (1)
(1) CREC, University of Florida, Lake Alfred, FL, U.S.A.; (2) Corpoica, Villavicencio, Colombia; (3) USDA-APHIS-PPQ-CPHST, Beltsville, MD, U.S.A.; (4) USDA-APHIS-PPQ-CPHST, Riverdale, MD, U.S.A.
Phytopathology 102:S4.24

Citrus leprosis caused by *Citrus leprosis virus* cytoplasmic type (CiLVC), a member of *Cilevirus* genus, transmitted by mite vector *Brevipalpus* spp. CiLVC was previously identified in south and Central America and is rapidly spreading and threatens introduction into US citrus industry. CiLVC detection has been reported by symptom analysis, transmission electron microscopy, reverse transcription polymerase chain reaction (RT-PCR) and polyclonal antibodies. For the routine diagnosis of CiLVC serological diagnosis using monoclonal antibodies would ensure a constant source of uniform reagents. The putative coat protein gene of CiLVC cloned in pDEST17 expression vector and protein was expressed in *Escherichia coli.* The *E. coli* expressed coat protein was purified using Ni-NTA resin and used to immunize mice for monoclonal antibody (MAb) production. Four ascites fluids containing monoclonal antibodies 1D6C11, 1D6E10, 5F3G10, and 5F3F9 were obtained specific to *E. coli* expressed coat protein. The immunoglobulin G type (IgG) was purified from all four antibodies using Protein G agarose and conjugated with alkaline phosphatase. After initial screening, MAb 5F3G10 was selected in plate trapped (PTA) ELISA since it reacted positively with the total protein extract from the CiLVC infected tissue. MAb 1D6C11 was selected for detecting virus in double monoclonal antibody sandwich ELISA after 5F3G10 antibody used to capture virus. The antibodies titer (1:500) was determined for both MAbs in double monoclonal antibody sandwich- and PTA- ELISA and used for detection and identification of CiLVC infected citrus from Panama and Mexico.

Adaptation to heat stress in grapevine powdery mildew
R. A. CHOUDHURY (1), N. McRoberts (1), W. D. Gubler (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.24

Grapevine powdery mildew, caused by the ascomycete *Erysiphe necator* (Schwein.), is a major threat to grapes worldwide. Asexual spores proliferate rapidly during mild conditions, leading to epidemics and yield loss if the disease is left unchecked. However, *E. necator* is sensitive to adverse environmental conditions, such as excess heat, free water and UV radiation. The lethal effects of single exposures of heat have recently been explored. However, relatively little is known about the effects of consecutive exposures to lethal and sub-lethal temperatures. Using detached leaf co-culture assays, three-day-old single colonies of *E. necator* were exposed to one, two or three consecutive days of heat stress. While there was a consistent decrease in colony growth after a single heating event, each subsequent heating event had less of a detrimental effect on colony growth and sporulation date. Colonies appeared to adapt to heat stress rapidly, with no significant additional effects by the third treatment. Colonies also appeared to recover from consecutive heat stress, with no significant differences in colony diameter between treatments by eleven days after inoculation. These data suggest that powdery mildew is far more adaptable to environmental stress than previously thought. This adaptation to consecutive exposures of heat may also be a limitation to disease prediction in warm viticulture regions of the world.

Addressing uncertainty in powdery mildew epidemiology

R. A. CHOUDHURY (1), N. McRoberts (1), W. D. Gubler (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.25

Powdery mildew, caused by *Erysiphe necator* (Schwein.), is an important pathogen that affects cultivated grapevines around the world. Disease begins in the early season with the release of ascospores during rain events or with the emergence of hyphae that have overwintered in dormant buds. The pathogen rapidly multiplies during mild weather conditions, and is inhibited by excess heat or cold. Several epidemiological models have been developed to predict disease onset and disease intensity based on weather conditions, most notably the Gubler-Thomas (GT) model. The GT model uses hourly temperature data to predict the disease intensity based on optimum and lethal conditions for the pathogen, and results in an advisory forecast for disease management. However, scattered weather stations and microclimates lead to uncertainty in hourly weather data. There is also uncertainty of how the pathogen behaves in the field in response to unfavorable conditions, although recent and current studies are helping to fill these gaps. Fuzzy logic is a rule-based logic developed to help deal with uncertainty. In this study, we used fuzzy logic to modify the GT model to adapt to uncertainty in weather data and pathogen biology. We found that the fuzzy GT model performed as well as, or better than, the original GT model, and excelled in a location with a relatively distant weather station. The future prospects for such models are discussed.

Understanding the molecular mechanisms of *Maize fine streak virus* (MFSV) replication and infection

F. CISNEROS (1), Y. Chen (1), A. Michel (1), D. Willis (2), M. Redinbaugh (3)
(1) The Ohio State University, Wooster, OH, U.S.A.; (2) University of Wisconsin, Madison, WI, U.S.A.; (3) USDA-ARS, Corn and Soybean Research, Wooster, OH, U.S.A.
Phytopathology 102:S4.25

MFSV is a negative-sense RNA virus in the family *Rhabdoviridae* that is transmitted by the leafhopper *Graminella nigrifrons*. The virus replicates in both its maize host and insect vector. Similar to other rhabdoviruses, the MFSV genome encodes five structural proteins, the nucleocapsid protein (N), the phosphoprotein (P), the polymerase (L), the matrix protein (M) and the glycoprotein (G). In addition, MFSV has two extra ORFs, MFSV ORFs 3 and 4, for which functions remain unknown. Our goal is to define the roles of the proteins encoded by the MFSV ORFs 3 and 4 in replication and systemic infection of MFSV. Thus, we have initiated the development of a comprehensive protein interaction map. In yeast two-hybrid assays, the MFSV N and M, and the proteins encoded by genes 3 and 4 interact. Additionally, we are using RT-qPCR assays to determine the relative levels of gene expression of the MFSV genes in maize. Our preliminary results show that MFSV 3 and 4 are highly expressed in MFSV-infected plants, suggesting that their expression differs from the remaining MFSV ORFs. For animal-infecting rhabdoviruses, higher transcription of genes at the 3' end of the genome has been observed that reflects the amounts of each protein required for virion structure and function. Additional experiments are underway to corroborate our results. Our results indicate that the MFSV genes 3 and 4 are important for virus infection of its plant host, and suggest that their interaction may be important for function.

Effects of planting environment and seed quality on the field emergence of soybean

K. COCHRAN (1), J. Rupe (1), R. Holland (1), A. Steger (1), A. Palmer (2), S. Goeke (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Arkansas Soybean Promotion Board, Little Rock, AR, U.S.A.
Phytopathology 102:S4.25

Planting environment and seed quality affect field emergence of soybean. The objective of this work was to determine the effects of seed storage and planting environment on emergence (EM). In 2010 and 2011, seed was stored in four environments varying from climate controlled to on-farm storage. Two cultivars were used, one of higher vigor than the other. Seeds were sampled every 2 wks and subjected to standard germination (SG), accelerated aging (AA), Seed Vigor Imaging System (SVIS), and planted at the University of Arkansas Vegetable Research Substation at Kibler, AR and counted at 2 and 4 wks. Data were divided into an early period of high EM with some variability between seed lots and moderate temperatures, followed by a period of little variability between seed lots, lower EM, and high temperatures, and a period moderate temperatures and increasing EM with differences in seed lots. In the case of both years, drop off of EM was associated with increased temperatures, especially in 2011. In 2010, SVIS was positively correlated with EM in early and late plantings, and all plantings in 2011. In 2010, there was a positive but inconsistent correlation between AA and EM. In 2011, there was a positive correlation between AA and EM at the end of the season. SG was positively correlated with EM in 2010 early in the season, but was negatively correlated with EM in the second period of the season. SVIS was more consistently positively correlated with EM than AA or SG.

WITHDRAWN

Effect of temperature on natural colonization of *Guignardia psidii* on 'Kumagai' guava

A. R. COLLETTI (1), I. H. Fischer (2), S. d. Lourenço (1), L. Amorim (1)
(1) ESALQ/USP, Piracicaba, Brazil; (2) APTA Centro Oeste, Piracicaba, Brazil
Phytopathology 102:S4.25

Black spot is one of the main postharvest diseases on guavas, and little is known about the influence of environmental variables on its development and how to control the disease. Controlled environment experiments were conducted to determine the effect of temperature on disease incidence, disease severity, disease progress rate and incubation period of *Guignardia psidii*, causal agent of black spot on 'Kumagai' guava. Fruit, provided by field with high disease incidence, were incubated at six different temperatures (10, 15, 20, 25, 30 and 35°C) with wetness duration of 24 hours. Analysis of variance and regression analysis were used to determine the relationship among temperature and black spot incidence and severity. Ten days after incubation, black spot did not develop on fruit incubated at 10 and 35°C. Among the six temperatures tested, 25 and 30°C were most favorable to black spot development ($p \leq 0.05$). There was an increase on disease incidence, disease severity and disease progress rate as temperature increase from 15 to 30°C. Lesion diameter was greatest at 30°C, reaching 2.66 cm, 10 days after incubation. At this time and at the same temperature, the disease incidence was 100%. Fruit incubated at 15°C presented 6.3% of disease incidence. The lowest incubation period was 6 days for fruit stored at 30°C. Refrigeration can be very useful to control postharvest diseases caused by quiescent infections that can develop during the fruit maturation.

WITHDRAWN

Identification of genes at the Rhg1 locus of soybean that impact soybean cyst nematode development

D. E. COOK (1), X. Guo (1), M. Hudson (2), A. MacGuidwin (1), A. F. Bent (1)
(1) University of Wisconsin, Madison, WI, U.S.A.; (2) University of Illinois, Urbana, IL, U.S.A.

Phytopathology 102:S4.26

Soybean (*Glycine max*) is a staple of global agriculture, with the 2010 U.S. crop valued at over $38 billion. Soybean cyst nematode (SCN), *Heterodera glycines*, is an obligate endoparasite that is the most economically damaging pathogen of soybean in the U.S. SCN is currently controlled by crop rotation and host resistance. SCN invades both resistant and susceptible varieties and initiates establishment of a feeding site (syncytium), but resistant varieties often limit or kill the syncytium, reducing SCN development. SCN resistance is a quantitative trait (some nematodes mature even on resistant hosts), and is controlled by multiple loci, but the single locus termed Rhg1 (Resistance to *Heterodera glycines*) accounts for a significant portion of the resistance in commercially available SCN-resistant soybean varieties. Rhg1 has been fine mapped to a genetic interval corresponding to a 67kb region of the susceptible variety Williams82. Despite its economic importance and over 50 years of research, there are no confirmed reports that identify particular genes from Rhg1 that control SCN resistance. Using gene silencing strategies in transgenic soybean roots generated using *Agrobacterium rhizogenes*, we have identified genes at the Rhg1 locus that contribute to SCN disease resistance. Locus sequence and gene expression analyses from multiple soybean lines are consistent with the concept that multiple genes at the Rhg1 locus contribute to SCN disease resistance. Results from current experiments characterizing gene and protein function will be presented.

Downy mildew of spinach—An overview of resistance

J. C. CORRELL (1), C. Feng (1), K. E. Kammeijer (2), S. Koike (2)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) University of California-Davis, Salinas, CA, U.S.A.

Phytopathology 102:S4.26

Spinach downy mildew disease, caused by the oomycete pathogen *Peronospora farinosa* f. sp. *spinaciae* (Pfs), continues to be a major production constraint for commercial spinach (*Spinacia oleracea*). However, based on cooperation through the International Working Group on Peronospora (IWGP), spinach seed companies, and various researchers, a considerable amount of information has been learned about the genetics of resistance, the race diversity of the pathogen, and how to breed for more durable resistance to the downy mildew pathogen. Based on the disease reactions of a diverse group of open pollinated spinach cultivars and hybrid spinach cultivars, six downy mildew resistance loci, designated RPF1 to RPF6, have been hypothesized. In order to determine the genetics of resistance to Pfs, a consortium project, involving a number of spinach seed companies, was initiated to introgress each of the hypothesized loci into a common susceptible genetic background (Viroflay) to develop Near Isogenic Lines or NILs. Although this work is still underway, efforts with RPF1 and RPF2 have shown that each segregates as a single locus and the resistance is dominant. Interestingly, each of the six hypothesized loci controls resistance to multiple races including some of the newest races identified. Fortunately, no races have been found in recent years that defeat all six of the hypothesized resistance loci. Almost certainly, additional resistance loci will be identified in spinach as a wider set of spinach accessions and collections are evaluated.

WITHDRAWN

Prevalence of dodine resistance in *Venturia inaequalis* populations in the northeastern United States following renewed use of Syllit for the management of apple scab

K. D. COX (1), S. M. Villani (1), L. Ramaekers (2)
(1) Cornell University, Geneva, NY, U.S.A.; (2) Agriphar, Ougrée, Belgium

Phytopathology 102:S4.26

The development of multiple fungicide resistance to several site-specific fungicide chemistries in *Venturia inaequalis* populations in the Northeastern US have left apple producers with few fungicide chemistries with post-infection activity for managing apple scab. A survey of 93 commercial, 6 baseline, and 18 research apple orchards from 2007-2009 revealed that less than 27% of the orchards surveyed had *V. inaequalis* populations with practical resistance to dodine. To further investigate the reduced prevalence of practical resistance to dodine in regional orchards, we surveyed 35 commercial, 4 baseline, and 34 research apple orchards in 2010 & 2011 for sensitivity to dodine using microscopy-aided relative growth assays. Less than 4% of the orchards surveyed had *V. inaequalis* populations with practical resistance to dodine. In 2010 and 2011, field trials were also conducted in an orchard formerly resistant to dodine, but with a 2009 population just below the threshold for practical resistance. Dodine programs, even applied post-infection, were as effective or improved over standard programs of protectant and site-specific fungicides for managing apple scab. Following the field trials in 2010 and 2011, dodine insensitivity, expressed as population mean percent relative growth, declined to 38.0 ± 3.9% in 2010 and to 26.2 ± 3.1% in 2011. Although the orchard population was nearly resistant to dodine in 2009, moderate dodine use restricted to the primary infection cycle did not select for a higher proportion of isolates within sensitivity to dodine. Hence, a reversion to dodine resistance in regional *V. inaequalis* populations may not occur following moderate dodine use.

Ecology of *Bacillus amyloliquefaciens* on wheat florets in relation to biological control of *Fusarium graminearum*

J. M. CRANE (1), D. M. Gibson (2), G. C. Bergstrom (1)
(1) Cornell University, Department of Plant Pathology and Plant-Microbe Biology, Ithaca, NY, U.S.A.; (2) USDA-ARS, Robert Holley Center for Agriculture and Health, Ithaca, NY, U.S.A.

Phytopathology 102:S4.26

The TrigoCor strain of *Bacillus amyloliquefaciens* is a promising biological control agent (BCA) for Fusarium head blight (FHB) of wheat, caused by the fungus *Fusarium graminearum* (teleomorph *Gibberella zeae*). We are using TrigoCor as a model to understand why it, like many BCAs, provides consistent FHB control under controlled environment but not in the field. Although TrigoCor, applied at anthesis, is capable of surviving on heads at significant levels throughout grain maturation, the amount of a key *Bacillus*-produced antifungal compound decreases dramatically by 3 days post-application. The rapid decline of metabolites on heads post-TrigoCor application is especially relevant in the field because late-season *Fusarium* infections occur beyond the time when antifungal levels are high. It is likely that although *Bacillus* populations persist on the wheat surface, most cells are present in an inactive spore form that cannot replenish metabolites as they are depleted. Observation with epifluorescence microscopy of deposition and colonization of wheat floral structures by *Bacillus* and *Fusarium* will be presented. Results of experiments aimed at increasing the percentage of *Bacillus* cells present in the more active vegetative form, both in inoculum and on the wheat surface, will be discussed.

Real-time PCR detection of the boxwood blight pathogen *Calonectria pseudonaviculata*

J. CROUCH (1), R. E. Marra (2), A. Y. Rossman (1)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) Connecticut Agricultural Experiment Station, New Haven, CT, U.S.A.

Phytopathology 102:S4.26

Boxwood blight is a newly emergent, destructive disease of boxwood (genus *Buxus*), caused by the ascomycete fungus *Calonectria pseudonaviculata*.

Initially identified in Europe in the mid-1990s, the disease was first reported in the U.S. in CT, NC and VA during October 2011. In less than four months, boxwood blight appeared in six additional states along the eastern seaboard and in the Pacific Northwest. Substantial losses due to boxwood blight have been reported from wholesale nurseries in the states of CT and NC. Current identification protocols for boxwood blight rely upon assessment of macroscopic symptoms and microscopic examination of the cylindrical asexual spores. Because fungicide treatments may suppress outward disease symptoms and fungal growth, rather than providing complete eradication of the causal fungus, the pathogen may elude detection. Therefore, to reliably identify the presence of *C. pseudonaviculata* in boxwood tissue, a real-time PCR assay based on a 74-bp amplicon in the beta tubulin (Tub2) region was developed. Assay specificity relied on a 5-carboxyfluorescein (FAM) fluorophore-labeled hydrolysis probe modified with four locked nucleic acid bases. Species-specific, reproducible identification of the fungus was made from as little as 0.0050 fg of DNA. This assay will provide a useful tool for monitoring the distribution and incidence of boxwood blight in the U.S.

WITHDRAWN

The role of calcium in the regulation of *Xylella fastidiosa* twitching motility

L. F. CRUZ (1), L. De La Fuente (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.27

The xylem-limited plant pathogenic bacterium *Xylella fastidiosa* (XF) is the causal agent of a range of economically important diseases. Mineral nutrients present in xylem sap may play a role in modulating bacterial physiology and virulence. Previously, this research group demonstrated the effects of Ca on the formation of biofilm, cell attachment, aggregation, and motility. Further experiments have been conducted to establish the role of Ca in the mechanism of XF twitching motility. Other metals added to the standard XF medium had no effect on (Mg, Fe) or diminished (Cu, Zn) XF motility. The association of Ca and the bacterium quorum sensing signal, diffusible signal factor (DSF), was evaluated by testing the effect of Ca supplementation on movement of a DSF mutant. This showed no involvement of DSF in Ca-regulation of movement. Expression analyses of the type IV pilus genes *pilA*, *pilT* and *pilY1* were performed under high and depleted Ca concentrations in cells incubated in microfluidic chambers. Expression analyses indicated an increase in the expression of *pilA* in cells under high Ca concentrations, and no differences in expression were found for *pilY1* and *pilT*. These results indicate that Ca-dependent regulation of twitching motility is not associated with the production of the DSF but could be directly related to the regulation of structural genes associated with type IV pili.

Probability of *Magnaporthe oryzae* (*Triticum* pathotype) introduction into the United States: A quantitative pathway analysis

C. D. CRUZ (1), J. P. Stack (1), R. D. Magarey (2), G. A. Fowler (3)
(1) Kansas State University, Manhattan, KS, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.; (3) USDA-APHIS-PPQ-CPHST-PERAL, Raleigh, NC, U.S.A.
Phytopathology 102:S4.27

Wheat blast, caused by the *Triticum* pathotype of *Magnaporthe oryzae* (Mot), has not yet been reported outside of South America. Wheat is a major crop in the United States (U.S.) and blast can cause serious yield losses. Thus, it is important to understand the potential for its entry and establishment in the U.S. Historical data suggest that human activities are responsible for most introductions of plant pathogens. Since Mot is seed-borne, contaminated grain may be an important pathway for introduction. The purpose of this study was to estimate the probability of Mot entry and establishment in the U.S. associated with the importation of wheat grain from two at-risk areas in South America. Probabilistic models for Mot entry and establishment were constructed. Monte Carlo simulations were run using the @Risk software to determine the probability of wheat blast entry and establishment as a consequence of grain importation. The NAPPFAST modeling system was used to create risk maps based on climate to identify U.S. areas suitable to pathogen establishment. These maps were used to inform the probabilistic model for entry and establishment. Our model results can be used to inform regulatory policy for U.S. wheat imports from at-risk countries. Preparedness plans for early detection, accurate diagnosis, and effective mitigation should be developed.

Phylogenetic analysis, fumonisin production, and genetic variability of *Fusarium fujikuroi* strains isolated from rice in the Philippines

C. R. CUMAGUN (1), M. Gonzalez-Jaen (2), K. I. Aguilar (1), A. Cruz Varona (2), P. Marin (2)
(1) University of Philippines-Los Banos, Los Banos, Laguna, Philippines; (2) Universidad Complutense de Madrid, Madrid, Spain
Phytopathology 102:S4.27

Fumonisins are important mycotoxins which often contaminate several cereals such as maize, wheat or rice, although they can also occur in a wide variety of other commodities. Fumonisins are responsible of serious chronic and acute diseases in human and animals and their presence is under regulation in more than 100 countries. Fumonisin production is basically limited to the members of the formerly so-called *Gibberella fujikuroi* species complex. *Fusarium fujikuroi* has been described as a maize and rice pathogen causing important agricultural losses. However, little information is available about the phylogenetics of this species and its ability to produce fumonisins in rice. In this work, we studied 23 strains isolated from rice in the Philippines and performed a phylogenetic analysis using the partial sequence of the elongation factor-1 alpha including isolates belonging to closely related species. Fumonisin production was analysed in seven-day-old cultures grown in fumonisin-inducing medium by an ELISA-based method and by real time RT- PCR using primers for *FUM1* gene, a key gene in fumonisin biosynthesis. The results indicated the ability of *F. fujikuroi* isolates to produce fumonisin at low levels in the conditions tested and a good agreement between results obtained by ELISA and real time RT-PCR. Fumonisin production of the isolates was not associated with their pathogenicity. High degree of variation was observed among the isolates using universally primed- polymerase chain reaction (UP-PCR) analysis, suggesting that sexual reproduction could play a significant role in generating variation of *F. fujikuroi* populations in the field.

Rust and brown eye spot on center pivot irrigated coffee

A. P. Custódio (1), E. A. POZZA (2), L. S. Santos (2), C. N. Uchoa (3), P. E. Souza (2), A. A. Pozza (4)
(1) Engineering Department, Federal University of Lavras, Lavras, MG, Brazil; (2) Department of Plant Pathology, Federal University of Lavras, Lavras, MG, Brazil; (3) Instituto Federal de Ciência, Tecnologia e Educação do Ceará., Fortaleza, Brazil; (4) Federal University of Viçosa, Florestal, Brazil
Phytopathology 102:S4.27

Irrigation has been used widely on coffee growth in Brazil. Knowledge of water effects on plant diseases is necessary to better management of irrigation systems to improve the sustainable agriculture. This research evaluated the center pivot irrigation, with different water levels, based on the crop coefficient of culture or Kc and your effect in the temporal progress of leaf rust and brown eye spot of coffee plants. Number of spores dispersed in the air was also evaluated. It was observed that different water levels applied through center pivot irrigation influenced the intensity of coffee leaf rust and brown eye spot. Largest intensity of leaf rust was observed at non irrigated plants and the lowest at irrigated with water depths equivalent to 100%, 120% and 140% of Kc. On the other hand, leaf brown eye was larger when more water was applied, with largest incidences at plants irrigated with 100 and 140% Kc while the incidence was the lowest at non irrigated plants. The results had a correlation with a coffee production. It was observed that number of leaves on coffee increased linearly as water depth increased. Acknowledgments: Capes, Cnpq and Fapemig.

WITHDRAWN

Flooding-associated soft rot of sweet potato storage roots caused by *Clostridium*

W. L. DA SILVA (1), C. Clark (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.28

Sweet potato storage roots are commonly destroyed by a rapid soft rot that develops when fields are flooded. The purpose of this work was to identify the pathogens responsible for development of that soft rot. Samples were collected from storage roots with soft rot from intentionally flooded fields and decayed tissue was streaked on plates of nutrient dextrose agar plus 0.05% cysteine. Symptomless sweet potato storage roots were stab inoculated with bacterial isolates, then wrapped with moist paper towels, kept in plastic bags, and incubated at 25°C for 4 days. Ten Gram positive bacteria were re-isolated from rotting storage roots that were able to grow in anaerobic but not in aerobic atmospheres. Endospores were observed in all isolates by differential staining. Six of the isolates had pectinase activity as indicated by pit production in double-layer pectate medium and caused soft rot symptoms in storage roots. Genomic DNA was extracted from representative colonies and the 16s ribosomal RNA region was amplified using primers FD1 and RD1 and the PCR products were sequenced. BLASTn analysis of the 1425 bp sequence resulted in 98% homology with *Clostridium puniceum* strain BL 70/20 from rotting Irish potatoes (GenBank Accession No. NR_026105.1). Understanding the role of pectolytic clostridia in flood-induced soft rotting will be vital in managing sweet potato crop losses following hurricanes and other flooding events.

A role for mating type in *Aspergillus flavus* infection of corn and in biological control?

K. E. DAMANN (1)
(1) Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.28

An *A. flavus* collection from corn kernels from 11 fields revealed that ~98% (549/562) were mating type *Mat1-2*. However, 90% of the isolates were in two vegetative compatibility groups (vcgs) so it was unclear if the high infection frequency could be attributed to mating type or to other traits of the two vcgs. To test the hypothesis that mating type was causal, an isolate from the two predominant kernel infecting vcgs and from two vcgs not found in kernels were deployed on wheat seed between two rows of corn at silking. Ears from both rows were harvested and 20 kernel isolates per row per vcg treatment were obtained. These were tested for homology to the applied vcg and for mating type. For each of the vcgs homology ranged from 0-5% indicating that vcg was not a major contributor to infective ability from inoculum on the ground. However, 94% (150/160) of the isolates were *Mat1-2* mostly of undetermined vcg. Clearly *Mat1-2* strains appear to have much greater infective ability. *Mat1-1* and *Mat1-2* are transcriptional activators and it appears that genes activated by *Mat1-2* have a role in infection. It is also significant that biological control strains Afla-Guard, AF36, K49, and 5 of our Louisiana biocontrol strains of *A. flavus* are all *Mat1-2*. This is consistent with a role for infection by the biocontrol strains to allow expression of intraspecific aflatoxin inhibition of toxigenic wild-type strains in the infection court thereby mediating biological control.

Black leg and yield responses of winter canola cultivars to timing of inoculation with *Leptosphaeria maculans*

J. P. DAMICONE (1), T. J. Pierson (1), C. B. Godsey (1), M. C. Boyles (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.28

Canola cultivars reported to be susceptible (HC 107W), intermediate (DKW 46-15), and moderately resistant (HC 154W) to black leg were planted in the field. Oat kernel inoculum and naturally infested canola stubble were broadcast over plots at the seedling, early rosette, and late rosette growth stages in the fall; and at the first flower bud stage in the spring. Non-inoculated plots served as the untreated check. Disease incidence (% plants with black leg) and disease severity (stem girdling from 0-5) were determined on stubble soon after harvest. Averaged over inoculation timings, differences between cultivars were minimal as disease incidence ranged from 68 to 79% and disease severity from 1.5 to 1.7. Averaged over cultivar, both disease incidence (89%) and severity (2.2) were greater ($P<0.05$) for inoculation at the seedling stage compared to other inoculation times except for the early rosette timing. Yield trended lower for inoculation at the seedling stage, but the effect of inoculation timing on yield was not significant ($P>0.05$). The lack of a differential cultivar response to inoculation with *L. maculans* indicates that the cultivars have a similar level of disease resistance. In assessing the relationship between yield and disease for all plots, there was a 12.3 kg/ha reduction in yield for each percentage increase in disease incidence and a 351 kg/ha reduction in yield for each increase in disease severity from 0 to 5 ($P<0.01$).

Associations between the timing of fungicide application and percent control of Fusarium head blight and deoxynivalenol contamination in wheat

D. D'ANGELO (1), K. T. Willyerd (2), J. D. Salgado (3), L. V. Madden (3), P. A. Paul (3)
(1) Ohio State University, Orient, OH, U.S.A.; (2) Ohio State University, OARDC, Wooster, OH, U.S.A.; (3) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.28

Fungicides are most effective against Fusarium head blight (FHB) and deoxynivalenol (DON) contamination in wheat when applied at early anthesis. However, when frequent rainfall occurs during anthesis and a fungicide treatment is more warranted for FHB and DON control, it is often impossible to make ground applications due to wet, soggy field conditions. Field experiments were conducted in Ohio to evaluate the effects of post-anthesis fungicide applications on FHB and DON. Separate plots were treated with the fungicide Prosaro 421 SC (19% prothioconazole + 19% tebuconazole, 476 ml/ha) at early anthesis and at two, four, and six days post-anthesis. Untreated plots were left as checks, and all plots were spray-inoculated at late anthesis with a spore suspension of *Fusarium graminearum*. FHB incidence (INC) and index (IND) were estimated at three weeks post-anthesis and percent *Fusarium* damaged kernels (FDK) and DON were quantified post-harvest. Mean responses from treated and untreated plots were used to estimate percent control of INC (C_{INC}), IND (C_{IND}), FDK (C_{FDK}), and DON (C_{DON}) relative to the check, for each fungicide treatment. C_{INC}, C_{IND}, C_{FDK}, and C_{DON} ranged from 44 to 60%, 51 to 73%, 58 to 90%, and 68 to 77%, respectively. C_{INC} and C_{IND} were highest for the treatment made at anthesis, decreasing linearly as applications were delayed. However, relative to the treatment made at anthesis, post-anthesis treatments led to comparable or higher C_{FDK} and C_{DON} values.

Phenotypic and genotypic characterization of recent clonal lineages of *Phytophthora infestans* in the United States and Canada

G. DANIES (1), I. M. Small (1), K. L. Myers (1), P. A. Zuluaga (1), R. A. Childers (1), K. A. Bekoscke (1), S. E. Stead (1), A. Teeratananon (1), D. D'Attilio (1), W. E. Fry (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.28

Phytophthora infestans, the causal agent of late blight disease, has been detected in the United States and Canada since the mid XIX century. Due to the lack of, or very limited, sexual reproduction, the populations of *P. infestans* in the United States and Canada are reproducing mostly asexually and thus show a simple genetic structure. The objective of this study was to characterize the most prevalent clonal lineages of *P. infestans* responsible for

the epidemics in the northeastern region of the United States in the summers of 2009 and 2010. Characterization included analysis of genotypic (neutral) markers and phenotypic traits. A secondary objective was to establish whether individuals of *P. infestans* within clonal lineages have common and predictable phenotypic traits. Four clonal lineages (US-8, US-22, US-23, and US-24) were most predominantly found in 2009 and 2010. Both mating types, differences in sensitivity to mefenoxam, differences in pathogenicity on potato and tomato, and differences in speed of germination were detected among these four clonal lineages. It seems that the analysis of genotypic markers may predict phenotypic traits of importance to disease management.

'*Candidatus* Phytoplasma pruni' and its relatedness to phytoplasmas causing grapevine yellows disease in eastern United States

R. E. DAVIS (1), Y. Zhao (1), E. Dally (1), I. Lee (1), R. Jomantiene (2), S. M. Douglas (3)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) Nature Research Centre, Vilnius, Lithuania; (3) Connecticut Agricultural Experiment Station, New Haven, CT, U.S.A.
Phytopathology 102:S4.29

Phytoplasmal diseases are among the most damaging maladies known in fruit plants. In peach (*Prunus persica*) and cultivated grapevine (*Vitis vinifera*), X-disease and grapevine yellows can be major factors limiting production. Understanding the epidemiology of these diseases is a prerequisite for design of effective measures to control their spread and minimize their damaging effects. We investigated multilocus molecular genetic characterization of phytoplasmas associated with peach X-disease and propose the name '*Candidatus* Phytoplasma pruni' for the phytoplasma. Phytoplasma strains causing grapevine yellows in mid-Atlantic states were closely related to X-disease phytoplasma, but specific single nucleotide polymorphisms in the 16S rRNA genes consistently distinguished grapevine-infecting strains from strains in X-diseased peach. Ribosomal protein and protein translocase SecY genetic loci further distinguished the grapevine yellows strains from the peach X-disease strains. The close relatedness between the X-disease and grapevine yellows phytoplasmas raises important questions. For example, can grapevine-infecting strains infect peach or other stone fruit; can peach X-disease strains infect cultivated grapevine; and are the different phytoplasma genotypes transmitted by the same or different insect vectors? Research aimed at answering these questions should be aided by the present work's elucidation of multilocus molecular markers characterizing the genotypes.

Comparative genomic and biochemical analyses of the large pPANA1 plasmid of *Pantoea ananatis*

P. de Maayer (1), S. N. Venter (1), T. COUTINHO (1)
(1) University of Pretoria, Pretoria, Republic of South Africa
Phytopathology 102:S4.29

The enterobacterium *Pantoea ananatis* is commonly found as endo- and/or epiphytes on a wide range of plants. However, it is also an emerging pathogen on a number of agronomically important crops, including rice, onion, corn and *Eucalyptus*. Four complete and four draft genomes of *P. ananatis* strains are available. These include the genomes of *Eucalyptus*, corn, onion and rice pathogenic strains, a clinical strain as well as saprophytic strains. Common to all eight strains is a large plasmid, pPANA1, ranging in size from 281 to 352-kb. Comparative genomics on the *P. ananatis* plasmids were performed. This revealed that the plasmids share a highly syntenous and conserved core. Pairwise comparisons of the proteins encoded on the plasmids indicated that between 74 and 98% of the proteins are shared between two strains with an average amino acid identity of 99.5%. Comparison to the 530-kb pPag3 plasmid of *P. vagans* C9-1 and the 794-kb pPAt9B01 plasmid of *Pantoea* sp. At-9b indicated a shared backbone, suggesting a shared evolutionary origin. The analysis also revealed a number of proteins with a putative role in plant interactions and colonization. Genes encoding a cyclic B-glucan and a locus encoding a type VI secretion system suggests a further potential role in plant pathogenesis. Plasmid-free strains were produced and biochemical analyses performed using BIOLOGs. This indicated the role of the plasmid in the utilization of a number of carbohydrates, amino acids and organic acids. The analyses of the universal pPANA1 plasmid revealed that it plays an important role in colonization and survival by this pathogen in its plant hosts, and may contribute to plant pathogenesis.

Diversity of endophytic *Fusarium oxysporum* populations in tomato: An ecological perspective

J. DEMERS (1), B. K. Gugino (1), M. Jimenez-Gasco (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.29

The plant pathogenic fungus *Fusarium oxysporum* is widely found as an endophyte colonizing plants and as a saprobe in agricultural soils. While pathogenic populations have been well studied, there is a gap in our knowledge about the ecology of this fungus as an endophyte. We collected over 600 isolates from different tomato cultivars in two nearby fields. Samples were obtained from the stem and roots of 32 asymptomatic tomato plants and from cores of the soil surrounding each plant. Isolates were characterized to sequence type using the translation elongation factor 1a gene. A total of 26 sequence types were identified, although two sequence types predominated regardless of sampling location or substrate. Two to six sequence types were found within each plant, highlighting the diversity of endophytic *F. oxysporum*. Comparisons using nearest-neighbor statistics found that populations from each plant were not genetically differentiated from populations in the surrounding soil. However, populations from different tomato cultivars within the same field were genetically differentiated, suggesting that plant genotype may exert selective pressure on populations of endophytic *F. oxysporum*. These results show the ecological diversity of *F. oxysporum* endophytes and saprobes within one field.

Are endophytic *Fusarium oxysporum* host adapted?

J. DEMERS (1), M. Jimenez-Gasco (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.29

The fungus *Fusarium oxysporum* is a widely found as an endophyte. It is not known whether or not these endophytes are adapted to particular hosts, similar to pathogenic forms of *F. oxysporum*. To test the hypothesis of host adaptation, we amassed a global collection of 75 putative endophytes from chickpea. Phylogenetic analyses were done based on the translation elongation factor 1a gene (TEF), the β-tubulin gene, and the intergenic spacer region (IGS). Similar phylogenies were inferred from TEF and β-tubulin, which were both slightly discordant with the IGS phylogeny. However, in all gene trees, the chickpea endophytes tended to fall into only a few clonal lineages, suggesting some degree of adaptation to chickpea. Haplotypes were further characterized using four microsatellite markers. The majority of isolates had unique haplotypes not shared by other isolates. To correlate the ability to colonize chickpea tissues with phylogenetic placement, 38 chickpea endophytes and 20 isolates from other hosts were inoculated onto chickpea plants. Plants were harvested after seed set and tested for colonization. Preliminary results show that an isolate from tomato was equally competent at colonizing chickpea roots as an isolate from chickpea, but only the chickpea isolate was later recovered from the seeds of the inoculated plant. These results have implications for understanding the evolutionary ecology of host-specific pathogenic forms of *F. oxysporum*.

Changes in ROS and lignin associated with progression of *Plasmodiophora brassicae* (clubroot) from cortical to stele cells

A. Deora (1), B. D. GOSSEN (2), M. McDonald (1)
(1) Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada; (2) Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada
Phytopathology 102:S4.29

Plasmodiophora brassicae completes its life cycle in two phases; the first in root hairs and the second in the root cortex and stele. Differences in reactive oxygen species (ROS) and lignin in the root tissue of clubroot-resistant and susceptible cultivars of canola (*Brassica napus*) were assessed in sections stained with diaminobenzidiene (DAB) and toludiene blue O (TBO), respectively, at 5 wk after inoculation. Pathogen development was also assessed in comparable root sections stained with methylene blue. The pathogen was not observed in root sections of the resistant cultivars (secondary phase did not progress) or the susceptible controls. However, the pathogen preferentially colonized xylem parenchyma in the stele of susceptible cultivars. ROS accumulated primarily in the endodermis, pericycle and vascular cambium in both the resistant cultivars and the susceptible controls. In contrast, there was no accumulation of ROS in clubroot-infected roots of susceptible cultivars. Accumulation of ROS may create a chemical barrier that the pathogen is able to detoxify in susceptible plants. TBO staining showed that the walls of parenchyma cells stained blue in resistant and control plants; but were purple in infected areas of the roots of susceptible cultivars. The difference in color indicates an alteration in lignin composition in secondary cell walls.

***Phaeomoniella chlamydospora* on grapevine (*Vitis vinifera*) with black-vascular symptoms in Chile**

G. A. DIAZ (1), B. A. Latorre (1)
(1) Pontificia Universidad Catolica de Chile, Santiago, Chile
Phytopathology 102:S4.29

Young grapevines often show black-vascular streaking (BVS) in the arms or trunks that, in older plants, may progress to a brown-hard wood decay (BHD)

and/or a yellow-soft wood decay (YSD)+ necrotic line (NL). *Phaeomoniella* (*Ph.*) spp. isolated from BVS, BHD or BVS+BHD+YSD+NL were characterized and exhibited colonies that were yellow green to olivaceous, yeast-like, with white margins and a 1-cell conidia that was hyaline and ellipsoid. Analysis of the ITS region (ITS4 and ITS5) and beta-tubulin (Bt2a and Bt2b) of 22 *Ph.* isolates showed 98-100% and 98-99% identity with *Ph. chlamydospora* (*Ph.c*), GenBank nos. AF197973 and AF253968, respectively. The ITS sequences showed divergence between *Ph.c* isolates that was associated with their geographical origin. Of 590 *Ph.c* isolates, 89.8% were obtained from BVS. *Ph.c* (n=4) inoculated on grapevine plantlets produced slight chlorosis, reddish leaves and stunted shoots; the re-isolation of *Ph.c* from the diseased plantlets was successful. *Ph.c* (n=4) inoculated green shoots, spurs and root cuttings of grapevine showed mean BVS values of 4.1-4.4, 6.8-7.3 and 8.3-9.1 cm after 2, 7.5 and 15 mo, respectively, whereas non-inoculated controls remained symptomless. *Ph.c* was re-isolated only from inoculated tissues. Our results suggest that the BVS symptoms are caused by *Ph.c* that may be associated with slight chlorosis and reddening of the leaves. These results showed that *Ph.c* was the only pathogen associated with BVS in grapevines.

Frequency of isolation, aggressiveness, and impact on yield of Fusarium root rot species in soybean in Iowa

M. M. DIAZ-ARIAS (1), L. F. Leandro (1), G. P. Munkvold (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.30

Fusarium root rot is a widespread and common disease in soybean. Different *Fusarium* species have been reported to infect soybean roots but their occurrence, aggressiveness, and impact on yield have not been quantified. A three year survey, and greenhouse and field trials, were conducted to clarify the importance of Fusarium root rot in Iowa. Using morphological and molecular techniques, 15 *Fusarium* species were identified in association with soybean roots, a greater diversity than previously known. *Fusarium oxysporum*, *F. solani*, *F. acuminatum*, and *F. graminearum* were most frequently isolated and most prevalent at the county and field level. Aggressiveness of isolates representing the most frequent species was tested under greenhouse and field conditions. In the greenhouse, aggressiveness differed between species and among isolates at V3 stage; *F. graminearum* caused the most severe root rot and detrimental effects on root morphology, followed by *F. virguliforme* and *F. proliferatum*. Significant variation in aggressiveness was observed among *F. oxysporum* isolates, some of which caused severe damping off. In the field, low root rot severity was observed. Yield was not significantly reduced but significant linear relationships were found for some isolates between yield and root health measures. This study is the first to reveal a pathogenic association of seven different *Fusarium* species with soybean roots during vegetative and reproductive growth stages in Iowa.

Effects of conventional, organic, and no-till field soils on development of southern blight of tomato caused by *Sclerotium rolfsii*

J. Díaz-Pérez (1), V. PARKUNAN (1), M. Purvis (1), M. Finger (1), P. Ji (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 102:S4.30

Soils in vegetable fields in southern Georgia under different production systems had different effects on development of diseases on vegetables. Observations during the last several years indicated that disease incidences on tomato and pepper grown in conventional, organic, and no-till fields were high, medium, and low, respectively. The effect of soils from different fields on development of southern blight caused by *Sclerotium rolfsii* was further evaluated in greenhouse studies. Tomato seedlings (cv. FL47) were grown in soils collected from vegetable fields under long-term conventional, organic, and no-till production. The plants were artificially inoculated with *S. rolfsii* and disease severity was quantified using a 0-5 scale. Disease severities on tomato plants grown in organic and no-till field soils were 61.3% and 77.4% lower than in conventional field soil. Molecular and biochemical studies are being conducted to analyze diversity of soil microflora and microfauna and to determine functional microbial communities contributing to disease suppression. The studies will provide useful information for understanding the mechanisms of disease reduction associated with the disease suppressive soils and potential implementation for disease management in practical vegetable production.

Stress-induced response of prophage FP1 and FP2 in '*Candidatus* Liberibacter asiaticus'

F. DING (1), Y. Duan (2), S. Zhang (3)
(1) USDA-ARS-USHRL, IFAS-TREC, University of Florida, Fort Pierce, FL, U.S.A.; (2) USDA-ARS-USHRL, Fort Pierce, FL, U.S.A.; (3) IFAS-TREC, University of Florida, Homestead, FL, U.S.A.
Phytopathology 102:S4.30

'*Candidatus* Liberibacter asiaticus' (*C*Las), the prevalent bacterial pathogen associated with citrus huanglongbing (HLB), harbors at least two prophages named FP1 and FP2. Due to the fastidious nature of *C*Las, little is known about the prophage's response to stress conditions. In this study, we used real time PCR to investigate the potential conversions of the FP1 and FP2 prophages under stress conditions by comparing to the copy number of 16S rDNA in the HLB-affected periwinkle and citrus. When the HLB-affected periwinkles were exposed to heat stress for 4.0 hours, more FP1 and FP2 were released at 42? and 45? than at 37?. With a shift from 23? to 37?, the relative copy number of FP1 and FP2 doubled, while a shift from 23? to 42? or 45?, the relative copy number of FP1 and FP2 increased to more than 2.5~5-fold compared to initial samples. Meanwhile, HLB-affected citrus scions were treated with tetracycline at concentration 500ppm to 2500ppm by soaking for three days, and then grafted to rootstock for further evaluations. When treated by tetracycline for 7h to 9h, the relative copy number of FP1 and FP2 reached to the highest level with an increase of 2~3.7-fold compared to initial samples. The results indicated the prophages in *C*Las may be converted from a lysogenic cycle to a lytic cycle by stress induction, providing a potential mechanism to reveal why HLB-affected periwinkle became undetectable for *C*Las 3 months after a heat treatment.

Genetic diversity of international collections of *Puccinia striiformis* f. sp. *tritici*

D. Dipak (1), X. CHEN (2), D. R. See (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.30

Puccinia striiformis f. sp. *tritici* (Pst) causes stripe rust, one of the most important diseases of wheat worldwide. Co-dominant microsatellite markers were used to determine the population structures of 292 isolates collected from 18 countries (Algeria, Australia, Canada, Chile, China, Hungary, Kenya, Kyrgyzstan, Mexico, Nepal, Pakistan, the United States, Russia, Spain, Tajikistan, Turkey, Turkmenistan, and Uzbekistan). These isolates were separated into two genetic clusters and an admix group. Genetic cluster 1 consisted of 55% of the isolates from all of the countries. Cluster 2 consisted of 35% of the isolates, mostly from China and Uzbekistan. The admix group consisted of 10% of the isolates, mostly from Asian countries. Cluster 2 and the admix group shows some geographic structures in Asia. In general, results obtained from Bayesian statistics, principal component analysis, and cluster analysis consistently revealed the lack of geographical differentiation among the country-wise collections. Most molecular variation occurred within countries (96%) followed by among international regions (4%) and variation among countries was not significant. Collections from China and Central Asia had significant but low level of genetic differentiation with collections from North and South Americas. Either low or insignificant genetic differentiation between other regions may explain the low geographic structuring of the wheat stripe rust pathogen worldwide.

Screening of biological control agents from fresh produce against foodborne human pathogens

S. Dobhal (1), G. Zhang (2), L. M. Ma (1), M. ARIF (3)
(1) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.; (2) Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, U.S.A.; (3) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.30

Outbreaks of foodborne illness have been associated with consumption of contaminated fresh fruits and vegetables and effective control strategies including the use of biological interventions are needed. The objective of this study was to isolate microorganisms from fresh produce that were antagonistic towards foodborne human pathogens: *Salmonella*, *Escherichia coli* O157:H7 and *Shigella*. Fresh leafy green vegetables and tomatoes from various locations in Oklahoma were collected. The surface wash from vegetables were ten fold serially diluted and plated. The plates were overlaid with each pathogen independently and screened for colonies exhibiting inhibitory zone. The selected colonies were picked and streaked for isolation. The isolates showing antagonistic activity towards all three pathogens were selected and identified through 16S rDNA sequencing. More than 50 isolates were found to be inhibitory to all three pathogens tested. Majority of the isolates belong to genera of *Pseudomonas* and *Bacillis* including *Pseudomonas fluorescens*. Further studies are ongoing to evaluate the effectiveness of antagonists on the survival and growth of foodborne human pathogens on fresh produce during storage. The final results of these studies could be used as biological control agents in minimizing the risk of foodborne pathogen contamination in fresh produce.

First-year almond tree performance as affected by preplant soil steam, backhoe, and fumigation treatments in a replanted site with the presence of plant-parasitic nematodes

D. A. DOLL (1), G. T. Browne (2), B. Hanson (3), S. A. Fennimore (4)
(1) University of California, Merced, CA, U.S.A.; (2) USDA-ARS, Davis, CA, U.S.A.; (3) University of California-Davis, Davis, CA, U.S.A.; (4) University of California, Salinas, CA, U.S.A.
Phytopathology 102:S4.31

Successive generations of almond orchards are suppressed by replant problems, i.e., Prunus replant disease (PRD), a poorly understood soilborne complex, nematode parasitism (NP), and other factors. Preplant soil fumigation with methyl bromide (MB) or alternative fumigants can control PRD and NP, but due to the phase out of MB and various regulatory mandates, optimized fumigant and non-fumigant treatments are needed. In a replanted almond orchard, we are evaluating the efficacy of pre-plant treatments with: steam (applied with tractor-mounted soilaugers 90 cm diameter to a 60 cm depth, centered at tree planting sites [TPS]); soil mixing (achieved with backhoe in 1.5 x 1.5 x 1.5 m volumes centered at TPS); and soil fumigation (MB, or 1,3-dichloropropene [1,3-D], or 1,3-D pluschloropicrin [1,3-D:CP], applied with tractor-mounted shanks to row strips or broadcasted areas). Treatment efficacy was assessed as increases in stem diameter from Jan. 2011 (planting) to Feb. 2012 (end of tree dormancy). Sampling confirmed the presence of *Cylindrocarpon* sp., *Pythium* spp. (PRD associated) and *Mesocriconema xenoplax* (inflicts NP). Soil mixing and steam had little and no effect, respectively (stem diameter increases 102 and 100% of controls, respectively), whereas all fumigation treatments were clearly beneficial (stem diameters 117 to 123% of controls). Steam and soil mixing alone did not achieve the benefit of soil fumigation for first-year control of PRD and NP, but evaluations will be continued for several seasons.

Resistance of daylily cultivars to isolates of the daylily rust pathogen *Puccinia hemerocallidis*

W. Dong (1), J. BUCK (1)
(1) University of Georgia, Griffin, GA, U.S.A.
Phytopathology 102:S4.31

Daylily rust caused by *Puccinia hemerocallidis* was first detected in the Southeastern U.S. in 2000 and was subsequently spread via interstate transport on infected plants throughout much of the country. In our original study in 2002, eighty four daylily cultivars from commercial growers were evaluated for resistance to a single isolate of *P. hemerocallidis*. The objective of the current study was to determine if virulence differs among multiple isolates of *P. hemerocallidis*. Fifteen daylily cultivars from our first study and six additional cultivars were inoculated with seven isolates of *P. hemerocallidis* collected from various locations in Georgia in 2010 and the isolate of *P. hemerocallidis* used in our 2002 study. Four separate divisions of each daylily cultivar were inoculated with a urediniospore suspension from each isolate and reaction type (resistant, moderately resistant, moderately susceptible, and susceptible) was assessed after three weeks. Differences in virulence were observed between isolates of *P. hemerocallidis* on six of the daylily cultivars: three cultivars evaluated in 2002 (Hush Little Baby, Joan Senior and Hyperion) and three additional cultivars (Purple De Oro, Primal Scream, and Ruby Spider). These results suggest that different races of *P. hemerocallidis* are present in commercial nurseries which may result in daylily rust developing on cultivars previously thought to be resistant.

Efficacy of ethaboxam toward species of *Phytophthora* and *Pythium*

A. E. DORRANCE (1), M. L. Ellis (2), D. McDuffee (3), K. Arthur (4)
(1) The Ohio State University, Wooster, OH, U.S.A.; (2) The Ohio State University, OARDC, Wooster, OH, U.S.A.; (3) Valent U.S.A. Corporation, Indianapolis, IN, U.S.A.; (4) Valent U.S.A. Corporation, Plano, TX, U.S.A.
Phytopathology 102:S4.31

Stand loss due to seed and seedling pathogens is very common in Ohio for both corn and soybean. In many cases *Phytophthora* or *Pythium* were identified as the causal agent. One strategy to manage these pathogens is the use of seed treatments. The efficacy of ethaboxam was evaluated towards *Phytophthora sojae*, *P. sansomeana*, and eight different *Pythium* spp. in amended agar assays. This fungicide was also evaluated for efficacy as a soybean seed treatment in greenhouse assays, and field trials. In amended agar and greenhouse assays, ethaboxam provided greater, similar, or was less efficacious than metalaxyl towards the isolates of the different *Phytophthora* and *Pythium* spp. In field studies, seed treated with 7.5 to 15 g a. i./100 kg of seed had significantly higher stands and yield in 2008 and 2010 for the moderately susceptible variety. The moderately resistant variety had significantly higher stands and yield in the same trials when ethaboxam was applied at the same rates in 2010 and 2011 at two locations. Ethaboxam applied to soybean seed as a seed treatment will provide another tool to manage seedling diseases caused by *Phytophthora* and *Pythium* spp.

Comparison of amended agar and greenhouse assays to evaluate the efficacy seed treatment fungicide ethaboxam

A. E. DORRANCE (1), M. L. Ellis (2), C. B. Meador (3), K. Arthur (4)
(1) The Ohio State University, Wooster, OH, U.S.A.; (2) The Ohio State University, OARDC, Wooster, OH, U.S.A.; (3) Valent U.S.A. Corporation, Leland, MS, U.S.A.; (4) Valent U.S.A. Corporation, Plano, TX, U.S.A.
Phytopathology 102:S4.31

Amended agar assays are often used as a first step in evaluating compounds for efficacy towards plant pathogens. This can be challenging for some fungicides, as true fungi and oomycetes, may have alternative mechanisms for some physiological pathways. In addition, amended agar assays are often used as a means to measure limitation in mycelia growth as zoospores, conidia, or ascospores are challenging to produce in lab situations. This study compared the efficacy of ethaboxam on mycelial inhibition on amended agar plates and from treated seed planted into infested sand or vermiculite. Ethaboxam limited mycelia growth at 0.1 ppm for *Phytophthora sojae* and 100 ppm for *Py. sylvaticum* but had no effect on *Pythium aphanidermatum* at 100 ppm. Seed treated with ethaboxam significantly reduced damping-off at 7.5, 10, and 15 g a.i./100 kg of seed for *P. sojae*, but had no effect on *Py. aphanidermatum*. Ethaboxam limited mycelia growth and protected seed and seedlings from damping-off caused by *P. sojae* but not *Py. aphanidermatum*. Amended agar assays were a good predictor for the efficacy of ethaboxam as a seed treatment. This information indicates that additional isolates of these and other species from different geographic regions can be evaluated with the amended agar assays.

Thermal treatments eliminate or suppress the bacterial pathogen in huanglongbing-affected citrus

M. S. DOUD (1), M. T. Hoffman (1), M. Zhang (2), E. Stover (1), D. Hall (1), S. Zhang (3), Y. Duan (1)
(1) USDA-ARS-USHRL, Fort Pierce, FL, U.S.A.; (2) IFAS-IRREC, University of Florida, Fort Pierce, FL, U.S.A.; (3) IFAS-TREC, University of Florida, Homestead, FL, U.S.A.
Phytopathology 102:S4.31

The causal agents of citrus huanglongbing (HLB), a destructive disease of citrus, are '*Candidatus* Liberibacter asiaticus' (Las), '*Ca*. L. africanus' and '*Ca*. L. americanus'. Previous studies have found variations in temperature sensitivity and tolerance among these species. Here we described the use of thermotherapy to alleviate HLB caused by Las, the most prevalent and heat tolerant species. Using a temperature-controlled growth chamber, we evaluated the thermotherapy regime required to eliminate or suppress the Las bacterium in citrus, using 3 temperature treatments (40, 42 and 45°C) for time periods ranging from 2 to 10d. Results of qPCR after treatment showed significant decreases in the Las titer, reaching an undetectable level, combined with healthy vigorous tree growth. Repeated surveys confirmed previously infected plants show no detectable Las, while untreated plants remained infected. The results indicated continuous thermal exposure of 40-42°C for at least 48h was sufficient to significantly reduce Las in HLB-affected citrus. However, heat treatments yielded different results in dooryard citrus and commercial groves using portable greenhouses for 3-10d. Although temperatures within the greenhouse surpassed 40°C for multiple hours, the results indicated a suppression rather than elimination of Las in the treated HLB-affected plants. This variation may be due to fluctuating greenhouse temperature and lower soil temperature. Further optimizations of field trial heat treatments are needed.

Management of bacterial spot caused by *Xanthomonas euvesicatoria* in organic tomato production systems

J. G. DRIVER (1), M.C. Iott (1), C. T. Taylor (1), F. J. Louws (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.31

Foliar diseases present a severe challenge to organic tomato growers due to prevalence of warm temperatures and long periods of leaf wetness in Western North Carolina. An experiment was implemented on certified organic land in Waynesville, NC to assess the impact of host genetics and spray programs (using OMRI approved products) on foliar disease severity. The experiment included main plots of 4 heirloom/heirloom-type tomatoes with varying levels of early blight and late blight (LB) resistance: Cherokee Purple, NC144, NC244 and RG220. Sub-plots consisted of 6 different spray programs: DNP-03; Stimplex (*Ascophyllum nodosum* seaweed extract); Serenade or QRD-146 (*Bacillus subtilis*); rotated biweekly with Nordox (Cuprous oxide). These were compared to a standard (STD) control of Serenade/Regalia/Nordox and non-sprayed plots not treated with any products. Blight pressure was very low but

bacterial spot (BS) pressure was high and the causal agent was verified through culture-based assays. Tomato variety did not impact BS severity and spray interactions were not significant. AUDPC analysis demonstrated plots not sprayed had the greatest severity of BS. QRD-146 and Serenade treatments significantly reduced BS severity but not as much as the STD spray, DNP-03 and Stimplex treatments. Reduced BS severity translated into yield effects with DNP-03 and Stimplex generating the greatest yield of marketable fruit compared to the QRD-146 and Serenade treatments. Control plots had the lowest yield and the STD program was intermediate. These novel, OMRI-approved products offer significant opportunity for organic growers to achieve reduced disease severity and higher yields in heirloom production systems.

Comparative epidemiology of late blight and early blight of potatoes under different environmental conditions and fungicide programs in Brazil

H. S. DUARTE (1), L. Zambolim (1), F. Machado (1), H. Porto (1), E. Mizubuti (1), P. Paul (2)
(1) Universidade Federal de Viçosa, Viçosa, Brazil; (2) The Ohio State University/OARDC, Wooster, OH, U.S.A.
Phytopathology 102:S4.32

Among the diseases that affect potatoes, late blight (LB) caused by *Phytophthora infestans* and early blight (EB) caused by *Alternaria grandis* are considered of great importance because of the losses they cause. Three field experiments were conducted in Viçosa, MG, Brazil, with the objective of comparing LB and EB epidemics under different environmental conditions and fungicide application programs. Potato cultivar 'Ágata', susceptible to both LB and EL, was used in all experiments. Each experiment consisted of two side-by-side trials, and each trial was arranged in a randomized complete block design, with 5 treatments and 5 replications. At 30 days after planting, plots intrials 1 and 2 were spray-inoculated with an isolate of the A2 mating type of *P. infestans* (200 sporangia/mL) and an isolate of *A. grandis* (200 conidia/mL), respectively. Fungicide applications were initiated seven days after inoculation and repeated at 7- or 15-day intervals. LB and EB severity were assessed every two days and AUDPC was estimated for both diseases. Yield was quantified and the association between yield and AUDPC was evaluated. Based on AUDPC, LB was more aggressive than EB under all environmental conditions, and this was reflected in the yield. LB caused yield losses as high as of 82.4%, while EB caused yield losses of up to 44.9%. Fungicide effects on LB and EB and associated yield losses varied among environments, indicating the importance of using tools such as forecast systems to guide fungicide application decisions.

Regulation of expression of CorA, a virulence factor and magnesium, nickel, and cobalt transporter in the soft rot pathogen, *Pectobacterium carotovorum*

K. DUMENYO (1)
(1) Tennessee State University, Nashville, TN, U.S.A.
Phytopathology 102:S4.32

The soft rot pathogen, *Pectobacterium carotovorum* (formally *Erwinia carotovora* subsp. *carotovora*) causes disease on diverse plant species by synthesizing and secreting copious amount of plant cell wall-degrading enzymes. These enzymes include pectate lyases (Pel), polygalacturonases (Peh), cellulases (Cel), and proteases (Prt). Exoenzyme production and virulence are controlled by many factors of bacterial, host and environmental origin. The magnesium, nickel and cobalt transporting membrane protein, CorA is required for full virulence and enzyme production. CorA-deficient strains express corA-lacZ at a higher level than CorA+ strains indicating corA negatively affects its own expression. The competitive inhibitor of CorA, cobalt (III) hexaammine (Co(III)Hex) specifically inhibited the transport function of CorA by reversing the toxic effect of cobalt chloride in CorA+ strains but had no effect on enzyme production in both CorA+ and CorA- strains. The expression of corA-lacZ was about four-fold lower in HrpL-deficient strains lacking the hrp-specific extracytoplasmic sigma factor. The promoter region of corA contains sequence with high similarity to the consensus Hrp-box sequence. Put together, these data indicate that corA is part of the Hrp regulon and its effect on virulence is independent of intracellular magnesium concentration.

Incidence and impact of *Verticillium dahliae* in dirt associated with certified potato seed lots

J. K. DUNG (1), P. B. Hamm (2), J. E. Eggers (2), D. A. Johnson (1)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) Department of Botany and Plant Pathology, Hermiston Agricultural Research & Extension Center, Oregon State University, Hermiston, OR, U.S.A.
Phytopathology 102:S4.32

Verticillium dahliae (*Vd*), causal agent of Verticillium wilt of potato, can be found in the vascular system of infected seed tubers and has been found in dirt associated with certified potato seed. The purpose of this research was to quantify *Vd* in dirt associated with certified seed tubers and determine if this inoculum is related to the development of Verticillium wilt in the subsequent crop. *Verticillium dahliae* was recovered from 68% of dirt collected from the surfaces of seed tubers between 2009 and 2011. Over 82% of dirt samples collected from trucks and bags used to transport seed tubers contained *Vd*. Most dirt from seed lots exhibited *Vd* levels between 5 and 50 CFU/g but *Vd* levels between 50 and 500 CFU/g were observed all three years. Over 93% of isolates recovered belonged to vegetative compatibility group 4A. Levels of *Vd* in stem sap increased with increasing levels in dirt from seed tubers but only when levels of the pathogen in field plot soils were low ($P<0.01$). Postharvest levels of *Vd* increased in all field plots compared to preplant levels and greater postharvest levels of *Vd* in the field soils were related to increased levels of *Vd* in dirt on seed tubers ($P=0.04$). The transport of infested soil with seed tubers provides a means to introduce *Vd* into fields not previously cropped to potato or have received preplant management practices such as soil fumigation and, once established, increase after the cropping of susceptible hosts.

Role of coinfection by *Pectobacterium* spp. and *Verticillium dahliae* in the development of early dying and aerial stem rot of potato

J. K. DUNG (1), D. A. Johnson (1), B. K. Schroeder (1)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.
Phytopathology 102:S4.32

Potato early dying (PED) can be caused by several pathogens including the soilborne fungus *Verticillium dahliae* (*Vd*) and pectyolytic bacteria in the genus *Pectobacterium*. *Pectobacterium* spp. can also cause aerial stem rot of potato and additive or synergistic interactions may increase disease severity when both pathogens are present. The objective of this study was to determine if co-infection of potato by *Vd* and *Pectobacterium* results in additive or synergistic increases in PED or aerial stem rot severity under greenhouse conditions. Real-time PCR was used to quantify pathogen and host DNA in stems and determine if relative pathogen DNA levels increased *in planta* following co-infection. PED symptoms caused by *P. carotovorum* subsp. *carotovorum* strain Ec101 or *Vd* strain 653 alone were similar and included wilt, chlorosis and senescence. Inoculations with *P. wasabiae* strain PwO405 caused aerial stem rot symptoms including water-soaked lesions and necrosis. Greater *Pectobacterium* DNA levels were observed in plants inoculated with PwO405 compared to Ec101, suggesting that aerial stem rot can contribute to increased levels of *Pectobacterium* inoculum. Although significant additive or synergistic effects were not observed in plants co-inoculated with *Pectobacterium* and *Vd* ($P > 0.05$), *Vd* DNA levels were 1.4 to 1.7 times greater in basal stems of plants inoculated with both pathogens and levels of Ec101 DNA were 2.8 times greater when plants were co-inoculated with *Vd*.

Genomic comparisons of two *Bacillus subtilis* biocontrol strains with different modes of actions

C. DUNLAP (1)
(1) USDA/NCAUR/CBP, Peoria, IL, U.S.A.
Phytopathology 102:S4.32

Bacillus subtilis strains AS 43.3 and OH131.1 were isolated from wheat anthers and shown to be efficacious in managing Fusarium head blight in greenhouse and some field trials. Chemical analysis of the cell-free culture supernatant identified *B. subtilis* strain AS 43.3 to be a potent producer of the antifungal lipopeptides: surfactin, iturin and fengycin. In addition, strain AS 43.3 demonstrated strong antibiosis of *Fusarium graminearium* in competition assays. Under the same conditions, *B. subtilis* strain OH 131.1 produced only a small amount of the lipopeptide surfactin and did not inhibit *F. graminearium* in competition assays. The lack of antifungal metabolites in OH 131.1 suggested it may utilize a different mode of action than AS 43.3 or only produced them in the wheat infection court. To determine the secondary metabolite potential of each strain, we used next generation sequencing to produce assembled genome libraries with greater than 30x sequence coverage. Sequence alignments of the two genomes identified differences in the secondary metabolite pathways between the organisms. Data analysis also identified previously unknown pathways in each strain and the data suggests strain AS 43.3 may need to be reclassified as a *B. amyloliquefaciens* strain.

Monitoring changes in population structure of an isolated research population of *Phytophthora capsici*

A. R. DUNN (1), C. D. Smart (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.32

The vegetable pathogen *Phytophthora capsici* (causal agent of Phytophthora blight) is moved between fields in soil, water, or infected plants but not wind. Previous work has shown that field populations of *P. capsici* are very diverse, with limited gene flow between fields and resulting in relatively isolated inbreeding populations. It is not known how this may affect pathogen aggressiveness or ability to adapt to resistant host varieties or new fungicide chemistries. To begin to answer these questions, a research field with no prior history of Phytophthora blight was inoculated in Fall 2008 with two single-spored isolates of *P. capsici* collected from nearby farms. In 2009 and 2010 susceptible vegetables were planted in the field and 47 and 59 isolates, respectively, were collected and genotyped using six microsatellite loci. A total of 26 and 30 unique multilocus genotypes were identified in 2009 and 2010, respectively. Neither the fixation index (F_{IS}) nor the pairwise F_{ST} between years were significantly different from zero, suggesting that the population in this field is randomly mating and that populations from the two years are not significantly differentiated. Characterizing this research population immediately after infestation will allow future comparisons to be made after years or decades of isolation and inbreeding. Potential implications for understanding *P. capsici* population dynamics on vegetable farms and effects on disease management will be discussed.

Heterologous expression and functional analysis of the wheat group 1 pathogenesis-related (PR-1) proteins

K. L. Dunnell (1), J. D. Faris (1), T. L. Friesen (1), M. C. Edwards (1), S. LU (1)
(1) USDA-ARS, Cereal Crops Research Unit, Fargo, ND, U.S.A.
Phytopathology 102:S4.33

The group 1 pathogenesis-related (PR-1) proteins have been widely used as hallmarks of plant defense pathways, but their biological functions are still unknown. We report here the functional analysis of two basic PR-1 proteins following the identification of the wheat *PR-1* gene family. The predicted mature PR-1 proteins were expressed in *Pichia pastoris* and both targeted proteins were detected in the yeast secretions as a single species of about 15 kDa in SDS-PAGE with a yield estimated at ~ 0.01 mg/ml. Identity of the expressed PR-1 proteins was confirmed by western blot and MALDI-TOF/TOF analyses. No apparent anti-fungal activities were observed in spore germination inhibition assays; both PR-1 proteins, however, appeared to be resistant to proteolytic attack by a bacterial subtilisin-like protease that completely digests several un-related proteins under the same conditions. Reverse transcriptase PCR indicated that both *PR-1* transcripts were induced in at least three different wheat lines upon infection by *Barley stripe mosaic virus* (BSMV), a tripartite, (+) sense RNA virus infecting monocot crops, suggesting that the two PR-1 proteins may play a role in basal defense in wheat. Further studies are needed to explore the possibility that PR-1 proteins may function as protease inhibitors or provide protection for other host proteins involved in plant signaling and/or defense pathways.

Acquisition and transmission of *Pantoea ananatis* and *Pantoea agglomerans* (causal agents of center rot of onion) by onion thrips (*Thrips tabaci*)

B. DUTTA (1), R. D. Gitaitis (1), R. Srinivasan (1), D. Langston (1), A. Barman (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 102:S4.33

Onion thrips (*Thrips tabaci*) and the onion pathogens *Pantoea ananatis* (Pna) and *P. agglomerans* (Png) were studied to determine if onion thrips could acquire and transmit bacterial pathogens associated with the center rot of onion complex. After surface-disinfested onion thrips (n =four/treatment) fed on onion leaves supporting epiphytic populations (~1 $\times 10^8$ colony-forming-units/ml) of either Pna or Png, thrips were sampled and bacterial acquisition after 0, 1, 6, 24, and 48-h feeding periods was determined. Thrips were crushed in 1 ml of 0.1M phosphate buffered saline. Aliquants of 100 µl of serial dilutions (1:9) were spread-plated on tryptic soy broth agar (TBSA) or PA-20 media and presence of bacteria determined. Transmission studies were conducted using thrips that fed for 48-h on onion leaves supporting populations of either Pna or Png as above. Thrips were removed and placed on healthy, 3-wk-old onion seedlings in the greenhouse (~70% RH and ~ 25° C). Plants were evaluated for symptom development for up to 14 days. There was an exponential positive relationship between thrips-feeding period and percent thrips acquiring Pna or Png ($R^2 \geq 0.95$; $P \leq 0.01$) with 100 and 91.7% of the thrips acquiring Pna and Png, respectively after a 48-h feeding period. In two independent trials, 60.0 and 75.0% of the seedlings exposed to thrips acquiring either Pna or Png, developed typical center rot symptoms, respectively. Results from this study demonstrate a potential role of *T. tabaci* in the transmission of both Pna and Png, causal agents of center rot of onion.

Ambrosia asymptomatic virus 1*—A novel *Mandarivirus

M. DUTTA (1), U. K. Melcher (1), N. S. Bashir (2)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) University of Tabriz, Tabriz, Iran
Phytopathology 102:S4.33

Virus research in agriculture has been focused mainly on the pathogenic nature of viruses. To understand the true nature of plant-viral interactions, the possibility of the presence of non-pathogenic viruses should be explored. To this end, The Plant Virus Biodiversity and Ecology project was undertaken. Plants from theTallGrass Prairie Preserve were systematically studied over several years for the presence of viruses. Here we present the genome, genomic organization and genetic variability of a novel member of *Alphaflexiviridae*. The virus has a single stranded RNA genome of 7280bp and has six open reading frames (ORFs). Coat protein sequence analysis of the virus exhibits similarities to the genus *Potexvirus* but whole genome and replicase sequence analysis strongly indicates that the virus belongs to the *Mandarivirus* group.

Irrigation management for the reduction of dollar spot disease of creeping bentgrass

N. DYKEMA (1), J. Vargas (1), K. Frank (1), W. Kirk (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.33

Dollar spot, caused by *Sclerotinia homoeocarpa*, remains a major disease of economic importance on golf course turf as most of fungicide budgets are spent to control it. Due to increasing regulatory restrictions by the EPA, alternatives to fungicides and integrated management approaches are being investigated to combat turfgrass diseases. This study compares different irrigation regimes based on frequency and timing while integrating the use of resistant cultivars to assess disease management. Three irrigation regimes were randomly assigned to three replicate 11 m x 11 m irrigation plots. Irrigation regimes included: light daily (approximately 0.25 cm) at 5:00 am, light daily (approximately 0.25 cm) at 10:00 pm, or infrequent (1-2 times weekly) to equal the total amount of irrigation applied to the daily treatments at 10:00 pm. Within each irrigation plot, three creeping bentgrass cultivars were randomly replicated four times in split-plots measuring 2.7 m x 3.7 m. Preliminary results from one year of field testing indicate that light irrigation applied at 10:00 pm on a daily basis resulted in less dollar spot in three creeping bentgrass cultivars than the same application volume applied at 5:00 am on a daily basis or the same total weekly volume of irrigation applied on an infrequent basis (1-2 times weekly) at 10 pm at night. In addition, differences among three creeping bentgrass cultivars were observed under each of three different irrigation regimes.

WITHDRAWN

Coat protein expression strategy of *Oat blue dwarf virus*

M. C. EDWARDS (1), J. J. Weiland (1)
(1) USDA-ARS, Fargo, ND, U.S.A.
Phytopathology 102:S4.33

Oat blue dwarf virus (OBDV) was the first *Marafivirus* (family *Tymoviridae*) to be sequenced and for which an infectious clone has been reported. Sequence data are now available for multiple marafiviruses, yet the expression strategy of these viruses remains uncharacterized. Translation experiments with OBDV suggest that a large 227 kDa polyprotein encoded by much of the genome is post-translationally processed into its functional components, in agreement with its tymoviral lineage. ORFs for the two coat proteins (CPs) of approximately 21 kDa and 24 kDa are encoded near the 3' terminus and are coterminal with the ORF encoding this large polyprotein. Although a marafibox analogous to the tymobox is presumed to serve as a promoter for a subgenomic RNA encoding the CPs, the expression strategy for the CPs has not been thoroughly investigated. We have developed a series of point and deletion mutants in an infectious OBDV clone in an effort to dissect and analyze CP expression strategy in protoplasts. The 21 kDa (major) CP appears to be the product of direct translation of a subgenomic RNA, while the 24 kDa (minor) CP appears to be a cleavage product derived from both the polyprotein and a larger ~26 kDa precursor translated directly from a subgenomic RNA.

Resolving the *Pythium ultimum* species complex

Q. A. EGGERTSON (1), C. A. Levesque (2), C. R. Buell (3), J. P. Hamilton (3)
(1) Carleton University, Ottawa, ON, Canada; (2) Agriculture and Agri-Food Canada, Ottawa, ON, Canada; (3) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.34

Pythium ultimum is comprised of two morphological varieties: *Pythium ultimum* var. *sporangiiferum* which readily produces zoospores at room temperature and *Pythium ultimum* var. *ultimum* which does not. Previous efforts to resolve this morphological species complex have been conflicting or inconclusive. Four hyper-variable gene regions were identified by comparing the number of single nucleotide polymorphisms between the annotated sequenced genomes of two isolates of var. *ultimum*. Primers were designed for these regions which were sequenced along with three other previously identified genes: internal transcribed spacer (ITS), beta-tubulin and *Ochromonas* mastigoneme protein (OCM1). Bayesian analysis using the program *BEAST (Bayesian Evolutionary Analysis Sampling Trees) was used to determine species limits using the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) technique. Four genetically distinct species have been identified which are not distinguishable by morphology.

WITHDRAWN

Pathogenicity of *Diaporthe* species associated with stem canker of blueberry in Chile

K. ELFAR AEDO (1), B. A. Latorre (1), R. Torres (1)
(1) Pontificia Universidad Católica de Chile, Santiago, Chile
Phytopathology 102:S4.34

Stem canker is a major disease of blueberry (*Vaccinium corymbosum*) in Chile and is associated with the *Diaporthe/Phomopsis* complex and *Neofusicoccum* spp. Symptoms include apical necrosis, reddish stem necrosis, internal vascular discoloration and dieback. The pathogenicity of *D. australafricana* (n=3), *D. ambigua* (n=1), *D. phaseolorum* (n=1), *Diaporthe* sp. (n=2) and *N. parvum* (n=1) was studied using the detached stems of blueberry, apple and pear and mature blueberry fruits in the laboratory. Field assays were conducted using the attached stems of mature blueberries 'O'Neal' and the shoots of 'Thompson Seedless' grapevines. The inoculations were performed with a mycelium plug inserted underneath the epidermis. The *Diaporthe* spp. were pathogenic in blueberry, apple, pear and grapevine, developing brown to reddish necrotic lesions of 3.0 to 42.2 mm. The inoculated fruits developed a brown soft rot that partially covered the fruit after 6 days at 20°C. *Diaporthe* spp. were less virulent than *N. parvum* on blueberries and the other hosts, but *D. australafricana* (isolate 24.1.1.p1) was the most virulent species on the grapevines. The age of the blueberry stems influenced the development of the necrotic lesions in that <1-year-old stems were more susceptible than 1- to 2-year-old stems. *D. australafricana*, *D. ambigua*, *D. phaseolorum* and *Diaporthe* sp. were pathogenic in blueberry, but they were significantly less virulent than *N. parvum*.

Temperature influences stem canker development in blueberry caused by *Neofusicoccum parvum*

K. ELFAR AEDO (1), B. A. Latorre (1), R. Torres (1)
(1) Pontificia Universidad Católica de Chile, Santiago, Chile
Phytopathology 102:S4.34

Stem canker of blueberry (*Vaccinium corymborum*) is associated with Botryosphaeriaceae spp. and frequently occurs in Chile in association with *Neofusicoccum parvum*. The effect of temperature on the infection caused by *N. parvum* was studied on detached 'O'Neal' stems. Actively growing <1-yr-old stems (n=12) and dormant 1-yr-old partially lignified stems (n=12) were inoculated with a mycelium plug that was inserted beneath the cortex. The stems were incubated at 0 to 35°C for 15 to 21 days in a humid chamber (>85%), and necrotic stem lesions of 8.7 to 100.3 mm were obtained. *N. parvum* was successfully re-isolated from the diseased stems. The temperature and the age of the stem had significant influences on the necrotic lesion development, and a significant ($p<0.0001$) interaction between the temperature and age of the stems was observed. The disease severity increased as the temperature increased between 5 and 30°C and was higher in the <1-yr-old stems than the 1-yr-old stems, whereas the length of the necrotic lesions decreased between 30 and 35°C. At 5°C, infection only occurred in <1-yr-old stems, and no infection occurred at 0°C. The optimal temperature for lesion development was 30°C. For both types of stem, a polynomial regression model best explained the relationship between the temperature and lesion length when the temperature ranged between 0 and 35°C. In conclusion, stem canker caused by *N. parvum* depends on the temperature and the age of the stem.

Influence of *Fusarium palustre*, drought, and DMSO on herbivory of *Spartina alterniflora* by marsh crabs

W. H. ELMER (1)
(1) The Connecticut Agricultural Experiment Station, New Haven, CT, U.S.A.
Phytopathology 102:S4.34

Recovery from Sudden Vegetation Dieback (SVD) in New England salt marshes has been hindered by the herbivorous purple marsh crab, *Sesarma reticulatum*. We hypothesized that inoculation of *Spartina alterniflora* (SA) with the stem rot pathogen, *Fusarium palustre* (FP), and/or drought may increase herbivory. SA were exposed to different irrigation regimes and inoculated with FP or left un-inoculated. Pairwise combinations of SA plants were placed in bins with marsh crabs and photographed over a 1-2 day period for visual estimates of loss due to consumption. Herbivory was greatest on drought-stressed plants and/or plants that were inoculated with FP. Plants exposed to the same treatments and transplanted into a crab-infested SVD site were similarly consumed. In other published studies, it was shown that stressed SA plants had higher levels of dimethylsulfoxide (DMSO) than healthy plants. To determine if DMSO was an attractant for the marsh crab, we drenched healthy SA and other grass species with DMSO (20 micromoles) to determine if it increased herbivory when compared to untreated plants. Consumption of SA was significantly greater in the first 4 hr. on DMSO-treated SA plants than on controls, but did not increase herbivory on other grass species. These findings suggest that plant stress, whether by disease or drought may increase the attractiveness of SA to herbivory by marsh crabs and that DMSO might function as a chemo-attractant in SA.

WITHDRAWN

Postharvest development of citrus black spot symptoms and the viability of conidia

H. ER (1), K. Hendricks (2), P. D. Roberts (2), A. H. vanBruggen (3)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) Southwest Florida Research and Education Center, IFAS, University of Florida, Immokalee, FL, U.S.A.; (3) Department of Plant Pathology, University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.35

Citrus black spot, caused by *Guignardia citricarpa*, was first detected in Florida in March 2010. Infection causes unsightly lesions on the peel, making the fruits unsuitable for fresh fruit market, but infected fruits can still be used for juice production. The objectives are: (i) to determine the percentage of asymptomatic fruits that develop black spot symptoms during storage, (ii) to determine the percentage of black spot lesions that harbor conidia and the germination and infection rate of the conidia. For objective (i), a total of 432 asymptomatic fruits from two different black spot positive groves were incubated at 4, 12, or 22°C and 80, 90, or 100% relative humidity for eleven weeks. For objective (ii), symptomatic oranges were incubated in crisper boxes with 100% relative humidity at ambient temperature. Two oranges were removed at weekly intervals and pycnidia on black spot lesions were isolated to check for presence of conidia and their germination rate. Eighteen out of 432 asymptomatic oranges developed black spot like symptoms during storage. Significantly more lesions were found at 12°C, compared to the other two temperatures (P=0.0002). Of the field-borne pycnidia, 17% harbored conidia with a germination rate ranging from 5% at week 2 (n=56) down to 0.08% (n=3430) at week 6. The experiments are currently repeated and infectivity of conidia will be determined on leaves and fruits. These results are important for estimating the likelihood of spread of the pathogen outside of the current quarantine area as result of transportation of asymptomatic and symptomatic fruits.

From peptidoglycan (PGN) perception to activation of innate immune responses in plants

G. ERBS (1), T. Sundelin (1), M. Newman (1)
(1) University of Copenhagen, Frederiksberg, Denmark
Phytopathology 102:S4.35

In plants, innate immunity is triggered through Pathogen Recognition Receptors (PRRs) in response to Microbe-Associated Molecular Patterns (MAMPs). We have already shown that the two lysine motif (LysM) containing plasma membrane proteins LYM1 and LYM3 in *Arabidopsis* interact with peptidoglycan (PGN) and that the transmembrane LysM receptor kinase CERK1 is involved in transmembrane signaling. We have preliminary data indicating that the perception and the following activation of immune responses in *Arabidopsis* occur differently for PGN and its breakdown product muropeptides. A transcriptome analysis shows early induction of genes in *Arabidopsis* in response to PGN whereas a late induction of genes was observed in response to the muropeptides. A greater insight into the mechanisms of MAMPs perception by plant PRRs and the following signaling transduction pathway will have considerable impact on the improvement of plant health and disease resistance.

A new pest: *Fusarium* sp. and its vector tea shot-hole borer (*Euwallacea fornicatus*) causing Fusarium dieback on avocado in California

A. ESKALEN (1), D. H. Wang (1), M. Twizeyimana (1)
(1) Department of Plant Pathology and Microbiology, University of California-Riverside, Riverside, CA, U.S.A.
Phytopathology 102:S4.35

The Asian ambrosia beetle (*Euwallacea fornicatus*) forms a symbiotic relationship with *Fusarium* sp. This beetle has recently been threatening the Israeli avocado industry. The beetle also causes severe damage on tea (*Camelia sinensis*) branches in Sri Lanka and India. In California, the beetle was first reported on black locust (*Robinia pseudoacacia*) in 2003, but there was no record of symbiotic fungus damage. Symptoms of white powdery exudate either dry or surrounded by wet discoloration of the outer bark in association with a single beetle exit hole were observed on several backyard avocado trees in South Gate, Downey and Pico Rivera, Los Angeles County, California in February and March 2012. While no visible injury to the bark was observed, examination of the cortex and wood under the infested spot bored by the beetle(*E. fornicatus*) revealed brown discolored necrosis caused by a fungus. Symptomatic cortex and sapwood tissues were plated onto PDA medium amended with tetracycline (0.01%). After 4 to 5 days of incubation at room temperature, fungal colonies akin to *Fusarium* sp. were produced. Fungal identification was determined by using the rDNA internal transcribed spacer (ITS) and elongation factor (EF1-α) primers. Pathogenicity tests were conducted by inoculating detached green shoots of healthy avocado trees. Lesions were observed on all inoculated shoots except for the control. Mean lesion lengths were 12.8 cm 3 weeks after inoculation. This is the first report of symbiotic *E. fornicatus* and *Fusarium* sp. on avocado in California.

Validation of EDNA, a newly developed bioinformatics tool, for detection of *Phakopsora pachyrhizi* from metagenomic samples

A. S. ESPINDOLA (1), C. D. Garzon (1), J. Fletcher (1), W. L. Schneider (2)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) USDA, Fort Detrick, MD, U.S.A.
Phytopathology 102:S4.35

Accurate and sensitive pathogen detection is needed to prevent devastation of economically important crops. Molecular based diagnosis includes end-point and real-time PCR which, although specific, have some limitations. Next Generation Sequencing offers new and exciting alternatives for pathogen detection from metagenomic data. Unlike PCR primers binding to one locus, this technology analyzes a great proportion of the pathogen genome. The objective of this study was to assess EDNA, a recently developed massive parallel sequencing based protocol for detection of plant pathogens, on *Phakopsora pachyrhizi*, the soybean rust fungus. Whole nucleic acids were obtained from infected soybean plants and analyzed by 454 sequencing. Metagenomic data was analyzed with EDNA. The sample sequence database (SSD) contained more than 9 Mb sequences clustered in 30,426 reads with an average read length of 309.04 bp. The pathogen read abundance ranged from 0.158% to 1.63% (very low to low abundance). E-probes were blastn searched in the SSD, resulting in 1902 hits at e-values of $1x10^{-3}$ and as low as 268 hits at an e-value of $1x10^{-9}$. The specificity of the detection method was checked using shuffled e-probes. Specificity ranged from 99.85 to 100%. The use of e-probes (up to 146,000) designed specifically for *P. pachyrhizi* permitted the unequivocal detection of soybean rust in infected samples, confirming the usefulness of EDNA for fungal detection from infected plant material.

Validation of EDNA, a newly developed bioinformatics tool, for the detection of *Pythium ultimum* from metagenomic samples

A. S. ESPINDOLA (1), C. D. Garzon (1), J. Fletcher (1), W. L. Schneider (2)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) USDA-ARS, Fort Detrick, MD, U.S.A.
Phytopathology 102:S4.35

Pythium ultimum (Oomycota) is responsible for root roots, fruit roots, and damping off of multiple crops, with universal distribution. *P. ultimum* is the only species of the genus with a sequenced genome; hence we used it as model system for validation of the EDNA (E-probe diagnostic nucleic acid analysis), a recently developed massive parallel sequencing based protocol for detection of plant pathogens. Rapid detection of oomycetes may contribute to a timely eradication of infected plant materials and effective control/management of diseases. The development of a new generation of molecular techniques has led to new approaches for diagnosis. The objective of this study was to assess the EDNA protocol for detection of *P. ultimum*. Whole nucleic acids were obtained from infected potato plants and analyzed by 454 sequencing. The presence of the pathogen was verified by end point PCR before sequencing. The sequencing output or sample sequencing database (SSD) was analyzed using unique e-probes (up to 500,000) designed specifically for *P. ultimum*. The sequencing output produced 61,728,898 of total bases arranged in 145,922 sequencing reads. A blastn search was carried out and parsed data were analyzed. Positive hits ranging from 1,070,606 ($1x10^{-3}$ e-value) to 70,250 ($1x10^{-9}$ e-value) were assessed. Detection specificity ranged from 99.76% to 100%, with a sensitivity of 72.4 %. Consequently *P. ultimum* can be detected with high sensitivity and specificity using EDNA.

Virulence traits in *Xylella fastidiosa* strains are modulated by calcium
M. R. EVANS (1), L. Cruz (1), L. De La Fuente (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.36

Xylella fastidiosa (XF) is a bacterial plant pathogen that infects a variety of economically important crops in the US. XF forms biofilms that are hypothesized to clog host plant xylem vessels, resulting in a lack of water and nutrient depletion. Previous research in our laboratory has shown that calcium added to culture media increases biofilm formation and twitching movement for the XF wild-type Temecula strain. In order to investigate if this effect is widespread among diverse XF strains, *in vitro* assays for these virulence traits were performed on 13 additional strains of XF. Serial dilutions of calcium in PD2 medium displayed a positive correlation between calcium levels and biofilm formation for all strains. Assays for twitching movement showed increased movement for most strains when grown on PW medium supplemented with 2mM calcium and a decrease in movement when grown on PW medium supplemented with 1.5mM EGTA (a calcium chelator). Our results show that calcium is an important regulator of virulence traits across diverse XF strains. Based on genomic analysis, the genes *mopB* and *msrA*, coding for an outer membrane protein and the enzyme methionine sulfide reductase respectively, were selected as potential calcium related candidates for a site-directed deletion mutagenesis. By analyzing the phenotype of mutant bacteria deficient in these genes, the role of calcium in XF infection can be elucidated and utilized in future disease control methods.

Evaluation of lima bean germplasm for resistance to *Phytophthora capsici*, the causal agent of lima bean pod rot
T. A. EVANS (1), N. F. Gregory (1), E. G. Ernest (2)
(1) University of Delaware, Newark, DE, U.S.A.; (2) University of Delaware, Georgetown, DE, U.S.A.
Phytopathology 102:S4.36

Lima bean (*Phaseolus lunatus*) is an important processing vegetable crop in Delaware and the Mid-Atlantic region (MAR) with approximately 5,670 ha planted each year. In 2002, *Phytophthora capsici* was determined to be the causal agent of a new and threatening disease in Delaware, lima bean pod rot (LBPR). In 2008, it was determined that mefenoxam resistance was present in populations of *P. capsici* from lima bean fields in the MAR. Recently, LBRP has increased in incidence and is impacting lima bean growers and processors in the region. Because of the increased incidence of LBPR and mefenoxam resistance, we began evaluations of lima bean accessions for resistance to *P. capsici* for use in our breeding program. In 2010 and 2011, 31 distinct lines of lima bean were evaluated in the field for resistance to *P. capsici*. Two lines showed no signs or symptoms of LBRP in 2010 or 2011. They are PI 347826, a landrace collected in California, and PI 477041, a landrace collected in Arizona. In the fall of 2011, a greenhouse evaluation of lines that did not produce flowers and pods in the field under Delaware conditions was carried out. Of the 19 distinct lines evaluated in greenhouse mist chambers, 17 produced pods and signs and symptoms of LBPR. Two lines produced pods and did not show signs or symptoms of LBPR after being inoculated twice. They are PI 256450, a landrace from El Salvador and PI 362772, a landrace from Brazil.

Fine-scale genetic structure of *Monilinia fructicola* populations within peach tree canopies
S. E. EVERHART (1), H. Scherm (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 102:S4.36

We examined the spatio-temporal dynamics of brown rot disease (caused by *Monilinia fructicola*) and the structure of the corresponding pathogen population within individual, intensively mapped peach tree canopies. Across 3 years, a total of six trees were monitored for disease development throughout the season, tagging each individual symptom (blossom blight, green fruit rot, preharvest fruit rot, and twig canker) and mapping it in three dimensions using a magnetic digitizer. In addition, *M. fructicola* was isolated from each of the mapped symptoms. Trees had between 250 and 866 fruit total, with a final preharvest fruit rot incidence of 13 to 36%. DNA from all 718 single-spored isolates obtained from these trees was evaluated with 20 polymorphic SSR markers developed previously. For one tree analyzed at the time of this writing, haploid diversity for isolates collected in the early-season blossom blight, mid-season green fruit rot, and late-season preharvest fruit rot epidemics showed decreasing diversity over time (h = 0.520, 0.470, 0.466, respectively). No spatial genetic structure was detected for either early- or mid-season periods, whereas significant autocorrelation among genetic distances was observed at spatial distances up to 1.25 m during the preharvest interval. The presence of such fine-scale patterns of *M. fructicola* populations within individual trees is likely due to a localized zone of influence in pathogen spore dispersal.

WITHDRAWN

Detection and characterization of boscalid resistance in *Alternaria solani* causing early blight on potatoes in Idaho
K. L. FAIRCHILD (1), L. A. Miles (1), T. D. Miles (1), P. S. Wharton (1)
(1) University of Idaho, Aberdeen, ID, U.S.A.
Phytopathology 102:S4.36

Field isolates of *Alternaria solani* which causes early blight of potato in Idaho were evaluated in vitro for their sensitivity towards the fungicide boscalid. A total of 20 isolates were collected from foliar-infected tissue in 2009, 35 in 2010, and 59 in 2011. Fungicide sensitivity was tested using the spiral-gradient endpoint dilution method. The frequency of boscalid-resistant isolates (EC50 = 10,000 mg/L) went from 15% in 2009 to 66% in 2010. In 2011, the number of resistant isolates increased to over 70%. The target site of boscalid is the succinate dehydrogenase (SDH) enzyme complex, which is vital for fungal respiration. Sequence analysis of the Sdh gene revealed mutations in the subunits SdhB, SdhC and SdhD that were responsible for the emergence of boscalid resistance in potato fields in Idaho. The identification of these mutations will allow for the development of a rapid method to assess boscalid sensitivity in *A. solani* populations.

Pathogenicity and virulence of *Bipolaris* species and impact on switchgrass biomass
O. L. FAJOLU (1), M. M. Dee (1), K. Gwinn (1), P. A. Wadl (1), A. L. Vu (2), R. N. Trigiano (1), B. H. Ownley (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.; (2) University of Massachusetts, Amherst, MA, U.S.A.
Phytopathology 102:S4.36

Switchgrass (*Panicum virgatum* L.) has potential as a biofuel crop; however, little attention has been directed toward switchgrass pathogens and their impact on feedstock biomass. Information on pathogenicity and virulence of fungi isolated from switchgrass will aid in development of disease management strategies. The objectives of this study were to determine the pathogenicity and virulence of *Bipolaris sorokiniana*, *B. oryzae*, *B. victoriae* and *B. spicifera* isolates recovered from commercial switchgrass seed and naturally infected field plants, and determine the impact of these fungi on feedstock biomass. Isolates were pre-screened for pathogenicity and virulence with a detached leaf assay on 'Alamo'. Eighteen of the most virulent isolates were evaluated on whole plants, or applied to surface-sterilized seeds. The aggressiveness of each isolate and their effects on stand establishment, plant health, and biomass yield were measured. Tests were arranged in a CRD and data were analyzed with SAS. All isolates of *Bipolaris* were pathogenic on switchgrass, but there were significant differences in pathogen aggressiveness (P<0.0001) among and within species. Nine virulence groups were identified; *B. sorokiniana* and *B. oryzae* were the most virulent, whereas *B. spicifera* was the least and *B. victoriae* was moderately virulent (F-LSD, P=0.05). Losses in

biomass were 15.2% to 69.7% for plants inoculated with 10^1 CFU/ml, and 25.7% to 72.5% for plants inoculated with 10^5 CFU/ml.

Assessing anthracnose symptoms in Andean lupin *Lupinus mutabilis*
C. E. FALCONI (1), A. W. van Heusden (2)
(1) ESPE University, Conocoto, Pichincha, Ecuador; (2) Wageningen University, Wageningen, Netherlands
Phytopathology 102:S4.37

Anthracnose, caused by *C. acutatum*, is the most devastating fungal disease in lupin. The fungus spreads through the main stem and produces necrotic spots and orange spores. It can also grow on leaves and terminal branches. The level of tolerance was studied under greenhouse conditions and depended on plant stage and inoculation method. Lupin plants of the cultivar I-450 ANDINO were grown and plant-pathogen interactions were determined in five different phenological stages (0.5, 1.0, 1.5, 2.0, 2.5-month-old plants). Three isolates of *C. acutatum* were used for inoculation on the meristematic section of the main stem either by spraying or pipetting on an artificial wound. A scale (1-6) was used to score disease severity. There were significant differences between the two inoculation methods and the five phenological stages. Plants that received the inoculum by pipetting after artificial wounding showed significant ($P<0.05$) more anthracnose symptoms than those that were sprayed. One and a half-month-old plants were the most tolerant and spraying appears to be the best method for a preliminary screening of large lupin populations, but artificial wounding is more reliable when screening potential resistant genotypes. We recommend to do the first screening in young plants (1.0-months old) and to confirm tolerance when flowering starts (2.5-months old) in this way the overall host reaction can be determined. Seeds of selfed lupin plants were partly used for replanting and disease evaluation and partly for measuring alkaloid content. There was no correlation between disease severity and alkaloid content. Project financially supported by TELFUN project /WUR and ESPE University, Ecuador.

First report of *Meloidogyne enterolobii* on Noni, Chinese Eaglewood, and Clove in China
W. H. Fang (1), F. M. Ying (2), C. M. CAI (2)
(1) Institute of Environment and Plant Protection, Haikou, Peoples Republic of China; (2) Hainan Academy of Agricultural Sciences, Haikou, Peoples Republic of China
Phytopathology 102:S4.37

Root-knot nematode *M. enterolobii* has a high reproductive rate and a wide host range, and is currently spreading in many agricultural crops around the world. From 2007 to 2010, we surveyed the incidence of root-knot nematodes in Hainan Province of China on three important Chinese medicinal crops: noni (*Morinda citrifolia*), Chinese eaglewood (*Aquilaria agallocha*) and clove (*Syzygium aromaticum*). Samples were collected from roots of each crop and nematode species were identified by morphology, isoenzyme technology and mtDNA marker. Measurements of the second-stage juveniles and morphological characteristics of the female perineal patterns coincided with the descriptiton of *M. enterolobii*. The perineal patterns were variable, with moderately high to high dorsal arch and with no lateral lines. Isoenzyme experiments showed a strong malatedehydrogenase (Mdh) band (N1a) and a weaker esterase (Est) band (VS1-S1). PCR amplification of the mtDNA between *COII* and *lrRNA* gene was accomplished with primers #C2F3 (5'-GGTC AATGTTCAGAAATTTGTGG-3') and #1108 (5'-TACCTTTGAC CAATC ACGCT-3'). A product of 0.7 kb was obtained and the fragments could not be digested with restriction enzyme *Hinf*I. All these results confirmed the presence of root-knot nematode. To our knowledge, this is the first record of *M. enterolobii* parasitizing noni, Chinese eaglewood and clove in Hainan, China.

The epidemiology of *Bean golden mosaic virus* in transgenic bean lines
J. C. FARIA (1)
(1) EMBRAPA-CNPAF, Santo Antonio De Goias, Brazil
Phytopathology 102:S4.37

Bean golden mosaic virus (BGMV) is the causal agent of a destructive disease of common beans causing losses of up to 100% in a singly early affected field. A transgenic bean line, namely Embrapa 5.1, with resistance to BGMV was developed using the siRNA strategy. In the years of 2007 to 2011 field experiments were conducted to evaluate the resistance of the transgenic line and potential commercial lines derived by four backcrosses, after the initial cross to Embrapa 5.1. The experimental plots consisted of five replications in a randomized block design using a total of five bean lines (including non-transgenic controls), in 2007-2008. Each plot had five rows of five meters in length. In 2009-2011 twenty six inbred/homozygous lines were evaluated in replicated trials. The plots had four four-meter rows, and were replicated twice in a randomized block design. The non-transgenic commercial lines were included as controls. Disease progress was evaluated weekly by the incidence of symptomatic plants since disease onset. BGMV incidence varied from 4.6 to 87% of the plants in the control cultivars. There were no transgenic infected plants. In each of the three years more than 4000 transgenic plants were evaluated. It was concluded that Embrapa 5.1 and their derivative homozygous lines were equally and completely resistant to BGMV and will help growers in the management of the disease in the coming years. Embrapa 5.1 has been approved for commercial release.

Genetic diversity and whitefly transmission of *Tomato apex necrosis virus*
R. Felix (1), A. M. Cochran (2), N. Yu (2), G. H. Rodriguez (1), S. A. Trinh (2), Z. XIONG (2)
(1) University of Occidente, Los Mochis, Sinaloa, Mexico; (2) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.37

Tomato apex necrosis virus (ToANV) is an emerging virus on tomatoes. The virus contains two genomic RNA molecules of approximately 7 kb and 5 kb, and is closely related to viruses in Sequiviridae. Infections by ToANV produce necrosis of growing tips and young leaves of tomato plants, resulting in severe stunting and severe yield losses. Early infections may destroy the entire crop. To better understand the virus, twenty samples exhibit typical ToANV symptoms were collected near Los Mochis, Sinaloa, Mexico for molecular characterization and transmission studies. Plants sampled included cultivated tomato and tomatillo, and two weeds, *Datura inoxia* and *Solanum rostratum*. Two pairs of PCR primers were designed according to the sequences conserved between ToANV and three other closely related viruses to amplify a 0.7 kb fragment from RNA-1 and a 0.6 kb fragment from RNA-2. These primers easily amplified DNA fragments specific to ToANV in tomatoes, tomatillos, and datura, but not from *S. rostratum*. To characterize the genetic diversity of ToANV, PCR-amplified fragments of RNA-1 and RNA-2 cloned and sequenced from isolates collected at different locations. Preliminary sequence analysis suggests genetic variations among different isolates. To identify the dominant vector for the field transmission of ToANV in the Sinaloa State, two species of native whiteflies were collected and used to transmit ToANV from tomatillos to a number of solanaceous species.

WITHDRAWN

Disease reactions of IRRI near-isogenic rice to U.S. isolates of *Magnaporthe oryzae*
C. FENG (1), F. Rotich (1), J. Correll (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 102:S4.37

Rice blast, caused by *Magnaporthe oryzae*, is a destructive disease of rice. The use of resistant cultivars is the most effective way to manage this disease. However, to be effective, it is necessary to know how specific resistance genes respond to the pathogen population. Two sets of near-isogenic lines

(NILs), each containing a target resistant gene, in either a Japonica cultivar (Lijiangxintuanheigu -LTH) or an Indica cultivar (CO39) background, have been developed by IRRI. Twelve U.S. reference isolates were tested on 31 LTH NILs and 20 CO39 NILs containing 25 targeted resistance genes. NILs containing genes Pia and Pi3(t) were susceptible to all reference isolates tested whereas NILs containing Pi9(t) or Pi12(t) were resistant to all isolates. Lines containing genes Pib, Pi11(t) and Pita-2 were resistant to 9 or 10 isolates. Four loci provided resistance to reference isolate 49D (race IB-49) or IB33 (race IB-33), 7 loci were resistant to isolate TM2 (race k), and 14, 16, and 17 loci were resistant to isolate IB-54, isolate #24 (race IG-1) and isolate ID-13, respectively. Pi19(t) and Piks were only resistant to one isolate. PiI, Pikh, Pikp were resistant to two isolates, five loci, including Pi1, Pi7(t), Pik, Pika, Pikm were resistant to 3 isolates. Thus, the five loci (Pi9(t), Pi12(t), Pib, Pi11(t) and Pita-2) were the most effective resistance genes to the panel of reference isolates evaluated and could be exploited to improve resistant to rice blast disease in the U.S.

Development of a multiplex real-time PCR assay for multiple seedborne spinach pathogens

C. FENG (1), J. C. Correll (1), L. J. du Toit (2), B. H. Bluhm (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Washington State University, Mount Vernon, WA, U.S.A.
Phytopathology 102:S4.38

Fresh market spinach is a highly nutritious vegetable, and dramatic increases in both production and consumption have occurred in the USA over the last two decades. The increase in production has resulted in an increase in disease pressure by a number of seedborne pathogens. Downy mildew, caused by the obligate pathogen *Peronospora farinosa* f. sp. *spinaciae* (Pfs) is the most important and destructive disease of spinach. *Verticillium dahliae* (Vd), although not a pathogen of the vegetative production crop, is an important disease during seed production and represents a potential concern if planted in non-infested soils. In addtition, Stemphylium leaf spot, caused by *Stemphylium botryosum* (Sb), and Cladosporium leaf spot, caused by *Cladosporium variabile* (Cv), are important foliar diseases of spinach that can be seedborne. Multiple seed detection assays for individual pathogens is less economically feasible than a multiplexed assay for several seedborne pathogens. DNA primers and probes targeting these four spinach pathogens were designed, and proved highly specific in TaqMan real-time PCR assays tested against 12 Pfs isolates, 20 Vd isolates, 6 Sb isolates, and 4 Cv isolates. The assays were very sensitive based on testing a range of concentrations of pathogen DNA. A rapid DNA extraction protocol from single seed was developed which enables individual and batches of seed to be examined for multiple pathogens. Optimization of the multiplex real-time PCR seed assay is in progress.

Characterization and management of *Botrytis cinerea* resistant to multiple fungicides

D. FERNANDEZ-ORTUNO (1), A. Grabke (1), X. Li (1), P. Bryson (1), G. Schnabel (1)
(1) Clemson University, Clemson, SC, U.S.A.
Phytopathology 102:S4.38

Botrytis cinerea Pers., is the most economically important pre- and post-harvest pathogen of strawberry. The main strategy to control the disease involves the application of different classes of fungicides despite that *B. cinerea* is considered a high-risk pathogen for resistance development. We collected 216 *B. cinerea* isolates from strawberry fields in the Carolinas during 2011 and determined in vitro fungicide sensitivity to seven different classes of fungicides currently used for gray mold control in the Southeastern United States. About 60% of all isolates were resistant to boscalid, pyraclostrobin and thiophanate-methyl and more than 40% and 15% were resistant to cyprodinil and fenhexamid, respectively. None of the isolates were resistant to fludioxonil and iprodione. Some isolates were resistant to five fungicides, each from a different chemical class. Resistance to boscalid, fenhexamid, pyraclostrobin and thiophanate-methyl was correlated with point mutations in the corresponding target genes (SdhB, erg27, cytb and B-tubulin). A regional resistance monitoring program was implemented to help growers determine location-specific resistance profiles.

Blackleg in canola seed and dockage: Can it cause plant infections?

D. FERNANDO (1), B. Demoz (1)
(1) University of Manitoba, Winnipeg, MB, Canada
Phytopathology 102:S4.38

Blackleg disease caused by *Leptosphaeria maculans* is one of the major diseases of canola in the world. The pathogen can infect all parts of the plant but mainly causes girdling at the stem base causing subsequent yield loss. This study assessed the level of blackleg infection in seed and dockage, the type of spores produced by dockage, and ability of seed and dockage to cause infection in Canadian grown canola. Canola seed samples from different areas of the Prairie Provinces of Canada were tested for the presence of the aggressive *L. maculans* and the less aggressive *L. biglobosa*. One thousand seeds per sample were assessed using blot test. Isolates collected from each infected seed were identified with PCR using species-specific primers. The most frequently isolated species was *L. biglobosa* (4.04%) compared to *L. maculans* (0.24%). Infected seeds that germinated could not grow into adult plants; the cotyledons rotted. DNA was extracted from dockage and PCR tests showed the presence of *L. maculans* in dockage samples. Dockage samples incubated at different temperatures produced pycnidiospores but not ascospores. The ability of dockage to cause infection was tested by growing wounded cotyledons on flats covered with dockage pieces. Two weeks after wounding, infections marked by the presence of pycnidia were evident on cotyledons. Although *L. maculans* can be found in seeds, it is present at very low levels. Dockage could produce pycnidiospores and cause infection on cotyledons.

Latent period and infectious period: Useful concepts or vague notions

F. J. FERRANDINO (1)
(1) Connecticut Agricultural Experiment Station, New Haven, CT, U.S.A.
Phytopathology 102:S4.38

For the past half century, the latent period and the infectious period introduced by Vanderplank have dominated any discussion of plant disease epidemics. In Vanderplank's model the reproduction curve is given by a square temporal flush of progeny production between time p and time p + i. Alternatively, the first two temporal moments of the reproduction curve provide a pair of reproductive time scales: the mean delay time between infection and progeny production and the standard deviation about this mean. For the simple case described above, in which every infection follows exactly the same reproductive time course, the values of the moments are uniquely determined by the values of p and i. In reality, there is considerable variability in these time scales among individual infections because of several factors including: somatic differences in host tissue, differences in microclimate within the plant canopy, as well as the inherent range of virulence within the pathogen population. For such a dynamic situation the meaning and use of latent and infectious period are shown to be ambivalent and their use can be misleading.

Genetic variation and evolutionary adaptability of *Rhizoctonia solani* AG-1 IA from soybean under stress conditions

C. G. Ferro (1), P. C. CERESINI (2), G. M. Ferraudo (3), P. C. dos Santos (4), D. Perecin (1)
(1) Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil; (2) FAPESP/Universidade Estadual Paulista (UNESP), Ilha Solteira, Brazil; (3) CanaVialis/Monsanto, Campinas, SP, Brazil; (4) Universidade Estadual Paulista (UNESP), Ilha Solteira, SP, Brazil
Phytopathology 102:S4.38

Stress due to environmental changes can impact quantitative traits through changes in genetic and environmental variances. In this study we tested the hypothesis that high temperature stress can increase the genetic variation for quantitative traits such as the tolerance to a broad-spectrum fungicide in the fungus *Rhizoctonia solani* AG-1 IA from soybean. We evaluated the in vitro mycelial growth of three Brazilian populations of *R. solani* AG-1 IA from soybean under two temperature regimes, optimal (25°C) and above optimal (33.5°C), and under three concentrations of cupper oxychloride: no fungicide, 0.42 and 0.84 $g.L^{-1}$. We determined the components of evolvability: genetic (I_G) and environmental (I_E) variances and the broad-sense heritability (h^2) for mycelial growth under these conditions. We also compared the phenotypic differentiation for quantitative traits (Q_{ST}) and neutral genetic differentiation (based on microsatellite data) between three pairs of populations (F_{ST}). In general, under temperature stress (33.5°C), there was an increase in the genetic variance with a positive effect on the heritability for tolerance to copper oxychloride. Genetic differences were the main determinants of tolerance to cupper oxychloride under temperature stress ($h^2 \geq 0.70$). Most of the Q_{ST} estimates did not differ significantly from F_{ST}, indicating that neutrality played an important role in regional adaptation of the pathogen populations.

Combining sclerotia germination stimulants and fungicides for control of white rot of onions and garlic

A. FERRY (1), R. M. Davis (2)
(1) University of California-Davis, Woodland, CA, U.S.A.; (2) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.38

White rot, caused by the soil fungal pathogen *Sclerotium cepivorum*, is a devastating disease of *Allium* crops worldwide. The fungus spreads and overwinters as sclerotia, which germinate in response to *Allium* root exudates. Current available controls are sclerotia germination stimulants and fungicides.

Sclerotia germination stimulants, such as diallyl disulfide (DADS) are chemical mimics of *Allium* root exudates. While DADS is very effective in reducing initial inoculum, very few sclerotia can cause significant disease. Therefore, this method is most effective when combined with other treatments. In this study, we examined the effect on disease levels when DADS and fungicides are combined. The fungicides tested were tebuconazole, fludioxonil, fluopyram, penthiopiyrad and picoxystrobin. Studies were conducted in a field with naturally high disease pressure. Sclerotia levels were measured over the course of the season and disease incidence was measured at harvest. The use of DADS significantly reduced initial inoculum and also significantly reduced disease by 35%. None of the fungicides significantly decreased disease when applied alone. However, when combined with DADS, all of the fungicides in the study significantly reduced disease when compared to a DADS application alone. This study confirms the added disease control obtained from combining a fungicide application with a sclerotia germination stimulant.

Use of film-forming polymers for management of olive knot disease

E. J. FICHTNER (1), G. Kasun (2), C. DeBuse (3), W. H. Krueger (4), B. Kirkpatrick (2)
(1) University of California Cooperative Extension, Tulare, CA, U.S.A.; (2) Departement of Plant Pathology, University of California-Davis, Davis, CA, U.S.A.; (3) University of California Cooperative Extension, Woodland, CA, U.S.A.; (4) University of California Cooperative Extension, Orland, CA, U.S.A.
Phytopathology 102:S4.39

A shift toward high density plantings and mechanized harvest in California has enhanced severity of olive knot caused by *Pseudomonas savastanoi*. Current disease management practices rely almost exclusively on use of copper (Cu) bactericides, and preliminary studies demonstrate decreased Cu sensitivity in pathogen populations. Objectives of this study include an assessment of the potential for film-forming polymers to prevent infection and/or enhance foliar Cu persistence. In vitro studies demonstrated that a polymer film of Anti-Stress 550 (AS), placed on agar medium, inhibited pathogen growth. In two field trials, three polymer treatments (AS, Vapor Gard, Nu-Film P) were applied in the presence or absence of Kocide 3000. In one trial, polymer treatments were applied to potted 'Arbequina' and 'Manzanillo' plants three times throughout the winter and plants were subjected to natural inoculum. In the second trial, exposed leaf scars on mature 'Manzanillo' were coated with polymer treatments and inoculated with either 104 or 108 cfu/ml. Polymers alone had no influence on disease incidence (DI) in both trials, but AS may increase Cu persistence. In potted plants, both cultivars exhibited similar levels of DI and Kocide reduced DI. On inoculated branches, Kocide only reduced DI at the higher inoculum level. Further research is needed to address the potential for polymer coatings to bar infection, and assess efficacy of Cu use at low inoculum levels.

Investigating the effects of irrigation regimes on the susceptibility of tomato fruit to sour rot

K. FIEDLER (1), S. Rideout (1)
(1) Virginia Tech, ES AREC, Painter, VA, U.S.A.
Phytopathology 102:S4.39

Geotrichum candidum is the causal agent of sour rot in tomato (*Solanum lycopersicum*) and other fresh produce. This disease is a limiting factor to tomato production on the Eastern Shore of Virginia (ESV) and other major tomato producing regions; causing major losses in the field and especially during post-harvest handling. *Geotrichum candidum* infections are predominant during wet harvest conditions, abrupt drops in temperature due to rainfall, and improper post-harvest handling procedures. In the ESV, irrigation levels vary widely among tomato growers and often leads to over-watering of plants, which can result in water congested and soft fruit. This research investigates varying irrigation regimes and the influence on tomato fruit susceptibility. Tomato plants were grown in a greenhouse with low (0.75 liter/day), medium (1.5 liters/day), and high (2.25 liters/day) levels of irrigation applied once fruit set initiated, and fruit were harvested when pink in color. Following harvest, fruit were inoculated with *G. candidum* spore suspension via vacuum, then incubated at 20°C for 7 days. The presented results show the rate of infection and severity after 5, 7, and 10 days of incubation.

Phylogenetic analysis of a group of species of the genus *Fusarium* using DNA microsequences

J. J. FILGUEIRA-DUARTE (1), M. Rincon (1)
(1) Universidad Militar Nueva Granada, Bogotá, Colombia
Phytopathology 102:S4.39

The result of phylogenetic analysis that uses molecular methods for the genus *Fusarium* is controversial by differences between obtained results when we analyzed amplicons from different genes; studying the DNA sequences, we obtain trees with splits that set different distribution for studied species. The present paper, explores the possibility of used micro-sequences of preserved genes for solving problems in the *Fusarium* phylogeny. Therefore, six *Fusarium* species isolated from the Bogota's savanna (Colombia), responsible for the vascular wilt (*F. oxysporum*) and carnation basal rot (*F. avenaceum*, *F. culmorum*, *F. foetens*, *F. graminearum*, *F. verticillioides*) were evaluated using different genes: ITSs, α-Actin, β-Tubulin, COX, EF and Fumonisin. We found micro-sequences in the amplicons with highly preserved regions which are not useful for making phylogeny, this region have a CI (change index) between 0 and 0.29. Others regions (micro-sequences), that had a low number of changes in the nucleotide sequence with a CI between 0.3 to 0.79, when are analyzed using Neighbour Joining algorithm present a major difference between its, that other unpreserved regions with high percentages of changes (transitions or transversions) and CI between 0.8 and 1.0, that ended up having high phylogenetic similarity levels, showed between micro sequences of a single gene and between micro-sequences of different genes. These results are only a first approach for developing a model capable of identifying the micro-sequences on different genes that we can used to do bioinformatics and that produce the same phylogenetics relationship pattern in different analyzed regions.

Cellular interactions and transcript profiling of '*Candidatus* Liberibacter asiaticus and solanacearum' during psyllid infection and vector-mediated transmission

T. W. FISHER (1), J. M. Cicero (1), J. K. Brown (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.39

Huanglongbing and zebra chip are important diseases of citrus and solanaceous crops, respectively. The putative causal agents are distinct '*Candidatus* Liberibacter' species, which are transmitted by psyllids in a circulative manner. Identification and functional characterization of the gene products specifically involved in the cellular and molecular aspects of infection and the 'transmission pathway' are crucial to the development of successful management strategies, which are currently lacking for both disease agents. The alimentary canal and salivary glands of the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama and of the potato psyllid (PP) *Bactericera cockerelli* Sulc were examined using electron microscopy and *in situ* hybridization to identify the specific organs and/or other locations in the body that are occupied by '*Ca.* Liberibacter' spp. during infection and acquisition-transmission processes. Results indicate that '*Ca*. Liberibacter' lyse through the midgut and establish thick biofilms on its outer surface, and then sprawl anteriorly to accumulate in the salivary glands and oral region. *In silico* transcript profiling of infected and uninfected ACP and PP identified a number of mis-expressed, unique transcripts (unitrans). Functional predictions (gene ontology associations) implicate certain of these unitrans in '*Ca*. Liberibacter' infection of the psyllid host and/or in psyllid-mediated '*Ca*. Liberibacter' transmission.

Outer membrane protein OmpA is required for disease symptom development and colonization of sugarcane by *Xanthomonas albilineans*

L. A. FLEITES (1), P. C. Rott (2), S. Zhang (1), D. Gabriel (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) CIRAD Biology and Genetics of Plant-Pathogen Interactions, Montpellier, France
Phytopathology 102:S4.39

Xanthomonas albilineans (Xa) is a systemic, xylem-invading pathogen that causes sugarcane leaf scald. Xa produces albicidin, the only known pathogenicity factor in Xa. To identify additional pathogenicity factors, 1,216 independent Tn*5* insertions in Xa strain XaFL07-1 were screened for reduced pathogenic symptoms and reduced capacity to multiply in stalks of cultivar CP80-1743. Five (8.2%) independent insertions with reduced symptoms and capacity to multiply in stalks were found in *XaompA1* (XALc_0557), predicted to encode an OmpA family outer membrane protein. One mutant, M768, was able to consistently colonize stalk tissue but at severely reduced levels. Additional studies showed that all 5 mutants 1) produced albicidin, 2) were less motile (except M768), 3) were unable to grow in the presence of SDS (except M768), and 4) were slower growing than the wild type *in vitro*. Complementation was confirmed by two constructs; one carrying only the OmpA domain of XALc_0557, which provided partial complementation and the other carrying the entire *XaompA1* gene, which provided full complementation. This work shows that *ompA* is required for disease symptom development and colonization of sugarcane by Xa.

Infection and colonization of bermudagrass by *Ophiosphaerella korrae*, a causal fungus of spring dead spot of bermudagrass

F. FLORES (1), N. Walker (1), T. Mitchell (2), S. Marek (1), J. Anderson (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) The Ohio State University, Department of Plant Pathology, Columbus, OH, U.S.A.
Phytopathology 102:S4.39

Bermudagrass (*Cynodon dactylon* and *C. dactylon* x *C. transvaalensis*) is a commonly used turfgrass in the Southern United States where it is severely affected by spring dead spot (SDS). SDS is caused by one of three fungi in the genus *Ophiosphaerella*, namely *O. herpotricha*, *O. korrae*, or *O. narmari*. Despite the importance of SDS, the biology and ecology of the causal agents and the etiology of the disease remain poorly understood. In this study, the infection and colonization of bermudagrass tissues by *O. korrae* were characterized using fluorescence microscopy. *Agrobacterium* mediated transformation was used to transform *O. korrae* to express the red fluorescent protein tdTomato (tdTom). Roots and stolons of the interspecific hybrid cultivars Midlawn and Tifway, and *C. transvaalensis* accessions Uganda and 3200 were inoculated with tdTom expressing *O. korrae* and observed from 2 to 22 days post infection (DPI). *O. korrae* colonized roots and stolons of all cultivars at a similar rate with necrosis evident as early as 2 DPI on Tifway and Midlawn, while on 3200 and Uganda necrosis appeared at 8 DPI. Root epidermal and cortical cells were colonized rapidly in all bermudagrasses. However, vascular colonization did not occur until 4 DPI in Uganda, 8 DPI in 3200 and Midlawn, and 14 DPI in Tifway. For stolons, necrotic lesions were evident on Midlawn and Tifway at 4 DPI while 3200 and Uganda stolons showed discoloration but not necrosis up to 22 DPI. For all cultivars, the fungus did not penetrate beyond the epidermis of the stolons. These differences in infection and colonization of bermudagrasses suggest the underlying host genetics can be exploited for effective management of SDS.

Effect of inoculum concentration on the development of anthracnose fruit rot on flowers and fruit of different strawberry cultivars

B. B. FORCELINI (1), F. P. Gonçalves (2), N. A. Peres (1)
(1) University of Florida, Wimauma, FL, U.S.A.; (2) University of São Paulo, Piracicaba, Brazil
Phytopathology 102:S4.40

Anthracnose Fruit Rot (AFR), caused by *Colletotrichum acutatum*, is a major disease of strawberry in Florida and the southeastern United States. AFR can cause up to 80% yield loss. The use of pathogen-free plants, fungicides and resistant cultivars are important tools to control disease and reduce dissemination. This study compared the resistance of three strawberry cultivars (Strawberry Festival, Camarosa and Treasure) using five inoculum concentrations (0, 103, 104, 105 and 106 conidia/ml) on flowers, greenfruit < 2cm, and green fruit > 2cm. The experiment was arranged in a completely randomized design with five treatments (inoculum concentrations) per cultivar and 10 plants/treatment. Plants were kept in humid chambers for 16 h after spray inoculation.The incidence of AFR was assessed on tagged fruit for 16 days starting 5 days after inoculation. Cultivar susceptibility, inoculum concentration and plant organ were compared using the area under disease incidence progress curve (AUDIPC) by Tukey's test. There was a significant interaction between inoculum concentration, cultivar, and plant organ. Disease incidence was lower in Strawberry Festival than on Treasure and Camarosa on both fruit stages and flowers independently of the inoculum concentration. For all cultivars and inoculum concentrations, green fruit > 2cm was more susceptible than flowers and fruit < 2cm. There was no significant difference in susceptibility between flowers and fruit < 2cm. Strawberry Festival should be used in areas where weather conditions are extremely favorable for AFR and *C. acutatum* is present.

Physiological and biochemical aspects of the resistance of banana plants to Fusarium wilt potentiated by silicon

A. Fortunato (1), F. RODRIGUES (1), K. Nascimento (1)
(1) Universidade Federal de Viçosa, Viçosa, Brazil
Phytopathology 102:S4.40

This study evaluated the physiological and biochemical mechanisms feasibly involved with an increase in resistance of banana plants against Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), by silicon (Si). Plants from the Grand Nain (resistant to *Foc*) and Maçã (susceptible to *Foc*) cultivars were grown in plastic pots amended with 0 (-Si) or 0.39 g Si (AgroSilício) (+Si) per kg of soil and inoculated with the race 1 of *Foc*. The concentrations of lipid peroxidation (expressed as equivalents of malondialdehyde), hydrogen peroxide (H_2O_2), total soluble phenolics (TSP) and lignin-thioglycolic acid (LTGA) derivatives, and the activities of the enzymes phenylalanine ammonia-lyases (PAL), peroxidases (POX), polyphenoloxidases (PPO), β-1,3-glucanases (GLU), and chitinases (CHI) were determined on root samples at different times after inoculation with *Foc*. Pigments were determined on leaves. The concentration of malondialdehyde significantly decreased for plants from cultivars Grand Nain and Maçã supplied with Si compared to the -Si treatment while the concentrations of H_2O_2 on roots and pigments on leaves significantly increased. The concentrations of TSP and LTGA derivatives as well as the PAL, PPO, POX, GLU, and CHI activities significantly increased on roots of plants from cultivars Grand Nain and Maçã from the +Si treatment compared to the -Si treatment. Results of this study suggest that Fusarium wilt intensity on roots of banana plants supplied with Si decreased due to an increase in the concentrations of H_2O_2, TSP, and LTGA derivatives and greater activities of PAL, PPO, POX, GLU, and CHI in a scenario where the damage to root tissue during the course of infection by *Foc* was minimal. Financial support: CAPES and Harsco Minerais.

Transcriptome analysis reveal differences in induced systemic defence responses to biotrophic and necrotrophic pathogens and to wounding in two aspen clones

C. FOSSDAL (1), N. Yaqoob (1), B. Albrectsen (2), H. Solheim (1)
(1) Norwegian Forest and Landscape Institute, Aas, Norway; (2) UPSC, Umea, Sweden
Phytopathology 102:S4.40

Aspen trees are exposed to a range of attackers and employ varied strategies to reduce their impact. The diversity of responses may have importance for resistance properties at the stand level, and justifies the search for varied defensive strategies in natural populations. We used transcriptomic tools to evaluate diverse responses at the gene activity level in *Populus tremula* in response to wounding, and to inoculation with two pathogenic fungi (*Melampsora magnusiana* vs *Ceratocystis* sp.) that differ in life style (biotroph vs necrotroph) and host tissue requirement (live leaf vs dead wood tissues). Two aspen genotypes from the SwAsp collection with differences in growth and phenolic composition were used to study differences in resistance properties. High defence gene induction, high growth and elevated defence properties toward the biotroph appeared to support each other in this study exemplified in the more resistant SwAsp clone, whereas the more susceptible SwAsp clone was much less responsive to infections, and displayed more symptoms when infected with *M. magnusiana*. Interestingly, in the more resistant clone wounding gave greater systemic activity of selected candidate genes than when combined with the necrotroph, suggesting that this pathogen has some ability to suppress the induction or translocation of the systemic defence signal in this particular clone.

WITHDRAWN

Identification of maize WRKY transcription factors responding to *Aspergillus flavus* infection and their roles in resistance to aflatoxin contamination

J. FOUNTAIN (1), Y. Raruang (1), M. Luo (1), R. L. Brown (2), Z. Chen (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.; (2) USDA-ARS, Southern Regional Research Center, New Orleans, LA, U.S.A.
Phytopathology 102:S4.40

The colonization of maize (*Zea mays* L.) by the opportunistic pathogen *Aspergillus flavus* and subsequent contamination of kernels with its secondary metabolites, aflatoxins, are responsible for significant crop losses and negative impacts to human and animal health. This study focused on the role of maize

WRKY transcription factors (WRKY TFs), which form a regulatory network for defense against biotic and abiotic stresses. Previously, seven WRKY TFs significantly regulated by *A. flavus* infection of maize kernels were identified using oligonucleotide microarray, including *Zm*WRKY21, *Zm*WRKY53 (maize and rice homologs), *Zm*WRKY67, *Zm*WRKY68, and two putative WRKY TFs. Expression of these WRKY TFs was evaluated from 1 to 18 days after inoculation in maize lines B73 (susceptible) and TZAR-101 (resistant) using quantitative real-time PCR (qPCR). Of particular interest were the homologs of *Zm*WRKY53. Given the differential expression of *Zm*WRKY53 homologs observed in this study, the expression of different hormone pathway associated genes were also examined. In addition, H_2O_2 and lipid peroxidation levels in kernels were also examined for possible correlations with aflatoxin biosynthesis in *A. flavus*. Analyses of these pathway components may shed light on the resistance mechanisms of maize germplasm in response to *A. flavus* infection and aflatoxin contamination.

Investigations into the molecular mechanisms responsible for the decline in sensitivity to DMI fungicides in *Mycosphaerella graminicola* populations

B. A. FRAAIJE (1), H. J. Cools (1)
(1) Rothamsted Research, Harpenden, United Kingdom
Phytopathology 102:S4.41

We describe the complex recent evolution of the azole target sterol 14α-demethylase (MgCYP51) enzyme in response to selection by the sequential introduction of progressively more effective azoles, and discuss the contribution of individual and combinations of MgCYP51 amino acid alterations to azole resistance phenotypes and intrinsic enzyme activity. In addition, the recent identification of mechanisms independent of changes in MgCYP51 structure correlated with novel azole cross-resistant phenotypes suggests the further evolution of *M. graminicola* under continued selection by azole fungicides could involve multiple mechanisms. The prospects for azole fungicides in controlling European *M. graminicola* populations in the future are discussed in the context of these new findings.

Characterization of *Phoma* and *Phytophthora* isolates from chicory root

R. A. FRANCE (1), P. A. Millas (1)
(1) Instituto de Investigaciones Agropecuarias, Chillán, Chile
Phytopathology 102:S4.41

Phytophthora cryptogea and *Phoma exigua* var. *exigua* has been identified as pathogens causing chicory root rot at field and post-harvest production. Chicory plants show different virulence at the field, indicating a possible variability of the pathogen populations. The objective was to determine the presence of variability and virulence in a collection of *P. cryptogea* and *P. exigua*, previously isolated from chicory plants showing root rot symptoms. Six *P. cryptogea* and 25 *P. exigua* isolates were transferred to PDA medium and incubated in growth chambers at 20, 25 and 30°C, colonies growth were measured every other day up to 7 days. Significative differences in response to the 30°C were observed only in *P. exigua*. Six isolated over 25 were able to growth at such temperature. Then, the same isolates were inoculated on chicory root discs with agar plug from an actively growing culture, then incubated in humid chamber at 25°C for 7 days. Diameter of rot was measured and a rot area calculated. Difference of 93% and 91% in root rot areas were detected among *P. exigua* and *P. cryptogea* isolates, respectively. Therefore, variability is present in both pathogens population affecting chicory root.

Genomic island-based plasticity among the genomes of rice-pathogenic *Burkholderia glumae* and *B. gladioli* strains

F. FRANCIS (1), J. Kim (2), J. Ham (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.; (2) Louisiana State University, Center for Computation & Technology, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.41

Phytopathogenic *Burkholderia* species are the etiological agents of diseases for diverse flora and cause symptoms such as blight, wilt and rot. *Burkholderia glumae* and *B. gladioli* are important causal agents of bacterial panicle blight of rice, which is becoming an increasingly significant problem in global rice production. To better understand its genome-scale characteristics, the genome of the highly virulent *B. glumae* strain 336gr-1, isolated from Louisiana, was sequenced using Illumina Genome Analyser II. The 336gr-1 genome was aligned and compared with the previously sequenced genomes of *B. glumae* strain BGR1 and *B. gladioli* strain BSR3, which were isolated from infected rice plants in South Korea. Comparative analyses among the three strains revealed unique regions present in their genomes. Many of these unique regions correspond to genomic islands that contain mobile elements such as phage-related genes. A significant amount of plasticity was observed in these closely related strains of *Burkholderia* that are capable of infecting the same host plant. This finding may explain the versatility of *Burkholderia* members under various environmental conditions.

Characterization of the *Magnaporthe oryzae* proteome during appressorium formation

W. FRANCK (1), E. Gokce (1), Y. Oh (1), D. C. Muddiman (1), R. A. Dean (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.41

The rice blast pathogen, *Magnaporthe oryzae*, remains one of the most important pathogens of rice worldwide. A complex developmental process occurs on the leaf surface following attachment of the conidium resulting in germination and appressorium formation followed by penetration of the host leaf. Appressorium formation can be induced on artificial hydrophobic surfaces or by addition of cAMP. In this study the proteomes of germinating conidia and cAMP elicited appressoria were investigated to better understand the physiological changes that accompany appressorium formation. Protein samples were collected during conidial germination at 0, 4, 8, 12, and 18hr in the presence or absence of 50mM cAMP and analyzed by nano-LC MS/MS. A total 3,170 proteins were identified from nine biological conditions including 2,225 proteins that were common to all samples. Proteins were quantified by spectral counting and proteins showing a 2-fold change in abundance at a p-value of 0.05 between two conditions were defined as differentially expressed. During the process of conidial germination in the absence of cAMP a total of 591 proteins showed differential expression when compared to conidia. Treatment with cAMP resulted in the identification of 493 differentially expressed proteins, including 166 up-regulated proteins, when compared to untreated samples. An examination of the proteins regulated during conidial germination and appressorium formation will be presented.

Detection of phytoplasmas in Cicadellidae morphotypes of Bogotá, Colombia

L. Franco-Lara (1), L. PERILLA (1)
(1) Universidad Militar Nueva Granada, Bogotá, Colombia
Phytopathology 102:S4.41

Phytoplasmas of groups 16SrI and 16SrVII are known to exist in ornamental urban trees in Bogotá, such as *Fraxinus uhdei*, *Liquidambar styraciflua*, *Pittosporum undulatum*, *Populus nigra* and *Croton* spp. Other species as *Magnolia grandiflora*, *Eugenia* sp. and *Acacia melanoxyon* display symptoms and are being tested for phytoplasmas. The insect vectors of these phytoplasmas are unknown in the region. In 2011, leafhoppers were collected and grouped in 12 groups according to morphological characteristics; these groups varied in abundance and frequency of collection. Pooled samples from 1 to 5 insects were tested by nested PCR and 16SrDNA was amplified with primers P1A/P7A - R16F2n/R16R2 -fU5/rU3. So far, 3 morphotypes were positive and 9 were negative for the presence of phytoplasmas. Positive amplicons were cloned, sequenced and contigs were aligned; dendograms were built using previously reported sequences. Sequences are related with Group 16SrI phytoplasmas strains. All three leafhopper morphotypes carrying phytoplasmas belong to subfamily Deltocephalinae; further taxonomic identification is in process, but there is little information about these taxa in Colombia. Group 16SrVII phytoplasmas have been elusive but more extensive tests are being applied. Previous evidence indicates that insect vectors are polyphagous and very efficient vectors. Currently transmission trials are being carried to evaluate the vector ability of these morphotypes.

Survival of *Colletotrichum acutatum*, the causal agent of citrus postbloom fruit drop, on weeds

G. F. FRARE (1), L. Amorim (1)
(1) Escola Superior de Agricultura Luiz de Queiroz/Universidade de São Paulo, Piracicaba, Brazil
Phytopathology 102:S4.41

Citrus postbloom fruit drop (PFD), caused by *Colletotrichum acutatum* is an important disease that cause significant yield reduction. *C. acutatum* can survive on the surface of citrus leaves, in the form of quiescent appressoria. The weeds can act as alternative hosts of diverse pathogens, serving as inoculum sources and playing an important role in the disease epidemiology. The objective of this work was to verify if weeds frequently found in citrus orchards in São Paulo State may serve as inoculum source of *C. acutatum* for this crop. Seven weed species were inoculated with a conidial suspension of *C. acutatum*. Thirty six hour after the inoculation, samples of all weeds were collected and observed in optical microscope to verify the conidia germination. Thirty, sixty and ninety days after inoculation, *C. acutatum* was isolated from the inoculated leaves and inoculated on citrus flower. Conidia germination and appressoria formation of *C. acutatum* were observed in all inoculated leaves. There was no statistical difference in the *C. acutatum* survival rate during 30, 60 and 90 days, for each weed species. No symptoms were

observed in the inoculated leaves. Every isolated obtained from the weeds presented typical PFD symptoms when inoculated on citrus flowers. The results showed that weeds can serve as alternative hosts of *C. acutatum*, and may contribute as primary inoculum source of this pathogen to the citrus crop.

Practical and qualitative resistance to trifloxystrobin in populations of *Venturia inaequalis* in the northeastern United States

Z. FREDERICK (1), K. D. Cox (2)
(1) Cornell University, Trumansburg, NY, U.S.A.; (2) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.42

The development of widespread resistance to demethylation inhibitor fungicides led to increased use of quinone outside inhibitor (QoI) fungicides to manage apple scab in the northeastern United States. We monitored the development of qualitative resistance to the QoI fungicide trifloxystrobin in regional populations of *Venturia inaequalis* from 2007 to 2012. 139 orchard populations (n > 25) were tested for quantitative and qualitative resistance to trifloxystrobin using microscopy-aided relative growth/germination assays. Out of 139 orchards, 35 were found to have members with qualitative resistance to trifloxystrobin, which comprised 2.5 to 95.8% of the population. In 2011, trials were conducted in an apple orchard in Geneva NY to develop a ground truth standard for determining practical resistance to trifloxystrobin. In this population, 49.5% of the members had qualitative resistance. When trifloxystrobin was applied under standard conditions, the incidence of apple scab on mature 'Empire' fruit was higher (49.5 ± 5.7%) than that of a succinate dehydrogenase inhibitor (SDHI) standard program (20.0 ± 4.6%). On 'Jonagold', a less susceptible cultivar, the incidence of apple scab was slightly higher for the trifloxystrobin program (18.5 ± 11.1%) compared to that of the SDHI standard program (5.0 ± 1.3%). This information provides the basis for ascertaining the development of practical resistance in orchard populations of *V. inaequalis*.

***Triticum mosaic virus*: Genetic evidence for recent population expansion and balancing selection**

R. FRENCH (1), D. Seifers (2), S. N. Wegulo (3), S. Tatineni (1)
(1) USDA-ARS, Lincoln, NE, U.S.A.; (2) Kansas State University, Hays, KS, U.S.A.; (3) University of Nebraska-Lincoln, Lincoln, NE, U.S.A.
Phytopathology 102:S4.42

Triticum mosaic virus (TriMV), a mite-transmitted pathogen of wheat, was first discovered in Kansas in 2006, and is a novel species in the family *Potyviridae*. The P1 and coat protein (CP) coding regions of 14 isolates from Colorado and 18 isolates from Nebraska were amplified by RT-PCR and sequenced. Average pairwise sequence diversity was 0.4% for P1 and 0.2% for CP. The ratio of non-synonymous to synonymous substitutions was 0.19 for P1 and 0.12 for CP coding regions indicating that both cistrons are subject to strong purifying selection. Comparison of sequence diversity within and between the CO and NE samples revealed little population subdivision between geographical regions (Fst = 4.0%). Mismatch analyses of both coding regions provided evidence for population expansion. Temporal analysis using the BEAST software package suggests that this population expansion began circa 1997. Almost all polymorphic sites occurred at low frequencies, however, one site at amino acid residue 17 of the CP cistron occurred at an intermediate frequency. Half of the isolates had a threonine at this position while the other half had an alanine. Polymorphisms that are maintained at intermediate frequencies are generally believed to be a genetic signature of balancing selection. Potential selective forces acting on TriMV CP may be maintenance of virus in different alternative hosts or virus transmission by different mite biotypes.

Race diversity of *Puccinia helianthi* (sunflower rust) in the Northern Great Plains in 2011

A. FRISKOP (1), T. Gulya (2), M. Acevedo (1), R. Harveson (3), R. Humann (1), S. Markell (1)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS, Sunflower Research Unit, Fargo, ND, U.S.A.; (3) University of Nebraska, Scottsbluff, NE, U.S.A.
Phytopathology 102:S4.42

Sunflower rust, caused by the macrocyclic heteroecious pathogen *Puccinia helianthi*, is an economically important disease in the Northern Great Plains, particularly in North Dakota, where approximately 40 – 50% of the U.S. crop is produced. In 2008, the first documented sexual recombination event in the Northern Great Plains occurred, which coincided with localized epidemics and subsequent yield loss. Race determination is essential for breeding for resistance, and no race assessment of single pustule isolates in North Dakota has been done. The objective of this study is to determine the races of single pustule isolates collected in the Northern Great Plains. In 2011, a minimum of two single-pustule isolates were collected from 37 discrete locations in ND. In addition, a limited number of single-pustule derived isolates were created from bulk samples collected in other Northern Great Plains states. To determine races, single-pustule isolates were increased on a susceptible hybrid, fresh urediniospores were inoculated onto the standard set of nine sunflower differentials and infection types were evaluated 13-15 days later. Nine races were identified from 100 isolates with races 300 and 304 comprising ~80% of tested isolates. The most virulent race detected was 776, which is virulent on the resistance genes in eight of the nine differentials. The differential lines CM29 and HAR3 conferred resistance to ~98% of the races identified. Races identified in other Plains states were similar to those in North Dakota. Results from this survey will be used to aid resistance breeding efforts in the future.

Optimization and application of a chemiluminescent dot-blot immunoassay for detection of potato viruses

A. C. FULLADOLSA (1), R. Kota (1), A. O. Charkowski (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 102:S4.42

Potato is a host for many viruses and vegetative propagation of the crop leads to their accumulation, resulting in significant yield losses and reduced quality. The most widely used method of diagnosis of viral infections is the post-harvest test, for which the enzyme-linked immunosorbent assay (ELISA) is used. This method is efficient, and relatively inexpensive, but the large number of samples processed means that even small improvements can result in significant savings. Others have modified the ELISA by substituting microtiter plates for polyvinylidene fluoride (PVDF) membranes to develop a more flexible and inexpensive assay. We optimized a dot-blot immunoassay with viral proteins bound to a PVDF membrane and detection of the proteins with alkaline phosphatase labeled antibodies and a chemiluminescence reagent. The assay was tested for detection of viruses of seven genera. The cost of this assay is 85% less than that of a standard ELISA. We have also altered the assay by spotting an antibody array onto a PVDF membrane and tested it for its potential uses as a diagnostic tool for plant viruses. We used the dot-blot immunoassay for detection of *Potato virus Y* (PVY) in 1,530 samples from a post-harvest test to determine virus incidence in tubers after a field trial. Of the 110 PVY-positive samples, 101 were detected by using the assay, while the remaining 9 were not detectable due to high background signal or blotching, and were identified by using ELISA.

Interactions between winter chilling, asynchronous crop phenology, ontogenic resistance, and the risk of disease in grapevine and other perennial fruit crops

D. M. Gadoury (1), R. C. Seem (1), W. F. Wilcox (1), A. Stensvand (2), A. Ficke (2), M. M. MOYER (3)
(1) Cornell University, Geneva, NY, U.S.A.; (2) Bioforsk, Aas, Norway; (3) Washington State University, Prosser, WA, U.S.A.
Phytopathology 102:S4.42

Minimum chilling requirements of perennial fruit crops have been extensively studied, but little is known of how the degree and depth of winter chilling affects synchronization of host regrowth upon emergence from dormancy. The European grapevine species *Vitis vinifera* is a useful model system for studying the interactions between chilling, asynchronous phenology, development of ontogenic resistance, and the consequent risk of disease. The mean winter temperature ranged from -4.1 to 11.8C among 15 vineyard sites on 3 continents, and was associated with duration of bloom at each site: 2 d at the coldest sites, and > 2 wks at the warmest sites. This 7-fold increase in the duration of bloom directly translated to protracted susceptibility of grape berries due to their delayed acquisition of ontogenic resistance to major fungal pathogens, including *Erysiphe necator* and *Plasmopara viticola*. Downstream effects of asynchronous bloom such as asynchronous ripening and sugar accumulation also were recorded. Asynchronous regrowth following unusually warm winters has been noted in grapevine, apple, and stone fruits. This asynchrony could prolong the risk of disease in many pathosystems typified by phenology-defined windows of susceptibility to infection. The impact of climate change on the foregoing can be projected by examining these interactions across extant climatic gradients.

RNA-seq comparison of tuber and foliage transcriptome dynamics in response to late blight pathogen attack

L. GAO (1), Z. Tu (2), F. Katagiri (3), J. M. Bradeen (1)
(1) Department of Plant Pathology, University of Minnesota, St. Paul, MN, U.S.A.; (2) Minnesota Supercomputing Institute, University of Minnesota, St. Paul, MN, U.S.A.; (3) Department of Plant Biology & Microbial and Plant Genomics Institute, University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 102:S4.42

Cultivated potato is the world's number one non-grain food commodity. The late blight pathogen *Phytophthora infestans* has the capacity to attack both potato foliage and tubers. Importantly, foliar resistance against late blight does not guarantee tuber resistance, contrasting phenotypes can happen even within the same genotype. Most potato transcriptome studies targeted foliage and few studies target tuber-microbe interactions. We conducted a time-course RNA-seq study consisting of two genotypes (wild type susceptible and transgenic resistant lines), two treatments (with and without *P. infestans*), three time points, and three bio-reps for both tuber and foliage samples (72 samples in total, 36 for tubers, 36 for foliage). Around one billion paired-end Illumina Hi-Seq reads were generated; the majority of them were mapped uniquely to one location in the reference genome. We analyzed the transcription levels of over 30,000 potato genes using various software packages. We discovered marked transcriptome differences among genotypes and organs (tuber and foliage). Various regulatory and metabolic pathways were identified to distinguish transgene and organ specific defense responses.

Identification and characterization of *Pythium* species present in floricultural crops from Long Island, New York

P. A. Garrido (1), C. A. Salazar (1), C. I. Diaz (1), S. Posey (1), G. K. Orquera (1), H. A. Castillo (1), M. Daughtrey (2), C. D. GARZON (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) Cornell University, Riverhead, NY, U.S.A.
Phytopathology 102:S4.43

Several species of the genus *Pythium* cause damping-off of seedlings, seed disease, stem lesions, and root rots in greenhouse floral crops, where great losses occur every season. The aim of this study was to determine the predominant species in floricultural greenhouses from New York, and to analyze their population structure. Species identification was conducted using DNA sequences of the ITS region (including the 5.8S rDNA), and population structure of the dominant species was analyzed using Simple Sequence Repeats (SSRs). DNA sequence analyses of 264 *Pythium* isolates identified 16 species, of which the predominant species was *P. irregulare* sensu lato, including isolates of *P. irregulare*, *P. cryptoirregulare*, and *P. cylindrosporum*. A representative sample of *P. irregulare* s.l. was used to evaluate the genetic diversity and genetic structure of the samples. Principal component analysis (PCO) and UPGMA of SSR data defined four groups (A: *P. cryptoirregulare/P. cylindrosporum*; B: *P. irregulare* sensu stricto; C: *P. irregulare*; D: *P. irregulare/P. cryptoirregulare*), and AMOVA identified significant genetic differentiation between the groups (FPT= 0.47; p = 0.001). Group A, which includes isolates identified as either *P. cryptoirregulare* or *P. cylindrosporum* (Max Ident <96%), appears to be the most genetically distinct. Our results show that floricultural crops host a great diversity of *Pythium* species, of which those in the *P. irregulare* complex are the most relevant.

Population structure and genetic diversity of three species of *Pythium* isolated from forest tree nursery soils in Oregon and Washington

P. A. Garrido (1), C. D. GARZON (1), N. J. Grünwald (2), J. Weiland (2)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 102:S4.43

The genus *Pythium* includes some of the most important soilborne pathogens that cause damping-off and root rot of conifers, resulting in high seedling mortality in forest tree nurseries. The aim of this study was to analyze the diversity and population structure of three species of *Pythium* (*P. irregulare*, *P. sylvaticum*, and *P. ultimum*) isolated from soil at three forest nurseries in Oregon and Washington. Three molecular marker methods were used: Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeats (SSR), and Amplified Fragment Length Polymorphisms (AFLP). AMOVA, based on geographic distribution, identified significant genetic differences among nursery populations of *P. sylvaticum* and *P. irregulare*, whereas no significant differences among *P. ultimum* populations were found. Isolate distribution by Principal Coordinate analysis and UPGMA were congruent among methods, but provided no clear evidence of geographically-defined populations in either species. Instead, significant intraspecific differentiation, unrelated to nursery of origin, was detected in *P. irregulare* (2 groups), *P. sylvaticum* (3 groups), and *P. ultimum* (2 groups). The evolutionary processes determining the population structure observed in the samples of these species remain to be determined. Their discovery may provide useful information for disease prevention and management of *Pythium* diseases in forest nurseries.

Rapid and specific detection of *Pencillium* species causing blue mold decay on pome fruit in storage using molecular tools

V. L. GASKINS (1), K. A. Peter (1), I. Vico (1), W. J. Janisiewicz (2), W. M. Jurick (3)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) USDA-ARS, AFRS, Kearneysville, WV, U.S.A.; (3) USDA-ARS, Food Quality Laboratory, Beltsville, MD, U.S.A.
Phytopathology 102:S4.43

Blue mold is one of the most prevalent and economically important diseases of pome fruits worldwide. It is a continuous problem for growers and packers during long term storage and is caused mainly by *Penicillium expansum*. However, other *Penicillium* spp (*P. solitum*, *P. crustosum*, *P. commune*, *P. griseoroseum* etc.) have also been reported to cause blue mold decay, although at much lower frequencies. Therefore, a molecular-based diagnostic assay that is specific to blue mold fungi, like PCR, would be extremely helpful in making an accurate and timely identification. We have modified a set of primers based on the β-tubulin gene sequence from *P. expansum* that can detect multiple *Pencillium* species. Both spores and mycelium from fungal cultures can be used to extract genomic DNA and amplify the ~150 bp β-tubulin amplicon using both conventional and real time PCR. We have demonstrated the specificity of both primer sets at the genus and species levels using several *Pencillium* isolates and other common fungal pathogens causing postharvest decay of pome and stone fruit including: *Monolinia fructicola*, *Rhizopus stolonifer*, and *Botrytis cinerea*. Detection of *Penicillium* spp. from infected fruit samples, water in dump tanks, and walls and equipment from packinghouses will also be discussed.

Sensitivity of *Penicillium* spp. from decayed apple fruit to postharvest fungicides and identification of a new codon associated with thiabendazole resistance

V. L. GASKINS (1), I. Vico (1), K. A. Peter (1), W. J. Janisiewicz (2), W. M. Jurick (3)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) USDA-ARS, AFRS, Kearneysville, WV, U.S.A.; (3) USDA-ARS, Food Quality Laboratory, Beltsville, MD, U.S.A.
Phytopathology 102:S4.43

Blue mold decay is an important disease of stored apples worldwide. It is caused by *Penicillium* spp. among which *P. expansum* has developed resistance to benzimidazole fungicides. During 2010 and 2011, 238 isolates were collected from several packinghouses which store organic and conventionally grown apples either not treated or treated with fungicides for postharvest disease control. *Penicillium* spp. were isolated from decayed Pink Lady, Red Delicious, Golden Delicious, Fuji, and Honey Crisp apples. Isolates previously collected from decayed fruit (11) and others obtained from ATCC (8) were included for comparison. Fungicide sensitivity of *Penicillium* isolates to thiabendazole (Mertec), fludioxonil (Scholar) and pyrimethanil (Penbotec) was assessed and minimal inhibitory concentration (MIC) was determined. Thirty one out of the 238 isolates (22 resistant and 9 sensitive to thiabendazole) were selected and identified as *P. expansum* using the β-tubulin gene sequence. Twenty thiabendazole resistant and 1 thiabendazole sensitive isolate displayed a point mutation in the β-tubulin gene sequence at codon 198. A new point mutation in the β-tubulin gene sequence at codon 374 was discovered. Substitution of CTC to TTC was evident for all of the thiabendazole resistant isolates except one, and there was no change in sensitive isolates except for two.

Effects of micronutrients on *Fusarium oxysporum* f. sp. *spinaciae* and limestone-mediated suppression of spinach Fusarium wilt

E. W. GATCH (1), L. J. du Toit (1)
(1) Washington State University, Mount Vernon NWREC, Mount Vernon, WA, U.S.A.
Phytopathology 102:S4.43

The maritime Pacific Northwest is the only region of the US suitable for production of spinach seed, a cool-season, daylength-sensitive crop. However, the acidic soils of this region are highly conducive to spinach Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *spinaciae*. Rotations of 10 to 15 years are necessary to reduce the risk of losses to this disease. Raising soil pH with limestone partially suppresses spinach Fusarium wilt in these acid soils. Research on other Fusarium wilts suggests a reduction in availability of Fe, Mn, and/or Zn may partly explain this pH-mediated suppression. Experiments were completed to assess: 1) the effects of a range of concentrations (0 to 2 mg/liter) of Fe, Mn, and Zn on pathogen growth and sporulation *in vitro*; and 2) whether the disease-suppressive effect of soil limestone amendment is negated by adding chelated micronutrients. Increasing the concentration of Fe, Mn, or Zn in a defined liquid medium significantly increased fungal biomass and sporulation. The most significant effect on fungal biomass occurred with Zn, and on spore production with Mn. Adding Zn or Mn to naturally infested soil counteracted the suppressive effect of limestone on Fusarium wilt development. These results provide evidence of direct relationships between micronutrient availability and conduciveness of soils to spinach Fusarium

wilt, and could be utilized to optimize soil amendments for more effective management of this disease.

Deoxynivalenol concentration in primary spikes and tillers of barley and wheat

P. GAUTAM (1), S. Halley (2), J. M. Stein (3)
(1) South Dakota State University, Brookings, SD, U.S.A.; (2) Langdon Extension Research Center, North Dakota State University, Langdon, ND, U.S.A.; (3) Monsanto Company, Waterman, IL, U.S.A.
Phytopathology 102:S4.44

Little is known about the extent to which primary and secondary tillers contribute to mycotoxin contamination of grain in the Fusarium head blight (FHB) pathosystem. Experiments, split-split plot with five replications, were established at the SDSU Plant Pathology Research Farm in Brookings, SD in 2011 to examine this relationship. Main plots were two lines or cultivars each of spring malting barley, hard red spring wheat (HRSW), and hard red winter wheat (HRWW); which had similar agronomic characteristics but differed in their level of FHB resistance. Inoculation timings (Feekes 10.5 and 11.2) were on the sub-plots, and sub-sub plots included *Gibberella zeae* or mock inoculation. Twenty-five primary or secondary tillers were tagged in plots that were inoculated at Feekes 10.5 and Feekes 11.2, respectively. FHB severity was assessed on 25-tagged heads 18 days after inoculation. These were hand harvested at maturity. Heads from individual plots were bulked and analyzed for percent *Fusarium* damaged kernels (FDK) and analyzed for DON. The rest of the plots were mechanically harvested at maturity and analyzed similarly. Levels of DON were statistically higher in grain from the primary heads of barley and HRSW in each cultivar compared to its tillers. In contrast, there was numerically, but not statistically, higher DON level in tillers than primary heads in each cultivar for HRWW. Our results suggest that primary tillers probably contribute most to overall DON concentration in the grain, although secondary tillers may be important and should be included in management schemes for FHB.

***Salmonella* colonization of cantaloupe fruits, alone or together with *Erwinia tracheiphila*, following flower inoculation**

D. Gautam (1), M. Payton (1), J. Fletcher (1), L. M. MA (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.44

Salmonella spp. have been associated with cantaloupes and caused human illnesses, but whether the bacteria can contaminate the fruit or internalize into edible tissues through the flowers, or whether other microflora affect the process, is not known. We hypothesized that *Salmonella* Poona (*S*P) can invade fruit after introduction into the flower, and that the cucurbit pathogen *Erwinia tracheiphila* (*Et*) may influence *S*P survival. Peptone water, suspension (5µl; 10^7 cfu/ml) of an *S*P+*Et* mixture, or of each pathogen alone was dropped into pollinated flowers. Flowers were sampled at day 0 and blossom ends at 43 day post inoculation (DPI). Fruit interiors were examined at 15 and 43 DPI by direct plating and enrichment. At 15 DPI the interior tissue of one *S*P-treatment fruit was *S*P positive after enrichment but at 43 DPI all samples were negative. However, blossom end surfaces of all samples from *S*P and *S*P+*Et* treatments were *S*P positive, with 39% (*S*P alone) and 45% (*S*P+*Et*) increase in *S*P population from the initial inoculum level, even though the flowers had dehisced. *Et* was detected only at 0 DPI. Neither bacterium was detected from controls or non-inoculated fruits on the same plant. Our data suggest that, following flower inoculation, *S*P internalization of cantaloupe is a rare event; however, *S*P can survive on the blossom end surface until fruit maturity. Absence of either pathogen on or in non-inoculated fruit within the same plant, suggests lack of systemic movement.

Partial characterization of a new *Potyvirus* species infecting soybean plants in Brazil

P. d. Geraldino Duarte (1), S. B. Galvino Costa (1), A. R. FIGUEIRA (1)
(1) Universidade Federal de Lavras, Lavras, MG, Brazil
Phytopathology 102:S4.44

The *Soybean yellow shoot virus* (SYSV) was first detected in Lavras – MG-Brazil in 1984, infecting soybean plants in experimental field. The preliminary studies using electron microscopy, mechanical inoculation in host plants and serology showed that it was a *Potyvirus* member, able to induce more severe symptoms and different from any other virus already described for soybean plants. Since then, the molecular characteristics of this virus remained unknown. In this work the 3' end region of SYSV genome, including the poly A tail, 3'UTR, coat protein and part of the NIb and CI regions, was sequenced and analyzed. This region was amplified by RT-PCR, using the RNA extracted from partially purified virions and universal primers, designed to amplify the regions of CI, NIb and CP genes. The nucleotide and amino acid sequences of CI region revealed that SYSV is a distinct member of the *Potyvirus* genus, with an identity and similarity ranging from 51 to 63% and from 44 to 47%, respectively, when compared to other potyviruses from GenBank. The same was observed for the sequence of the 3' end region, which presented nucleotide identity between 55 and 59% and amino acids similarity between 29 and 32%. The highest amino acid similarity (61%) was seen with the partial sequence of NIb gene of *Glycine virus Y*, which only occurs in Australia. The results showed the uniqueness of SYSV, indicating that it is a *Potyvirus* species not yet described in Brazil and worldwide.

Isolation of *Cowpea mosaic virus* movement tubules and identification of host proteins involved in the viral movement from cell to cell

P. S. GERALDINO DUARTE (1), P. W. den Holander (2), A. R. Figueira (1), J. W. van Lent (2)
(1) Universidade Federal de Lavras, Lavras, MG, Brazil; (2) Wageningen University, Wageningen, Netherlands
Phytopathology 102:S4.44

The *Cowpea mosaic virus* (CPMV) moves from cell to cell using tubular structures formed by its movement protein. These tubules are tightly surrounded by the host plasma membrane and it is clear that plant factors are involved in the anchoring of the movement structure at the plasma membrane or possibly even as a structural component of the tubules. Due to this evident viral dependence of the host proteins for movement from cell to cell, in this work the tubules formed in protoplasts of *Vigna unguiculata* were used to identify possible host factors involved in the virus transport. Therefore, a protocol for tubules isolation was developed using protein A conjugated magnetic beads coated with antibody against the movement protein. The isolated proteins were analyzed using tandem mass spectrometry (MS/MS). A group of 55 proteins was identified as potential host conserved proteins that may interact with the movement protein of CPMV. Three proteins identified as belonging to the group of AAA-ATPases, chaperonin HSP 60, and chaperonin HSP 70 have already been identified as involved in different steps of the plant virus infection including the cell-cell movement. The identification of these proteins could help the understanding of the viral movement process, still not well known for most of the viral families.

Evaluating fungicide programs for potato early blight control and fungicide resistance management in Wisconsin

A. J. GEVENS (1), S. A. Jordan (1), K. M. Cleveland (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.44

Potato early blight (EB), caused by the fungus *Alternaria solani*, is a perennial and potentially destructive disease in WI. There are many registered fungicides effective in controlling EB. However, several have single-site modes of action with high risk for pathogen resistance. To prolong efficacy, fungicides must be used in programs which include broad spectrum protectants as mixes and in alternation. We evaluated 36 foliar fungicide programs for EB control on 'Russet Burbank' potatoes at the University of WI Agricultural Research Station located in Hancock WI. Among our 8-week-long, 7-day spray programs was a grower standard, an untreated control, a full-season application of chlorothalonil, and various iterations of the standard program with different single site fungicides. EB pressure was moderate with late onset and aerial blackleg caused by *Pectobacterium carotovorum* was prevalent throughout the trial. Plots were harvested on 14 September with no tuber EB observed. While all treatments had significantly less foliar disease than the untreated control, reduction was not correlated with high yields. Only 8 treatments yielded significantly better than the untreated control. Of these, 6 included a newly registered or soon-to-be registered fungicide, and 7 included a routine alternation with a chlorothalonil fungicide. Due to variability in the data as a result of blackleg, few other generalizations can be made of comparative program performance.

Formation of *Phytophthora infestans* oospores in planta of potato, tomato, and solanaceous weeds

A. J. Gevens (1), A. SANCHEZ PEREZ (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.44

Phytophthora infestans, causal agent of tomato and potato late blight, is a heterothallic oomycete. Both A1 and A2 types have been isolated from WI fields in 2009-2011 presenting risk for oospore production. However, to date, mating types have been geotemporally isolated. The use of resistant cultivars can aid in management of this disease, yet the potential for oospore production on resistant cultivars has not been well studied. *P. infestans* genotypes identified as US-22 (A2), -23 (A1), and -24 (A1) were evaluated for their ability to form oospores in potato, tomato, and solanaceous weeds. Two combinations were evaluated, US-22x23 and 22x24, and a single mating type US-22 as control. Four plants of each type were inoculated with

sporangial suspensions of each inoculum and incubated under 100% relative humidity at 18°C. At 8 days post inoculation (dpi), no oospores were found in all treatments. At 15 dpi, high numbers of oospores were produced with the mating type pairs in leaf tissue and in the epidermal tissue of stems of all susceptible and moderately resistant varieties of potato and tomato. No oospores were found in tomato carrying *Ph2* and *Ph3* resistant genes, and potato and tomato carrying the *RB* resistant gene. Low numbers of oospores were produced in solanaceous weeds. We demonstrated the potential for oospore production *in planta* with newly introduced *P. infestans* genotypes, which may play a significant role in the epidemiology of late blight.

Frequency of fungi associated with giant miscanthus in 2011

M. D. GILLEY (1), M. Tomaso-Peterson (1), T. W. Allen (1), B. S. Baldwin (1)
(1) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 102:S4.45

Giant miscanthus (*Miscanthus* x *giganteus* Greef et Deu.) is a tall perennial grass with potential for production as a biomass feedstock crop for alternative energy solutions. In 2010 foliar disease symptoms were observed on giant miscanthus in Starkville, Mississippi. In an effort to better understand the patho-system, a study was initiated to monitor fungal occurrence in naturally infected giant miscanthus fields. 'Freedom', 'Illinois' and 'Nagara' giant miscanthus varieties were observed monthly throughout the growing season in 2011. Lesions were excised from mature leaves, surface disinfested and rinsed prior to plating on water agar. Fifty lesions were plated per giant miscanthus variety each month and incubated at room temperature for 21 days under continuous fluorescent light. Fungi were identified based on fruiting structure and colony morphology using light microscopy. The internal transcribed spacer (ITS) regions of ribosomal DNA of selected fungi were amplified with PCR primers ITS1 and ITS4 to confirm identity. Predominant fungi observed among giant miscanthus varieties included *Alternaria*, *Phoma*, and *Stagonospora* species. *Bipolaris*, *Colletotrichum*, *Leptosphaeria*, *Nigrospora*, *Pithomyces* and some unknown species were isolated at lower frequencies. The isolation of gramineaceous pathogens from foliar lesions indicates a pathogenic relationship may exist among giant miscanthus varieties.

Diseases of giant miscanthus and switchgrass in Mississippi

M. D. GILLEY (1), M. Tomaso-Peterson (1), T. W. Allen (2), B. S. Baldwin (2)
(1) Mississippi State University, Mississippi State, MS, U.S.A.; (2) Mississippi State University, Starkville, MS, U.S.A.
Phytopathology 102:S4.45

Perennial grasses such as giant miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*) have the advantages of high yields and low input costs making them suitable for production as biomass feedstock crops. A disease monitoring program was initiated at Mississippi State University to observe 'Alamo' switchgrass and 'Freedom' giant miscanthus varieties for foliar diseases. Monthly assessments were made throughout the 2011 growing season. Foliar lesions were excised and surface disinfested, plated on water agar, and incubated for 21 days under continuous fluorescent light and room temperature to facilitate isolation of pathogenic fungi. Fruiting structure morphology was used for fungal identification. The internal transcriber spacer of rDNA was amplified by PCR using ITS1 and ITS4 primers, and sequences were compared to those in GenBank to confirm fungal identification. Leaf spot (*Bipolaris oryzae*) was observed throughout the growing season on both giant miscanthus and switchgrass. Anthracnose (*Colletotrichum navitas*) and leaf rust (*Puccinia* sp.) were only observed on switchgrass. Anthracnose appears mid-season and activity continues until natural senescence. Leaf rust, observed during early and late season, is more dependent upon environmental conditions. Comparison of foliar diseases among giant miscanthus and switchgrass will continue through the 2012 growing season.

***Acidovorax avenae* subsp. *avenae*: An emerging bacterial pathogen on creeping bentgrass**

P. R. GIORDANO (1), G. Sundin (1), M. Chilvers (1), B. Day (1), K. Frank (1), N. Mitkowski (2), A. Chaves (2), J. M. Vargas (1)
(1) Michigan State University, East Lansing, MI, U.S.A.; (2) University of Rhode Island, Kingston, RI, U.S.A.
Phytopathology 102:S4.45

A common plant pathogen, *Acidovoraxavenae* subsp. *avenae* (*Aaa*) has recently been found in association with an emerging disease on creeping bentgrass (*Agrostis stolonifera* L.) causing unique symptoms of etiolation and decline. First reported in 2010, *Aaa* has since been isolated out of more than 20 different symptomatic golf course samples. Identification via 16S rDNA sequencing and inoculation studies have confirmed pathogenesis of *Aaa* on creeping bentgrass. Temperature range studies show disease progression to be most severe at 30-35°C under high relative humidity. All creeping bentgrass cultivars inoculated thus far with 2.0 x 10^8 CFU of *Aaa* have been susceptible to infection when compared to sterile buffer control inoculated plants. Field studies and subsequent growth chamber studies on creeping bentgrass were conducted in order to elucidate potential chemical management options. Oxytetracycline pre-treated at 200 ppm significantly reduced leaf necrosis, discoloration, and thinning after inoculation with *Aaa* at 2.0 x 10^8 CFU compared to an untreated, inoculated control ($P < 0.001$). This is the first study elucidating *Aaa* infection on a commodity turfgrass in the United States. Genetic characterization of *Aaa* to closely related plant pathogenic *Acidovorax* spp. via multi-locus sequence typing (MLST) is underway with plans for genome sequencing of the turfgrass pathogen and the development of a molecular diagnostic assay.

Comparison between *avrGf1* and *avrGf2* which elicit hypersensitive reactions (HR) in grapefruit and sweet orange

A. M. GOCHEZ (1), G. Minsavage (2), N. Potnis (2), B. I. Canteros (1), R. E. Stall (2), J. B. Jones (2)
(1) EEA INTA Bella Vista, Bella Vista, Corrientes, Argentina; (2) Department of Plant Pathology, University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.45

Citrus canker is caused by two species, *Xanthomonas citri* subsp. *citri* (Xcc) and *X. fuscans* pv. *aurantifolii* (Xfa). The former species is associated with Asiatic citrus canker and the strains are designated A-group strains (Xcc-A). It is the primary pathogen where citrus canker occurs. The C-type canker, caused by C-group strains (Xfa), has been reported on Key lime (KL, *Citrus aurantiifolia*). The A^w strain of Xcc and the C strain of Xfa contain effectors (*avrGf1*, *avrGf2*, respectively) that cause HR on grapefruit (GF, *C. paradisi*) leaves. After cloning and sequencing *avrGf2*, we compared it with the previously sequenced *avrGf1* and determined that the two avirulence genes have 45% identity at the amino acid level and that both contain a chloroplast localization signal. Based on sequence comparison, two C-terminal motifs on each were observed that also appear in other effectors found in *Xanthomonas* and *Pseudomonas*. We compared *avrGf1* and *avrGf2*, both members of the XopAG family, by infiltrating leaves with suspensions of transconjugants of Xcc-A (Xcc-A306) containing each gene and screening for electrolyte leakage and population growth in KL and GF. There was no observable effect on virulence when transconjugants containing either gene was inoculated on KL. Expression of either gene in Xcc-A resulted in a similar phenotype following infiltration into GF leaves although *avrGf2* transconjugants elicited a faster HR and lower populations than those containing *avrGf1*.

Commercial-scale soil test *Verticillium dahliae* and *Pratylenchus penetrans* counts as influenced by long-term crop rotation and fumigation history

N. GOESER (1), P. J. Mitchell (2), A. J. Gevens (2), D. I. Rouse (2), A. MacGuidwin (2)
(1) Alsum Farms and Produce, Inc., Arena, WI, U.S.A.; (2) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 102:S4.45

The potato early dying complex (PED) is an economically important yield limiting disease predominantly caused by *Verticillium dahliae* and *Pratylenchus penetrans*. Potato growers often utilize soil test *V. dahliae* and nematode count data to influence soil management decisions. Soil fumigation is the most common management strategy to mitigate the risks of PED, but soil fumigation is environmentally hazardous and economically costly. Evaluating long-term crop rotation and soil fumigation history effects on soil test *V. dahliae* and nematode counts could result in crop rotation recommendations to reduce the reliance on soil fumigation. Soil samples were collected on a commercial potato farm located in Wisconsin in the fall of each year prior to potato production from 1999 to 2011. Soil test *V. dahliae* and *P. penetrans* count data were analyzed using generalized linear mixed model ANOVA techniques to evaluate the influence of crop rotation, crop frequency history and soil fumigation history. This analysis provides insight into commercial scale crop rotation and fumigation effects on soil test counts, but is limited to one grower in Wisconsin. Data pooling by growers, industry representatives and academic researchers can allow broader analyses to further refine crop management recommendations and increase productivity within a growing region.

Fungal and bacterial community responses to fallow period in the Bolivian highlands

L. GOMEZ-MONTANO (1), A. Jumpponen (1), M. A. Gonzales (2), J. Cusicanqui (3), C. Valdivia (4), P. Motavalli (4), M. Herman (1), K. A. Garrett (1)
(1) Kansas State University, Manhattan, KS, U.S.A.; (2) Fundacion PROINPA, La Paz, Bolivia; (3) Universidad Mayor de San Andres, La Paz, Bolivia; (4) University of Missouri, Columbia, MO, U.S.A.
Phytopathology 102:S4.45

Traditional fallow periods in the Bolivian highlands are being shortened in an effort to increase short-term crop yields, with potential long-term impacts on soil communities. Using 454-pyrosequencing, we characterized fungal and bacterial community responses to (1) the length of fallow period and (2) the presence of the plants *Parasthrephia* sp. or *Baccharis* sp. (both locally known as 'Thola'). Thola is widely considered by farmers as beneficial to soil health, although it is frequently harvested as a source of fuel by farmers. Soils in one study area, Ancoraimes, had higher levels of organic matter, nitrogen and other macronutrients compared to the other study area, Umala. In our analyses, Ancoraimes soils supported more diverse fungal communities, whereas Umala had more diverse bacterial communities. Unexpectedly, the longer fallow periods were associated with lower fungal and bacterial diversity. Fungi such as *Bionectria*, *Thelebolus*, *Acremonium* and *Chaetomidium*, and bacteria such as *Thermofilum*, *Paenibacillus*, and *Gemmata* decreased in abundance with longer fallow period. The presence of Thola did not significantly affect overall soil fungal or bacterial diversity, but did affect the frequency of some taxa such as *Alternaria* and *Bradyrhizobium*. Our results suggest that fallow period has a wide range of effects on microbial communities, and that the removal of Thola from the fields impacts the dynamics of the soil microbial communities.

Soil microbes in organic vs. conventional vegetable production: Capturing the active players through soil RNA analysis

L. GOMEZ-MONTANO (1), A. Jumpponen (1), M. Kennelly (1), K. A. Garrett (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 102:S4.46

Soil microbes are fundamental to the productivity of agricultural systems. Organic management may foster more diverse soil microbial communities beneficial for crop production, with the potential to reduce losses to pathogens. We evaluated active microbial community responses in a six-year field experiment with two-year rotation of tomato and pac choi. We compared microbial communities in organic vs. conventional nutrient management with low and high fertility levels. Both low fertility treatments had added nutrients only through a cover crop. The conventional high fertility treatment was supplemented with potassium nitrate, calcium nitrate, and inorganic pre-plant fertilizer. High organic fertility was supplied by fish hydrolyzate and compost pre-plant fertilizer. Using 454-pyrosequencing and DNA-tagging, we compared *total* resident fungal, bacterial, and archaeal communities using extracted DNA and the *actively metabolizing* microbial communities using extracted RNA. Using Inverse Simpson's Dominance as a measure of diversity, we found the highest bacterial diversity under organic management for the high fertility treatment. We recovered a number of bacterial genera that have important agroecological roles, such as *Nitrospira*, *Rhizobium*, and *Desulfovibrio*. Bacterial diversity was higher in DNA samples compared to RNA samples, indicating that the active microbial community is a subset of the DNA-inferred total community.

Simulated rainfall to evaluate removal of pyraclostrobin applied for control of postbloom fruit drop of citrus

F. P. GONCALVES (1), B. B. Forcelini (2), N. A. Peres (3), L. Amorim (1)
(1) Escola Superior de Agricultura Luiz de Queiroz, Universidade Sao Paulo, Piracicaba, Brazil; (2) University of Florida, GCREC, Wimauma, FL, U.S.A.; (3) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.46

Postbloom fruit drop (PFD), caused by *Colletotrichum acutatum*, is one of the most serious diseases of citrus in southwestern São Paulo State, Brazil. In this area, the weather conditions are extremely favorable for PFD and fungicide applications are often made during rain events. Nevertheless, no studies have been conducted to determine the retention of fungicides applied to citrus flowers just before rain. An experiment was carried out in a randomized block design with 3-yr-old sweet orange trees. The following treatments were applied: four rainfall simulations - 0, 4.3, 13 and 50 mm/h each with or without pyraclostrobin application. Branches 20 to 60 cm long containing flower buds were used as replicates. Simulated rainfall was applied 30 min after pyraclostrobin (0.25g a.i./L) sprays. After the simulated rainfall, the flowers were inoculated with 5 mL suspension of $2.5x10^5$ conidia mL^{-1} and were kept in humid chambers for 16 h. The experiment was performed twice. Treatments from both experiments were compared using the area under disease progress curve (AUDPC) by Tukey's test. Pyraclostrobin showed excellent PFD control in rainfall simulation with less than 13 mm/h. The reduction of incidence of symptomatic flowers ranged from 80 to 94% on sprayed trees. Fungicide wash-off was not observed on trees under rainfall simulation at 4.3 and 13 mm/h. However, with rainfall simulation of 50 mm/h, the amount of disease was similar on sprayed and non-sprayed trees. Our results indicate that wash-off of pyraclostrobin is influenced by rain intensity.

Electrophoretic profiles of peroxidases and polyphenol oxidases in jalapeño pepper plants inoculated with nonpathogenic rhizobacteria

A. GONZALEZ-FRANCO (1), L. Robles-Hernandez (1), E. Gonzalez-Gamez (1), E. Sanchez-Chavez (2)
(1) Universidad Autonoma de Chihuahua, Chihuahua, Mexico; (2) Centro de Investigación en Alimentación y Desarrollo, A.C. Unidad Delicias, Ciudad Delicias, Mexico
Phytopathology 102:S4.46

Jalapeño pepper plant assays were carried out to study the effect of *Streptomyces lydicus* 5US-PDA8 and *Streptomyces* sp. PRIO-41 on electrophoretic profiles of peroxidases (POX) and polyphenol oxidases (PPO). The bacterial spores or mycelia were used to coat pepper seeds, the plantlet roots or both. Non-inoculated plantlets were used as control. The enzyme activity was analyzed in roots and leaves at 40 and 60 days after transplanting. The enzymatic profiles were obtained after native PAGE electrophoresis. POX patterns from root extracts treated with any bacterial strain showed shifts in the dominant bands, but, treatments with 5US-PDA8 induced more dominant active ones. A unique band with low electrophoretic mobility was detected with 5US-PDA8 seed coated treatment. Since earlier determinations, treatments with 5US-PDA8 showed the strongest PPO patterns compared with those of PRIO-41 treatments and the control, though same complexity was observed. All the bacterial treatments induced different POX banding patterns from leaf extracts, but mainly faint bands were observed. The PPO patterns from leaf extracts of all the bacterial treatments were generally similar, except for PRIO-41 inoculated on seed, which showed one unique active band with low mobility. These results suggest that PRIO-41 and 5US-PDA8 strains are potentially effective to induce certain plant protection and may keep the plant in a state of alert during the growth cycle.

Effect of soil type and compaction on severity of clubroot (*Plasmodiophora brassicae*)

B. D. GOSSEN (1), H. Kasinathan (2), M. McDonald (2), G. Peng (1)
(1) Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada; (2) Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada
Phytopathology 102:S4.46

Studies were conducted under controlled conditions to examine the effect of soil type on clubroot caused by *Plasmodiophora brassicae*. Combinations of three factors: soil type (muck soil, pH 6.2; mineral soil, pH 6.8; non-calcareous sand, pH 6.5; soil-less mix, pH 6.0), pathotype (P3, P6; William's system), and biofungicide (Serenade, a.i. *Bacillus subtilis*; Prestop, a.i. *Gliocladium catenulatum*) were examined in a trial on canola (*Brassica napus*) and another on Shanghai pak choy (*B. rapa* subsp. *chinensis* var. *communis*). Seedlings were treated with Serenade (5% v/v) or Prestop (7.5 g L-1) at 5 days after germination and inoculated with *P. brassicae* 3 days later. Clubroot symptoms were assessed at 6 wk after inoculation. A third trial examined the impact of compaction on clubroot severity on canola. Each experiment consisted of four replicates, and each was repeated. Clubroot incidence and severity were slightly but consistently higher in Shanghai pak choy than canola, and inoculation with P3 resulted in slightly more clubroot than P6. Clubroot levels in soil-less mix were lower than in the other soil treatments, but there was little difference among the three soils. The biofungicides reduced clubroot, but the reduction was generally small and inconsistent. Clubroot severity increased substantially with increasing soil compaction. This indicates that soil type likely has a smaller impact on clubroot that level of compaction when the soil is saturated, e.g., after heavy or prolonged precipitation.

Identification, phylogenetic relationships, and biological properties of large satellite RNAs associated with *Grapevine fanleaf virus*

J. GOTTULA (1), D. Lapato (2), M. Fuchs (1)
(1) Cornell University, Geneva, NY, U.S.A.; (2) University of Virginia, Charlottesville, VA, U.S.A.
Phytopathology 102:S4.46

Large satellite RNAs associated with *Grapevine fanleaf virus* (GFLV) from the genus *Nepovirus* in the family *Secoviridae* were identified in a naturally GFLV-infected vineyard in California and in a national clonal germplasm repository in New York by IC-RT-PCR and specific primers. Analysis of the genetic variability showed nucleotide sequence similarities between large satellite RNAs of GFLV and *Arabis mosaic virus* (ArMV), a closely related virus. Two distinct phylogenetic groups, each with GFLV and ArMV satellite RNAs, were characterized, indicating no genetic distinction with respect to the identity of the helper virus. Nucleotide sequence analyses predicted that GFLV satellite RNAs originated from a recombination event between an ancestral GFLV genomic RNA and another RNA sequence of unknown origin. Assessing the biological properties of GFLV strains with and without

satellite RNAs showed no apparent effect of satellite RNAs on disease progress, symptom expression and virus titer in *Chenopodium quinoa*, a systemic host. This study sheds light on the origin and ecology of large satellite RNAs associated with GFLV.

Detection and molecular characterization of fenhexamid resistance in *Botrytis cinerea* isolates from strawberries

A. GRABKE (1), D. Fernández-Ortuño (1), G. Schnabel (1)
(1) Clemson University, Clemson, SC, U.S.A.
Phytopathology 102:S4.47

Gray mold is one of the most important diseases of strawberry. In the southeastern United States, the disease is primarily caused by *Botrytis cinerea* and to a much lesser degree by *B. caroliniana*. The control of gray mold is mainly based on the application of fungicides during bloom and fruit maturation. Several fungicides from different modes of action are registered for gray mold control, including the hydroxyanilide fenhexamid. This fungicide specifically inhibits the 3-ketoreductase of the ergosterol biosynthesis pathway and is therefore prone to resistance development. In order to determine levels of fenhexamid sensitivity in North and South Carolinian strawberry fields, 217 single-spore isolates were collected and subjected to a spore germination assay that distinguishes sensitive from resistant isolates. Fenhexamid resistance was found in three of four locations from North Carolina and in four of seven locations from South Carolina. Approximately 20% of all isolates were resistant to fenhexamid. Detached fruit studies demonstrated that field rates of Elevate controlled sensitive but not resistant isolates. Resistance was associated with three mutations in the *erg27* gene, encoding 3-ketoreductase, at position 412 leading to amino acid changes F412S, F412C, and F412I. A rapid polymerase chain reaction method was developed to distinguish sensitive from resistant genotypes.

WITHDRAWN

Integration of soil-applied neonicotinoid insecticides and acibenzolar-S-methyl for systemic acquired resistance (SAR) control of citrus canker on young citrus trees

J. H. GRAHAM (1), M. E. Myers (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.47

Soil application of systemic neonicotinoid insecticides for control of psyllid vectors of Huanglongbing disease on young citrus trees also produce season-long SAR control of citrus canker caused by *Xanthomonas citri* subsp. *citri*. Neonicotinoids, imidacloprid (IMID; Admire, Bayer) and thiamethoxam (THIA; Platinum, Syngenta), were compared with soil or microsprinkler applications of the commercial SAR inducer acibenzolar-S-methyl (ASM; Actigard, Syngenta) and foliar sprays of copper hydroxide (CH; Kocide 3000, Dupont) or streptomycin (STREP; FireWall, Agrosource) to evaluate their effects on the percentage of canker-infected leaves on 2 yr-old Vernia orange and 3 yr-old 'Ray Ruby' grapefruit trees in Ft. Pierce, FL. All treatments significantly reduced incidence of foliar canker compared to the untreated check. Soil drenches of ASM and season long rotations with IMID and THIA were highly effective for suppressing foliar canker on young grapefruit and orange trees under weather conditions absent of high intensity rains or tropical storms. Microsprinkler application of ASM was less effective than soil drench. The level of control for SAR treatments was comparable to eleven 21-da interval sprays of CH or STREP. SAR induced by soil-applied insecticides provides substantial benefits for canker disease management that can be augmented with ASM.

Morphological and physiological variation within *Phytophthora capsici* isolates from a worldwide collection

L. GRANKE (1), L. M. Quesada-Ocampo (1), A. Lebeis (1), L. Henderson (1), M. VanOverbeke (1), M. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.47

Sporangial length and width, pedicle length, oospore diameter, sporangia and chlamydospore production, and growth at 32, 35, and 38°C was determined for 124 *Phytophthora capsici* isolates from 12 countries. Sporangial length (38 to 60 µm), sporangial width (23 to 35 µm), length:breadth ratio (1.34 to 2.07), pedicle length (20 to 260 µm), and oospore diameter (22 to 37 µm) varied widely among isolates. Differences in sporangia production were observed among isolates, and isolates from non-vegetable hosts produced fewer sporangia than isolates from vegetable hosts. When cultures were incubated in liquid medium, 35 *P. capsici* isolates formed chlamydospores; all of the isolates that produced chlamydospores were originally isolated from vegetable hosts. Most (122 of 124) of the isolates were able to grow at 35°C, but all of the isolates grew poorly at 38°C. The results of this study indicate substantial variation in morphological and physiological characteristics among *P. capsici* isolates.

***Pospiviroid* detection in greenhouse and field crops: A sensitive RT-PCR assay for genus- and species-level identification**

D. L. GROTH-HELMS (1), S. Zhang (1), P. Russell (1), S. Juszczak (1)
(1) Agdia, Inc., Elkhart, IN, U.S.A.
Phytopathology 102:S4.47

Pospiviroids have been a focus of disease prevention strategies in potato and other solanaceous vegetable crops since their discovery. In recent years, this genus of plant pathogens has garnered increasing attention in greenhouse-grown ornamental plants and in plants subject to phytosanitary certifications. There are at least six species of pospiviroids found in greenhouse and field crops, including *Chrysanthemum stunt viroid* (CSVd), *Citrus exocortis viroid* (CEVd), *Potato spindle tuber viroid* (PSTVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd) and *Tomato planta macho viroid/Mexican papita viroid* (TPMVd/MPVd). Non-degenerate primers were designed from the conserved sequences of the members of this genus, allowing for detection of these six pospiviroids at the genus-level, and at the species-level through sequence analysis. The test was screened against a collection of previously characterized viroid species, including other members of *Pospiviroidae* and *Avsunviroidae*, to confirm genus-level specificity. The sensitivity of this two-step RT-PCR assay was demonstrated using composites of a single infected tomato leaflet among a pool of healthy leaflets, and by screening samples of quantified RNA. This reliable and sensitive approach to detect pospiviroids in foliar tissues can be used to prevent the spread of the pathogen and aid in its eradication. Validation of this assay to effectively screen tomato seed lots for seed-borne and seed-transmitted pospiviroids is underway.

Effects of multiple sources of seasonality on the risk of pathogen spread to vineyards: Vector pressure, natural infectivity, and host recovery

B. GRUBER (1), M. Daugherty (2)
(1) University of Florida/IRREC, Fort Pierce, FL, U.S.A.; (2) University of California, Riverside, CA, U.S.A.
Phytopathology 102:S4.47

For vector-borne pathogens, seasonality may manifest in the variability in vector abundance, vector infectivity, and host-infection dynamics over the year. The relative importance of multiple sources of seasonality affecting the possible spread of a plant pathogen, *Xylella fastidiosa*, into vineyards was explored. Observed seasonal population densities of the primary leafhopper vector, *Graphocephala atropunctata*, from 8 years of surveys in Northern California were incorporated into a model of primary spread to estimate the risk of pathogen infection under different scenarios regarding seasonality in vector natural infectivity (i.e. constant or increasing over the season) and grapevine recovery from infection (i.e. none or seasonal recovery). Seasonal natural infectivity, seasonal recovery, and especially the combination, reduced (up to 8-fold on average) within-season and cumulative yearly estimates of pathogen spread. Estimated risk of infection also differed greatly among years due to large differences in vector abundance, with wet and moderate winter and spring conditions favoring higher *G. atropunctata* abundance. Seasonal variation of the pathogen - vector interaction may play an important role in the dynamics of disease in vineyards, reducing the potential prevalence when these agents are less abundant. Moreover, climate, by affecting sharpshooter abundance or activity, may influence Pierce's disease dynamics.

How do *Phytophthora* pathogens emerge?

N. J. GRUNWALD (1)
(1) USDA-ARS, Corvallis, OR, U.S.A.

Phytopathology 102:S4.48

Plant pathogens appear to emerge at increasing rates, be it due to a combination of climate change, increased human travel and more frequent global trade among other factors. The genus *Phytophthora* comprises some of the most notable invasive and emerging plant pathogens. Notable examples include the sudden oak death pathogen *P. ramorum* and the potato late blight pathogen *P. infestans*. *P. ramorum* emerged repeatedly by at least four global migrations, three into North America and one into Europe. In both North America and Europe, *P. ramorum* populations remain clonal. Despite the fact that both mating types coexist in North America, sexual reproduction has not been observed. *P. infestans* populations show a similar pattern, but clearly undergo an apparently 'random' succession of clonal lineages as novel lineages are introduced and older lineages are displaced either due to stochastic effects or lower pathogen fitness. While in Europe populations have become sexual, populations in the US are still clonal despite presence of both mating types. Another mechanism of emergence has been observed for the South American species *P. andina*. This species emerged via hybridization between *P. infestans* and another unknown *Phytophthora* species that served as a hybrid parent. Hybridization, followed by host jump is hypothesized to have occurred in this case. The patterns of emergence observed can best be explained by repeated introductions of *Phytophthora* clones or clonal lineages, admixture of existing and new introductions, displacement of existing clonal lineages, occasional establishment of sexual populations, as well as hybridization and host jump. These combined efforts shed new light on mechanisms whereby plant pathogens in the genus *Phytophthora* emerge to become pathogens causing devastating diseases such as sudden oak death and potato late blight and continue to reemerge.

Ingress of *Salmonella enterica* Typhimurium into tomato leaves and soil management effect on its internal persistence

G. GU (1), J. M. Cevallose-Cevallos (1), A. H. van Bruggen (1)
(1) Emerging Pathogens Institute and Department of Plant Pathology, University of Florida, Gainesville, FL, U.S.A.

Phytopathology 102:S4.48

Several *Salmonella enterica* outbreaks have been traced back to contaminated tomatoes. In this study, ingress pathways and soil management effect on internal persistence of *S. enterica* Typhimurium in tomato plants were investigated. Tomato plants grown in conventional or organic soils were inoculated with a GFP-labeled *S. enterica* Typhimurium (10^9 CFU/ml) suspension by leaf dipping or through guttation droplets. Inoculated and adjacent leaflets were tested for *Salmonella* densities for 30 days after each inoculation. Ingress of *Salmonella* was detected under confocal microscope. Endophytic bacterial communities were characterized by PCR-DGGE two weeks before and after inoculation. Fruits and seeds were also examined for *Salmonella* incidence. *Salmonella* can enter tomato leaves through stomata and hydathodes and move into vascular system. More *Salmonella* survived in plants grown in conventional than in organic soil. The soil management effect on *Salmonella* survival was confirmed for tomato plants grown in two additional pairs of conventional and organic soils. Endophytic bacterial diversity of tomato plants grown in conventional soils were significantly lower than those in organic soils. All contaminated fruits (1%) were from tomato plants grown in conventional soils. About 5% seeds from infested fruits were contaminated. No *Salmonella* was detected in fruit-bearing tomato plants grown from internally contaminated seeds.

A TaqMan-based real-time PCR assay for specific detection and quantification of *Xylella fastidiosa* strains causing bacterial leaf scorch in oleander

W. GUAN (1), J. Shao (2), R. Singh (3), R. Davis (2), T. Zhao (4), Q. Huang (1)
(1) FNPRU, U.S. National Arboretum, USDA-ARS, Beltsville, MD, U.S.A.; (2) MPPL, PSI, USDA-ARS, Beltsville, MD, U.S.A.; (3) Plant Disease Diagnostic Clinic, Louisiana State University AgCenter, Baton Rouge, LA, U.S.A.; (4) Chinese Academy of Agricultural Sciences, Beijing, Peoples Republic of China

Phytopathology 102:S4.48

A TaqMan-based real-time PCR assay was developed for specific detection of strains of *X. fastidiosa* causing oleander leaf scorch. The assay uses primers WG-OLS-F1 and WG-OLS-R1 and the fluorescent probe WG-OLS-P1, designed based on unique sequences found only in the genome sequence of oleander strain Ann1. The assay is specific, allowing detection of only oleander-infecting strains, not other strains of *X. fastidiosa* nor other plant-associated bacteria, in cultured bacterium and in infected plant samples. The assay can also be applied to detect low numbers of *X. fastidiosa* in insect samples, or further developed into a multiplex real-time PCR assay to simultaneously detect and distinguish diverse strains of *X. fastidiosa* that may occupy the same hosts or insect vectors. Specific and sensitive detection and quantification of oleander strains of *X. fastidiosa* should be useful for disease diagnosis, epidemiological studies, management of oleander leaf scorch disease, and resistance screening for oleander shrubs.

WITHDRAWN

Comparative study of transcription regulation in genus of *Fusarium* using a multigenome microarray

L. GUO (1), X. Zhao (2), W. Jonkers (3), C. H. Kistler (3), J. Xu (2), L. Ma (1)
(1) University of Massachusetts-Amherst, Amherst, MA, U.S.A.; (2) Purdue University, West Lafayette, IN, U.S.A.; (3) University of Minnesota, St. Paul, MN, U.S.A.

Phytopathology 102:S4.48

The genus *Fusarium* contains many plant pathogens causing serious diseases on a wide range of crop plants globally as well as posing threat to human and animal health by producing mycotoxins. Genomes of four *Fusarium* species have so far been sequenced and annotated. Comparative gene expression and transcriptional regulation of *F. graminearum*, *F. verticillioides* and *F. oxysporum* is conducted using a multigenome comparative GeneChip that was designed containing a total of 961,400 probes covering up to 48,070 genes from the three *Fusarium* species. cDNA libraries of each sequenced *Fusarium* strains were prepared from RNAs isolation of vegetative hyphae in liquid cultures and several kinase mutants. The GeneChip was then hybridized with the cDNAs and gene expression signals in each library were detected, normalized and analyzed in Affymetrix microarray analysis package and GenePattern at Broad Institute. The results of differentially expressed genes and their function association will be discussed. Gene expression studies using *Fusarium* comparative GeneChip will provide greater insight on the global transcriptional regulation in the three *Fusarium* species and will aid identification of a number of fungal pathogenicity genes that are critical for our understanding of host-pathogen interactions.

***Verticillium* species recovered from commercial spinach in four valleys in California**

S. GURUNG (1), D. P. Short (1), K. Maruthachalam (1), S. T. Koike (1), Z. K. Atallah (2), K. V. Subbarao (1)
(1) University of California-Davis, Salinas, CA, U.S.A.; (2) Hartnell College, Salinas, CA, U.S.A.

Phytopathology 102:S4.48

Verticillum dahliae causes wilt on >300 plant hosts including spinach and lettuce. More than 78% of the total US spinach production occurs in California. Spinach seed from Washington State, Denmark, and The Netherlands has been implicated as a carrier of *V. dahliae* and as one of the causes of wilt on lettuce crops in California. The disease is currently restricted to the Pajaro and Salinas Valleys even though cropping patterns are similar in other valleys in California. The objective of this study was to examine why

Verticillium wilt of lettuce is confined to the above two valleys in CA, and to determine the frequency of isolation of different *Verticillium* species. Twenty plants from 60 commercial spinach fields in Salinas, Pajaro, Santa Maria and San Juan valleys of California were sampled, and the roots and petioles from each were plated on the NP-10 medium. More than 500 isolates recovered were characterized as *V. dahliae* or *V. tricorpus* like species based on colony morphology. Of these, 150 isolates were randomly selected and identified to species using the recently developed *Verticillium* species-specific primers and were predominantly composed of *V. isaacii* and *V.dahliae*. The frequency of recovery of *Verticillium* species did not explain the current distribution of the disease in California but the disease on lettuce was correlated with the magnitude of spinach production in the four valleys.

Genetic diversity of *Geosmithia morbida*, the causal agent of thousand canker disease in the southeastern United States

D. HADZIABDIC (1), L. M. Vito (1), P. A. Wadl (1), M. T. Windham (1), R. N. Trigiano (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 102:S4.49

The fungus *Geosmithia morbida* sp. nov, and the walnut twig beetle, *Pityophthorus juglandis*, have been associated with a disease complex of black walnut (*Juglans nigra*) known as Thousand Canker Disease (TCD). Disease is manifested as branch dieback and canopy loss, eventually resulting in tree death. In 2010 the disease appeared in the native range of black walnut including Tennessee, and subsequently in Virginia and Pennsylvania in 2011. This was the first known incident of TCD east of Colorado, where the disease has been established for more than a decade on indigenous walnut species. A genetic diversity study of 54 isolates of *G. morbida* throughout the native range of black walnut was completed using 15 polymorphic microsatellite loci. The mean number of alleles was 4.2 across five locations of *G. morbida*. Genotypic diversity parameter quantified by Shannon's Information Index was 1.12. Moderate genetic diversity (Fst=0.12) and analysis of molecular variance (AMOVA) revealed that the majority of genetic variation was within examined groups (88%). Understanding genetic composition and demography of *G. morbida* can provide valuable insight into understanding factors affecting the persistence and spread of an invasive pathogen, disease progression, and future infestation predictions.

Impact of cropping sequence on diseases, nematodes, and yield of peanut, cotton, and corn in Southwest Alabama

A. K. Hagan (1), H. L. CAMPBELL (1), K. L. Bowen (1), M. Pegues (2), J. Jones (2)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Gulf Coast Research and Extension Center, Fairhope, AL, U.S.A.
Phytopathology 102:S4.49

A study was conducted from 2003 through 2010 in Fairhope, AL to assess the impact of corn, cotton, and peanut cropping frequency on disease and plant parasitic nematodes activity, and yield. Disease activity in previous corn and peanut crops was minimal. Production and pest control were according to ACES recommendations. Disease activity in each crop was assessed prior to harvest using established rating scales. Soil samples for a nematode assay were taken after harvest, and processed using the sugar flotation method. Cropping frequency impact was greatest on peanut and to a lesser extent corn but not cotton. Lowest yields were noted for the peanut and corn monocultures in 2008, 2009, and 2010. Declining pod yields, which occurred when peanut followed peanut, closely mirrored increased leaf spot intensity. Yield for one and two yr out peanut rotations were similar. TSWV and stem rot incidence was not closely tied to peanut cropping frequency. While *Meloidogyne incognita* race 3 nor *M. arenaria* race 2 were not found, *M. incognita* race 1 was recovered from smallflower morning glory (*Jacquemontia tamnifolia*) infested plots. Declining yields in the corn monoculture were not tied to increased foliar diseases or root knot nematode. Similar yields across all cotton cropping sequences may be attributed to the absence of disease and plant parasitic nematodes. Corn, cotton, and peanut proved to be equally valuable rotation partners with one another.

Reliability and accuracy of SkyBit 2011 weather and disease forecasts in Pennsylvania

N. O. HALBRENDT (1), H. K. Ngugi (1), J. W. Travis (1)
(1) Penn State University, Biglerville, PA, U.S.A.
Phytopathology 102:S4.49

Weather and disease forecasts from SkyBit's E-Weather service were assessed for reliability and accuracy in comparison with data collected on-site at 10 orchards in Pennsylvania, or data obtained online from Accuweather.com. Overall, SkyBit slightly under-forecasts 'same-day' rainfall events and over-forecasts in the 24 h predictions. By contrast, Accuweather same-day predictions based on the local zip code had a high degree of over-forecasting (bias index = 1.34). SkyBit rainfall forecasts were more accurate than Accuweather predictions (sensitivity = 87 and 62%, respectively). Leafwetness, for which data was only available for SkyBit, was predicted with less accuracy (sensitivity = 81%). Temperature was the variable with best prediction statistics for both forecasters. Skybit apple scab and fire blight risk forecasts agreed strongly ($P < 0.001$), respectively, with modified MillsTable and Maryblyt predictions based on data collected on-site. However, the strong agreement in fire blight forecasts was only noted when 'High Risk' period in Maryblyt was counted as 'Infection Risk' indicating Skybit predictions are more precautious. These results indicate that: (i) information from SkyBit is as accurate and reliable as data collected on-site, and (ii) data obtained online using the local zip code lacks the spatial resolution needed for accurate orchard-specific forecasts. Because of convenience and the need for skills, growers may opt to subscribe to SkyBit than try to run disease forecasts themselves.

Progress in development of a Universal Plant Virus Microarray for the detection and identification of plant viruses

J. HAMMOND (1), D. C. Henderson (1), B. Bagewadi (2), K. F. Fischer (3), D. Wang (4), U. Melcher (5), K. L. Perry (6), R. L. Jordan (1), C. M. Fauquet (2)
(1) USDA-ARS FNPRU, Beltsville, MD, U.S.A.; (2) Danforth Plant Science Center, St. Louis, MO, U.S.A.; (3) University of Utah, Salt Lake City, UT, U.S.A.; (4) Washington University, St. Louis, MO, U.S.A.; (5) Oklahoma State University, Stillwater, OK, U.S.A.; (6) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.49

Microarrays based on oligonucleotides representing sequences conserved at the level of viral species, genera, and families are able to detect and identify both characterized and previously uncharacterized viruses infecting mammals and birds. Software initially developed for these animal virus microarrays has been further refined for both design and analysis of a Universal Plant Virus Microarray (UPVM). The UPVM is based on 9600 60-mer oligonucleotides, including at least four genus-level and four family-level probes per taxonomic group, and 44 control probes for highly conserved plant genes. These probes together represent all characterized plant viruses for which significant genomic sequence was publically available in GenBank as of December 2009, and additional sequences made available to us prior to public GenBank release. Associated methods have been developed for high quality total nucleic acid extraction, applicable to a broad range of plant tissues containing metabolites such as phenolics, polysaccharides, latex, and resins that can interfere with nucleic acid extraction or subsequent amplification. Validation of the UPVM with a broad range of DNA and RNA plant viruses is in progress. Many high-titer viruses can be detected by direct labeling of total RNA extracts. Amplification and subtractive hybridization protocols to increase the sensitivity of detection of low titer viruses are being examined.

Increased pepper yields following incorporation of biofumigation cover crops and their effects on soilborne pathogen populations and pepper diseases

Z. Hansen (1), A. KEINATH (1)
(1) Clemson University, Charleston, SC, U.S.A.
Phytopathology 102:S4.49

The use of brassica cover crops and their associated degradation compounds as biofumigants to manage soilborne pathogens could offer vegetable growers an alternative to the restricted broad-spectrum fumigant methyl bromide. Biofumigation was tested in two experiments to manage *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium* spp., and the diseases they cause in pepper. Field plots were seeded to one of three brassicaceous cover crops: oilseed radish, 'Pacific Gold' mustard, and 'Dwarf Essex' winter rapeseed. Cover crops were disked into soil and immediately covered with virtually impenetrable film (VIF) to reduce the escape of volatile pesticidal compounds. Controls included fallow plots with and without VIF. Treatments were followed by green bell pepper. All (+) VIF treatments reduced populations of *R. solani* compared to (-) VIF, with no differences between biofumigation treatments and fallow (+) VIF. Biofumigation treatments did not reduce populations of *Pythium* spp. or *S. rolfsii* compared to fallow (+) VIF. Pepper stunting was significantly lower in (+) VIF treatments compared to fallow (-) VIF, with no consistently significant differences between biofumigation treatments and fallow (+) VIF. *Pythium* isolated from roots of stunted peppers was identified as *P. aphanidermatum*. Biofumigation treatments did not reduce pepper death. Pepper yields were highest in biofumigation treatments compared to fallow (+) VIF, and (+) VIF yields were higher than (-) VIF yields.

Variability in *Phoma* species affecting sugar beet
L. E. HANSON (1), T. Mo (2), T. R. Goodwill (1)
(1) USDA-ARS, East Lansing, MI, U.S.A.; (2) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.50

Phoma betae can cause damage to sugar beet (*Beta vulgaris*) at multiple growth stages. It has historically been an important seedling disease, but this is largely managed by ensuring clean seed for planting. The pathogen also can cause a root rot, a leaf spot, and rotting of beets during storage. In the United States, the only pathogenic *Phoma* associated with beets has been *Phoma betae*. In Eastern Europe, some additional *Phoma* species have been reported to cause symptoms on sugar beet. Our aim was to investigate variability in *Phoma* isolates from sugar beet in the United States to determine whether there might be more than one species causing symptoms on beet. *Phoma* isolates had been collected as part of ongoing surveys for seedling diseases and root rot, and additional samples were collected from leaf spots. Isolates were examined for morphological characters on malt extract agar and oatmeal agar and a portion of the ITS region was sequenced for comparison. Of 14 isolates identified as *Phoma* by both morphological and molecular testing, six showed highest identity with *P. betae* while the remaining isolates showed highest identity with *Phoma* species other than *P. betae*. Leaf spots were observed on sugar beet treated with either *Phoma betae* or other *Phoma* isolates and pathogenicity screening is continuing.

Examination of race structure for *Verticillium dahliae* isolates affecting chile pepper production in New Mexico
S. HANSON (1), M. Radionenko (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 102:S4.50

Chile pepper production in the dessert South West has been greatly affected by the soil-borne fungus *Verticillium dahliae*. The lack of natural resistance in commercial peppers combined with few or no affective chemical and cultural controls makes *V. dahliae* a serious and persistent problem in chile pepper production. Tomato lines containing the Ve1 gene are resistant to race 1 of *V. dahliae* but not race 2. While races of *V. dahliae* affecting tomato have been well defined virtually nothing is known about the race structure of *V. dahliae* affecting chile peppers in the desert Southwest. Since transfer of the Ve1 gene from tomato to chile peppers is one potential option for deriving resistance to this pathogen, we set out to determine if *V. dahliae* strains present in NM could be controlled by the Ve1 gene. Approximately ninety independent isolates of *V. dahliae* were collected from major chile production regions in Southern New Mexico in 2011. These isolates were inoculated onto a susceptible tomato line lacking the Ve1 gene (Bonny Best) and Ve1 containing *V. dahliae* race 1 resistant tomato line (55 VF). In addition, molecular tests for defining the race of *V. dahliae* isolates were also performed. Our results indicate race 2 like *V. dahlia* strains that overcome Ve1 mediated resistance are common in NM and therefore that the Ve1 resistance gene will not be a good source of *V. dahliae* resistance for chile producers in the desert Southwest.

Screening for biocontrol agents for protection of chile peppers against *Phytophthora capsici* and *Verticillium dahlia*
S. HANSON (1), A. Garcia (1), J. Achata (1), R. Trejo (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 102:S4.50

Phytophthora capsici and *Verticillium dahliae* are among the leading causes of preventable yield losses in chile pepper production in the desert Southwest. The lack of natural resistance to these pathogens in commercial chile cultivars combined with few effective chemical and cultural controls make these two pathogens severe and persistent disease problems in chile pepper production. Biocontrol has shown promise for providing safe, economical, and "green" control of similar plant pathogens in other systems. In particular, various *Bacillus* spp. have proven to be effective against a range of soil-borne pathogens. This study focuses on isolating *Bacillus* strains from chile fields and evaluating their potential as biocontrol agents in both plate inhibition and plant protection assays. Results presented include analysis of over 200 *Bacillus* isolates and positive identification of isolates that are antagonistic toward *P. capsici* and *V. dahliae*.

WITHDRAWN

An improved method for DNA sequence-based identification of nematodes
S. HANSON (1), F. Solano (1), S. Thomas (1), J. Beacham (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 102:S4.50

The complexity of nematode communities and limited availability of morphological taxonomic expertise makes accurate identification of individual nematodes and characterization of communities a challenging problem. Several molecular based methods for nematode identification have been described. However, these rely on time consuming and inconsistent protocols for DNA isolation that often require manual lysis. We present here a method based on sonication, proteinase K treatment and quick freeze-thaw cycles that reliably produces PCR-suitable DNA from nearly 100% of *M. incognita* juveniles and over 94% of unknown nematodes randomly isolated from soil samples. Although it comprises several steps, the protocol is performed in a single tube, is straightforward and requires less than 60 min to complete. Further, this method produces enough DNA from a single nematode for 25 individual PCR reactions allowing multi-locus analysis, sample sharing, and repeat analysis. This method is also fully compatible with the long-term storage of specimens in DESS, a preservative solution that preserves both morphology and DNA, thus allowing complimentary analysis of morphology and DNA from either fresh or stored nematodes. The utility of this method is validated by its use in characterizing nematode diversity in soil samples from the New Mexico desert, demonstrating that the method is effective with a wide variety of soil inhabiting nematode taxa.

Novel autotransporters encoded by the citrus huanglongbing bacterium, '*Candidatus* Liberibacter asiaticus'
G. HAO (1), M. Boyle (2), L. Zhou (1), Y. Duan (1)
(1) U.S. Horticultural Research Laboratory, Fort Pierce, FL, U.S.A.; (2) Smithsonian Marine Station, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.50

Proteins secreted by the type V pathway are referred to as autotransporters, which are large extracellular virulence proteins localized to the bacterial pole. '*Candidatus* Liberibacter asiaticus' (Las) is the most destructive species causing citrus huanglongbing worldwide. Two hyper variable genes (hyv_{I} and hyv_{II}) were identified in the prophage regions of the psy62 Las genome. Bioinformatic analyses revealed that Hyv_{I} and Hyv_{II} share the characteristics of an autotransporter family, which contains large tandem repeats of a passenger domain and a C-terminal translocator domain. The Hyv_{I} C-terminus and full length Hyv_{II} were fused to GFP and expressed in *E. coli* driven by the T7 promoter. Confocal microscopy results show that both proteins are localized to the bacterial poles, similar to members of autotransporters from other bacteria. Despite the absence of signal peptides, Hyv_{I} was found to localize at the cell surface by immuno-dot blot using a monoclonal antibody against the Hyv_{I} protein, and its surface localization was also determined by removal of the Hyv_{I} antigen using protease treatment of intact bacterial cells. Co-inoculation of tobacco leaves with a P19 gene silencing suppressor and the GFP-Hyv_{I} C-terminal translocator domain construct facilitated GFP expression in *Agrobacterium*-mediated transient expression experiments. These results indicate that the Hyv_{I} protein did not target the cell nucleus, but did target organelles in tobacco leaves. This is the first evidence that '*Candidatus* Liberibacter asiaticus' may employ the type V pathway.

Pho P/Q-regulated genes are involved in *Salmonella enterica* root colonization
L. HAO (1), K. Cowles (1), J. Barak (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.50

Contaminated fresh produce has become the number one vector of non-typhoidal salmonellosis to humans. However, *Salmonella enterica* genes essential for the life cycle of this organism outside the mammalian host are for the most part unknown. Screening deletion mutants led to the discovery that mutants in STM1840 and STM2514 failed to colonize alfalfa roots. The STM1840 mutant had approximately 0.3 and 0.5 log (CFU/sprout) reduction in population compared to the WT at 48 and 72 h post inoculation (hpi) respectively, but no population difference from the WT at 24 hpi. The STM2514 mutant had approximately 0.5 log (CFU/sprout) reduction in population compared to the WT at 24 hpi but no population difference from the WT at 48 hpi. On tomato leaves, the STM2514 mutant had approximately 0.8 log (CFU/cm2) reduction in population compared to the WT. Further examination of the STM1840 mutant revealed a swimming defect on LB swim induction plates but no biofilm or pellicle formation difference in LB no salt media at 28°C compared to the WT. Both *STM1840* and *STM2514* are regulated by a PhoP/Q regulon, which responds to a variety of environmental stimuli. The function of both STM1840 and STM2514 in plants is still under study. Identification and characterization of essential genes and mechanisms that are needed by human pathogens to survive in/on plants will provide critical understanding of the basic biology of the pathogen and help to develop useful strategies to prevent plant contamination in the future.

Etiology and epidemiology of Alternaria leaf blotch and fruit spot of apple in Australia

D. O. HARTEVELD (1), O. A. Akinsanmi (1), A. Drenth (1)
(1) The University of Queensland, Brisbane, QLD, Australia
Phytopathology 102:S4.51

Alternaria leaf blotch and fruit spot of apple caused by *Alternaria*, are important diseases in the Australian apple industry. Control measures are inadequate, there is little information on the epidemiology and the identity of the pathogen(s) is still unknown in Australia. The aims of this study were to determine the identity, diversity and distribution of the pathogen(s) and determine essential features of the disease cycle such as sources of inoculum, overwintering, seasonal dynamics, timing of infection and infection process of *Alternaria* spp. in Australian apple orchards. DNA sequencing using endo-polygalacturonase and *Alternaria* allergen a 1 gene of 51 Australian isolates from symptomatic leaves and fruit of the six states of Australia identified 5 *Alternaria* species; *A. alternata*, *A. arborescens*, *A. tenuissima*, *A. mali* and *A. longipes*. *A. arborescens* isolates were the most prevalent and occurred in all the six states of Australia, while *A. alternata* and *A. tenuissima* occurred mostly in the regions where the fruit spot disease occurs. The sources and dynamics of *Alternaria* inoculum, the timing of disease development and climatic influences were investigated. Leaf residue provides the biggest contribution to *Alternaria* inoculum within the orchard, however conidia were also obtained from twigs during the year, indicating that leaf residue and twigs serve as overwintering sources. Timing of disease expression in the field relates to periods of warm temperatures and high rainfall. Our findings increase our understanding of *Alternaria* diseases in Australia and will aid in the development of improved control options.

The appearance of an unknown viruslike disease of sunflowers in Nebraska

R. HARVESON (1), T. Gulya (2), A. Karasev (3), S. Lenardon (4)
(1) University of Nebraska, Scottsbluff, NE, U.S.A.; (2) USDA, Fargo, ND, U.S.A.; (3) University of Idaho, Moscow, ID, U.S.A.; (4) CIAP-INTA, Cordova, Argentina
Phytopathology 102:S4.51

During the 2010 and 2011 growing seasons, sunflower plants exhibiting symptoms characteristic of viral infection were observed from two commercial fields in Box Butte County (one each year) and an experimental research plot in Scotts Bluff County. Symptoms, consisting of stunting, leaf distortion, and mosaic/mottle-type leaf patterns were first observed each year in early to mid-July and field symptoms tended to fade substantially over time. Mechanical transmission of field infections was accomplished on multiple occasions to seedlings in the greenhouse in both years suggesting the involvement of a pathogenic agent and not an abiotic cause. Symptoms on inoculated seedlings appeared after 12-15 days, beginning as small chlorotic spots followed after several weeks by the formation of ring spots in some instances. In Sept. 2011, leaf symptoms on field-infected plants were characterized by bright yellow ringspots on upper leaves. Flexuous rod particles were observed from electron microscopy leaf dips from samples collected from the 2010 field, but greenhouse-inoculated plants from this field tested negative for *Sunflower mosaic virus* (SuMV) and *Sunflower chlorotic mottle virus* (SuCMoV) utilizing ELISA and RT-PCR methods. Samples transmitted from the 2011 field were also tested by serological methods for SuCMoV and were also negative. To date the identity of the pathogen(s) is unknown and confirmation of the causal agent is ongoing.

Pentaplex Q-PCR quantifies DNA from fungi causing anthracnose, brown stem rot, and charcoal rot in field samples of soybean

J. S. HAUDENSHIELD (1), C. R. Bowen (1), G. L. Hartman (1)
(1) USDA-ARS, Urbana, IL, U.S.A.
Phytopathology 102:S4.51

Several stem pathogens of soybean (*Glycine max*) cause yield losses. By the end of the season, it is common to see signs (acervuli and microsclerotia) or symptoms (cankers and vascular discoloration). Initial infection of these stem pathogens often occurs early in the season without symptom development. To detect and monitor the colonization of younger soybean plants by these pathogens in the field, novel probe-based quantitative PCR (Q-PCR) assays were developed for *Colletotrichum* spp. and *Macrophomina phaseolina*, and optimized for combination in multiplex with a published probe-based assay for *Phialophora gregata*, and with an exogenous control reaction, to enable simultaneous quantification of the DNAs from these pathogens. Each assay was demonstrated to retain its detection limit and reaction efficiency in the presence of the primers and probes of the other assays, and in the presence of a 1000-fold excess of the other pathogen DNAs. The control reaction identified false-negatives. The pentaplex Q-PCR assay was field validated using 50 mg subsamples of dried, milled stem tissues from plants at different developmental stages. The combined assay allows simultaneous quantification of three different pathogens, reducing reagent cost and increasing diagnostic throughput.

Serological tests of transgenic crops learning module

M. C. HAYSLETT (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.51

Transgenic modification of crops is relevant to any biology, plant science, or plant pathology class. GMO crops illustrate several important concepts in biology and agriculture including gene expression, pest management, and biological technology. Several commercial lateral flow serological test strips are available to test plant tissues for proteins commonly expressed by GMO crops, including a variety of *Bacillus thuringiensis* (Bt) toxins, glyphosate resistance proteins, and the selectable marker neomycin phosphotransferase II. These strip tests are inexpensive and easy to use, so students can run the tests themselves. Our non-majors biology class students use Agdia ImmunoStrips to test Bt transgenic and non-transgenic corn plants and a few different cornmeal brands to see if the Bt toxin is present. In parallel, they treat plants with Bt spores to kill insect pests. These activities demonstrate insect pest damage and organic pest control and reinforce the concept of gene expression by showing that the bacterial Bt gene is expressed as the Bt toxin in transgenic plants. The activity also develops integrative critical thinking skills, launching discussions of GMO crop use in food and conventional versus organic agriculture.

Infectivity and synergism for two monopartite begomoviruses and a bipartite begomovirus isolated from endemic *Merremia* species in Puerto Rico

Z. He (1), A. M. Idris (2), Y. Tang (1), J. K. BROWN (3)
(1) Plant Protection Research Institute, Guangzhou, Peoples Republic of China; (2) Plant Stress Genomic Research Center, Thuwal, Saudi Arabia; (3) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.51

Begomoviruses are recognized as emerging pathogens of agronomic and horticultural crops in tropical and subtropical habitats worldwide. DNA extracted from *Merremia* species, common weeds in Puerto Rico (PR), exhibited mosaic symptoms was used to clone two monopartite begomoviruses, *Merremia leaf curl virus* (MeLCV), *Sweet potato leaf virus* (SPLCV-PR) and one bipartite begomovirus, *Merremia mosaic virus* (MeMV). No satellite DNAs were detectable in symptomatic plants from which monopartite genomes were cloned, indicating that these viruses were monopartite, making them the first to be found in wild plant species in the Western Hemisphere. Infectious clones of the three begomoviruses were constructed and cloned into a binary vector. Clones were used to inoculate separately or in mixtures *Nicotiana benthamiana* and *Ipomea setosa* plants by agro-infiltration. Results indicated that symptom severity and accumulation of the monopartite, or the bipartite viruses in inoculated plants varied with the particular combination of viruses. Southern blot analysis indicated that MeMV noticeably promoted the accumulation of MeLCV and SPLCV-PR, while MeLCV and SPLCV-PR presence reduced MeMV accumulation when all three viruses were present. These results indicate that MeMV is synergistic with MeLCV and SPLCV whereas, MeLCV and SPLCV interfere with

MeMV accumulation. This study system offers a unique opportunity to study monopartite-bipartite begomovirus interactions.

Performance of prebloom leaf removal for the control of Botrytis bunch rot of grapes in Pennsylvania

B. HED (1), H. Ngugi (2)
(1) Lake Erie Regional Grape Research & Extension Center, North East, PA, U.S.A.; (2) Pennsylvania State University, Biglerville, PA, U.S.A.
Phytopathology 102:S4.52

The severity of bunch rot disease caused by *Botrytis cinerea* is strongly related to cluster compactness. For two seasons, we evaluated cluster zone leaf removal at trace bloom (TBLR) for effects on cluster compactness and Botrytis bunch rot development on *Vitis vinifera* 'Chardonnay' in seven vineyards in Pennsylvania. The vineyard trials compared a 'grower standard' bunch rot management program (GS) to GS plus TBLR. TBLR reduced cluster compactness, measured as the number of berries per cm, by 11 and 10 % in 2010 and 2011, respectively, when averaged across all vineyards. However, significant reductions ($P \leq 0.05$) were achieved in only one vineyard in 2010 and three vineyards in 2011. TBLR reduced bunch rot severity by 37 % in 2010 and 55 % in 2011, when compared to GS with no leaf removal, but the reductions were significant in just one vineyard in 2010 and two vineyards in 2011. When compared to GS with leaf removal after fruit set, TBLR reduced bunch rot severity by an average of 20 % in 2010 and 30 % in 2011, but the reductions were significant in just one vineyard in each year. Over both seasons, TBLR reduced cluster weights in all but one vineyard, with reductions averaging 13 and 11 % in 2010 and 2011, respectively. There were no significant effects of TBLR on return fruitfulness (clusters per shoot) after year one. TBLR may represent an important option for integration into bunch rot management programs.

Evaluating seed treatments for their ability to control Fusarium root rot in legumes

N. HEGDE (1), D. Baer (1), P. Asija (1), K. Shetty (2), J. B. Rasmussen (1), R. S. Goswami (3)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) Syngenta, Greensboro, NC, U.S.A.; (3) DuPont Crop Protection, Newark, DE, U.S.A.
Phytopathology 102:S4.52

In recent years the prevalence of Fusarium root rot has been on the rise in North Dakota and since complete resistance to this disease is not available in commercial cultivars of legumes such as dry beans or peas, integration of chemical control is essential for effective disease management. This study focuses on evaluating commercially available seed treatment products for their ability to control damage due to two major root rot causing *Fusarium* species, *Fusarium solani* and *Fusarium avenaceum*. The products being evaluated in this study include Fludioxonil, Azoxystrobin, Thiabendazole, Ipconazole, Pyraclostrobin, Sedaxane and Trifloxystrobin which are being assessed both individually and as part of combinations. *In-vitro* and growth chamber assessments have been conducted and field trails are currently in progress. Inhibition of fungal growth and seed colonization was assessed in the laboratory using a petri-plate based assay and reduction in root rot was evaluated in the growth chamber using a modified sand-cornmeal inoculum layer method. The effect of these seed treatment products on root growth was also evaluated using a root scanner and the WinRhizo software. Initial results from *in-vitro* and greenhouse studies which suggest significant differences in the ability of these chemicals to control *Fusarium* species will be presented.

Sensitivity of *Guignardia citricarpa* Florida isolates to copper

K. E. HENDRICKS (1), P. D. Roberts (2)
(1) University of Florida, SWFREC, Immokalee, FL, U.S.A.; (2) University of Florida, Immokalee, FL, U.S.A.
Phytopathology 102:S4.52

Guignardia citricarpa, the fungus that causes citrus black spot was recently introduced into Florida, USA. The development of this disease in the presence of multiple applications of copper per year to protect against and manage citrus diseases warrants an investigation into the effect of copper on Florida isolates of *G. citricarpa*. *G. citricarpa* and *G. mangiferae* isolates, confirmed by internal transcribed spacer (ITS) sequencing of ribosomal DNA and DNA homology, were used to inoculate media amended with 50 and 500 µg ml^{-1} copper. Relative colony growth (amended/non-amended) was assessed on days 7 and 21. Each isolate of *G. citricarpa* was tested in triplicate. Copper reduced the growth of *G. citricarpa* isolates but had no effect on *G. mangiferae* isolates. By days 7 and 21, 58% of *G. citricarpa* isolates on media amended with 50 µg ml^{-1} copper were 80% that of the controls. Significant reduction in growth was seen at 500 µg ml^{-1}, however reported copper residues following copper application in the field are typically 100 times less than the concentration of copper used in this study. These results were compared to field studies examining the efficacy of fungicide sprays on CBS. This finding supports the need to find an alternative to alternating copper, copper/strobilurin regime for suppression and management of citrus black spot in Florida's groves.

Sensitivity of *Didymella bryoniae* isolates obtained from Florida greenhouses watermelon seedlings to boscalid

K. E. HENDRICKS (1), P. D. Roberts (2)
(1) University of Florida, SWFREC, Immokalee, FL, U.S.A.; (2) University of Florida, Immokalee, FL, U.S.A.
Phytopathology 102:S4.52

Didymella bryoniae, the fungus that causes gummy stem blight on cucurbits has been found to be resistant to boscalid in the Carolinas and Georgia. A few Florida isolates have been tested in the past and were found to be to be sensitive to boscalid. Recent reports of gummy stem on watermelon seedlings in greenhouses following application a 2:1 mixture of the fungicides boscalid and pyraclostrobin (Pristine), prompted a closer look at *D. bryoniae* isolates and their susceptibility to boscalid. Isolates of *D. bryoniae* were recovered from gummy stem lesions on transplant watermelon seedlings and placed on media amended with 3 mg ml^{-1} boscalid (boscalid dissolved in acetone) and 1 ml acetone (control). Isolates were grown in the dark and relative colony growth (amended/non-amended) was assessed on day 4. Resistance was defined as isolates with a relative colony growth greater than 0.2. Two replicate plates per treatment (amended and control) were tested per isolate. The study was repeated once. A total of 49 isolates were obtained from three greenhouses. Both boscalid sensitive and resistant isolates were found within a single greenhouse and approximately 40% of isolates within each greenhouse was resistant to boscalid. The mixture of resistant and sensitive isolates may indicate an introduction into the greenhouse through contaminated seeds obtained from varying sources.

Effect of temperature on latent period of wheat stem rust (*Puccinia graminis* subsp. *graminis* f. sp. *tritici*) isolates across different wheat cultivars

J. HERNANDEZ NOPSA (1), W. F. Pfender (1)
(1) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 102:S4.52

Experiments were conducted to describe the effect of temperature on duration of latent period (LP_{50}, time to reach 50% pustules erumpent) of wheat stem rust (WSR). LP_{50} of 744, 271, 251, 136, 183, and 275 h were observed for cv. Stephens at 5, 15, 20, 26, 29, and 33.5°C, respectively. LP_{50} for cv. McNair were 787, 206, 124, and 253 h at 5,15, 26, and 33.5°C, respectively. Data from both cultivars fit a similar polynomial model. A factorial experiment using four races of WSR (GCCNC, GCCSC, QFCSC, and GFCDC), and four wheat cultivars (Stephens, McNair, Scout 66, and Kingbird) that differ in level of resistance, was conducted at 15°C and 33.5°C. Averaged across cultivars, LP_{50} of the races at 15°C ranged from 206 h (QFCSC) to 280 h (GCCNC). LP_{50} at 33.5°C ranged from 183 h (GCCNC) to 261 h (GFCDC). Averaged across races, McNair consistently had the shortest LP_{50} at 15°C and 33.5°C (221 and 194 h), whereas Stephens (266 h at 15°C) and Kingbird (262 h at 33.5°C) had the longest. Of the 16 race-by-cultivar combinations, at 15°C Kingbird/QFCSC had the shortest LP_{50} (194 h) and Stephens/GCCNC the longest (314 h). At 33.5°C McNair/GCCSC had the shortest LP_{50} (167 h) and Stephens/GFCDC the longest (275 h). Kingbird was infected only by GCCSC at 33.5°C, whereas it was infected by all four races at 15°C. Across the 16 combinations, infection type was not related to LP_{50} duration. Data obtained will be useful in creating an epidemiological model to predict and mitigate WSR.

Two new highly divergent spinach curtoviruses that arose from recombination

C. HERNANDEZ-ZEPEDA (1), A. Varsani (2), J. K. Brown (3)
(1) Water Sciences Unit, The Yucatan Center for Scientific Research, Cancún, Mexico; (2) School of Biological Sciences, The University of Canterbury, Christchurch, New Zealand; (3) The University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.52

Two highly divergent new curtovirus (family, *Geminiviridae*) species were isolated from total DNA extracts from symptomatic spinach plants collected in south-central Arizona in 2009. The first virus, designated *Spinach severe curly top virus*-[AZ] (SSCTV-[AZ]), shared its highest nucleotide (nt) sequence identity percentage with *Horseradish curly top virus* (HrCTV). The second curtovirus, designated *Spinach curly top Arizona virus* (SCTAzV), shared its highest nt identity with *Beet curly top Iran virus* (BCTIV). Sequence comparisons between viral open reading frames (ORFs) indicated that the V1 and V2 ORFs were highly similar between SSCTV and SCTAzV, suggesting an interspecific recombination event. Further investigation using RDP3 analysis revealed an 887 nt recombinant fragment in the AV1and AV2 genes within the SSCTV genome, having SCTAzV and HrCTV as its minor

and major parent, respectively. Both spinach curtoviruses shared a low nt identity with respect to the C1 and C2 ORFs. The most divergent SCTAzV ORF was AC1, which was most closely related to the AC1 gene for the genus, *Mastrevirus*. *Nicotiana benthamiana* seedlings were inoculated with SSCTV and SCTAzV agro-clones. Results indicated that SCTAzV caused severe curly top symptoms (and 100% of infection) and SSCTV caused mild symptoms (and only 20% of infection). Co-inoculation of SCTAzV and SSCTV-AZ indicated that based on percentage infectivity and virus accumulation SCTAzV was the most 'successful' virus.

Role of the ABC transporter ATR1 on resistance to the toxin cercosporin in the cercosporin-sensitive organisms *Neurospora crassa* and tobacco
S. HERRERO (1), J. W. Gillikin (1), H. Eng (1), M. E. Daub (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.53

The toxin and virulence factor cercosporin is produced by many *Cercospora* species. Toxin photoactivation leads to production of toxic oxygen species able to cause ion leakage and cell death. *Cercospora* species are highly resistant to cercosporin, and our work focuses on elucidating cellular defense mechanisms in *Cercospora* species for application to disease control. Previous work in our laboratory identified the ABC-transporter, ATR1, from *C. nicotianae*. ATR1 acts as a cercosporin efflux pump and plays a role in conferring protection to cercosporin. In this work, we evaluated the potential of ATR1 to impart resistance to cercosporin and *Cercospora* species in two heterologous systems. First, we expressed *ATR1* in the cercosporin-sensitive fungus *Neurospora crassa* and have recovered strains (20%) that are significantly more resistant to cercosporin than wild type. We have also transformed haploid tobacco plants with an intronless version of *ATR1*. Regenerated plants were selected for resistance to bialaphos, and close to 96% tested positive for *ATR1*. Expression studies in a subset of plants using quantitative RT-PCR revealed that *ATR1* is expressed at different levels. Electrolyte leakage assays identified a range of responses to cercosporin toxicity that did not correlate with gene expression. We are generating homozygous diploid plants of selected high expression and highly resistant transformants. Inoculation experiments will be conducted with *C. nicotianae*.

A new generation of bacterial biofungicides based on the bacterium *Bacillus amyloliquefaciens* (strain D747) from Certis USA for use in vegetable and fruit disease control
H. HIGHLAND (1), S. Ockey (2), M. Dimock (3)
(1) Certis USA, Nokomis, FL, U.S.A.; (2) Certis USA, Yakima, WA, U.S.A.; (3) Certis USA, Columbia, MD, U.S.A.
Phytopathology 102:S4.53

In 2010, Kumiai Inc., Japan, licensed to Certis USA a new generation of bacterial biofungicides based on *Bacillus amyloliquefaciens* strain D747 (Ba D747) for global development under the experimental number CX-9032. Kumiai scientists discovered and patented the Ba D747 strain, the active ingredient used in Kumiai's Ecoshot® product in Japan. Ba D747 is a broad-spectrum, high potency, preventative biofungicide/bactericide for control or suppression of fungal and bacterial plant diseases. Ba D747 kills pathogenic organisms on foliage and other plant parts by producing antibiotic compounds (iturins) which disrupt pathogen cell wall production. Ba D747 also colonizes plant root hairs, preventing establishment of disease-causing fungi and bacteria. The U.S. EPA approved registrations of several Ba D747-based products in December 2011. The first product was launched in the U.S. in April 2012 under the name of "Double Nickel 55™" for control of powdery mildew, *Botrytis* and bacterial diseases of fruiting and leafy vegetables, grapes, strawberries and tree fruit. Compared to other bacterial-based biofungicides, Double Nickel 55 is highly potent which allows growers to use lower and more economical application rates. Ba D747, when applied to the soil, colonizes plant root hairs, preventing establishment of disease-causing fungi and bacteria. Application for a European Union Annex-I registration was submitted in 2011, and an approved provisional national registration in Italy occurred in February, 2012. The product, named "Amylo-X®" was launched in Italy by Intrachem Bio Italia SpA. In New Zealand, Etec Crop Solutions is launching a Ba D747 product this season under the name of "Bacstar®".

Efficacy of a *Vicia villosa* green manure and *Streptomyces lydicus* for management of Fusarium wilt of watermelon in the greenhouse and in vitro
J. C. HIMMELSTEIN (1), K. Everts (1), Y. Balci (2)
(1) University of Maryland, Salisbury, MD, U.S.A.; (2) University of Maryland, College Park, MD, U.S.A.
Phytopathology 102:S4.53

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON) reduces yield in watermelons in the mid-Atlantic region of the U.S., where the pathogen is widespread and few disease management practices are available. The legume cover crop, *Vicia villosa*, significantly suppressed Fusarium wilt when incorporated into soil as a green manure in previous trials. The biocontrol product Actinovate, whose active ingredient is *Streptomyces lydicus*, was evaluated alone or in combination with green manure cover crops. The cover crops: *V. villosa*, *Trifolium incarnatum*, *Secale cereale* or no cover crop, were amended to field soil in amounts that represented the biomass present during field incorporation in the spring. *Vicia villosa* was also amended at 1.5x, 2x and 3x concentration. Amended soils were either inoculated with FON or not inoculated and Actinovate was applied or not applied. Vine length of watermelon and Fusarium wilt were evaluated at 6 intervals. Inhibition of FON by *S. lydicus* was also evaluated in an in vitro plate assay. FON inoculation decreased watermelon vine growth across all treatments except in one trial where watermelon grown following *V. villosa* 3x and *T. incarnatum* amendments had similar vine length whether inoculated or not. In no one trial did watermelon grown in *V. villosa* amended soils have significantly higher wilt than plants in all nonamended soil inoculation treatments. However, contradictory to other studies neither the *V. villosa* cover crop biomass nor an Actinovate biocontrol significantly reduced Fusarium wilt or improved watermelon vine growth compared to nonamended treatments. Actinovate's active ingredient *S. lydicus* inhibited FON growth rate in vitro. Mechanisms of suppression that occur in the field may be due to factors that were not present in greenhouse conditions.

Baseline sensitivity of *Guignardia citricarpa*, the causal agent of citrus black spot, to strobilurin fungicides
M. HINCAPIE (1), N. Peres (1), M. Dewdney (2)
(1) University of Florida, Wimauma, FL, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.53

Citrus Black Spot (CBS), caused by *Guignardia citricarpa* was first identified in Florida and the United States in March 2010. The economic impact of the disease is yield loss caused by premature fruit drop and external blemishes on the fruit, making them unsuitable for the fresh market. Fungicide applications are the main control measures used in other citrus production areas around the world. The purpose of this project was to evaluate strobilurins fungicides for in vitro activity and to determine the baseline sensitivity of *G. citricarpa* isolates. The effective concentration needed to reduce growth by 50% (EC_{50}) was determined for 50 isolates from the two Florida counties where CBS is found. Ten-fold serial dilutions from 0.001 µg/ml to 10 µg/ml of azoxystrobin and pyraclostrobin were mixed into ½ strength PDA. Mycelium plugs of each isolate were placed on the center of three plates per concentration and the colony diameter change was measured 14 days later. EC_{50} values for azoxystrobin ranged from 0.01 µg/ml to 0.12 µg/ml and from 0.001 µg/ml to 0.07 µg/ml for pyraclostrobin. The average EC_{50} for pyraclostrobin was 0.01 µg/ml, and for azoxystrobin was 0.04 µg/ml. Research on the inhibition of spore germination using both fungicides is still in progress. Results from this study will give the baseline sensitivity to strobilurins and establish discriminatory doses for future resistance monitoring of this newly introduced pathogen.

TaqMan qPCR detection of three berry fruit ilarviruses
T. HO (1), I. E. Tzanetakis (1)
(1) Department of Plant Pathology, Division of Agriculture, University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 102:S4.53

Knowledge of the population structure of three berry-infecting ilarviruses, namely *Blackberry chlorotic ringspot virus* (BCRV), *Strawberry necrotic shock virus* (SNSV) and *Tobacco streak virus* (TSV), has grown considerably in the past few years. Sequence data, obtained in our laboratory or GenBank, were used for the development of TaqMan® quantitative PCR (qPCR) assays with absolute quantification. These assays were developed on the premise that they can detect all isolates of the three viruses studied to date. Total nucleic acids were extracted from virus-infected materials, digested with DNase and used for reserve transcription with specific and random primers, followed by qPCR. Standard curves were constructed and verified consistent detection of as low as 30 copies of the respective viruses. The assays, reproducible for both Ct values and calculated copy numbers, have been successfully tested on many berry fruit isolates.

The usefulness of concurrent, alternating, and mixture use of two high-risk fungicides as resistance management strategy
P. H. HOBBELEN (1), N. D. Paveley (2), F. van den Bosch (1)
(1) Rothamsted Research, Harpenden, United Kingdom; (2) ADAS UK Ltd., High Mowthorpe, Duggleby, Malton, United Kingdom
Phytopathology 102:S4.53

We adjusted a successfully tested fungicide resistance model to describe the development of resistance in pathogen populations of cereal crops in two sets

of fields, which are connected through spore dispersal at the end of growing seasons. We evaluated the usefulness of concurrent, alternating and mixture use of two high-risk fungicides as resistance management strategy using a modelling approach. We determined the effect of fitness costs of resistance, partial resistance to fungicides and differences in the dose-response curves and decay rates between fungicides on the usefulness of each strategy for different frequencies of the double resistant strain at the start of a treatment strategy. We used *Mycosphaerella graminicola* on winter wheat as a model system for fungal pathogens on cereal crops in general. We used the QoI (quinine outside inhibitor) pyraclostrobin as model for a high-risk fungicide. As a criterion for the usefulness of a strategy, we used the maximum number of growing seasons that an average epidemic can be sufficiently controlled in both sets of fields by fungicide doses, which are high enough to control a severe epidemic. For all scenarios, the maximum effective life of the mixture strategy was (one of the) highest.

The effect of the dose rate of a fungicide on the emergence of resistance

P. HOBBELEN (1), N. D. Paveley (2), F. van den Bosch (1)
(1) Rothamsted Research, Harpenden, United Kingdom; (2) ADAS UK Ltd., High Mowthorpe, Duggleby, Malton, United Kingdom
Phytopathology 102:S4.54

The evolution of fungicide resistance can be divided into an emergence phase and a selection phase. In the emergence phase, the resistant strain arises through mutation and its spread in the pathogen population depends on stochastic processes. Fungicide applications will decrease the size of the pathogen population and therefore the rate at which resistant mutants arise, but mutants will experience less competition for healthy host tissue due to the smaller pathogen population. To the best of our knowledge, all published experimental and modelling work on fungicide resistance concerns the selection phase. This study aims to determine the effect of the dose rate of a high-risk fungicide on the emergence time of a resistant pathogen strain. We use *Mycosphaerella graminicola* on winter wheat as model for a fungal pathogen on a cereal crop. The simulation model describes the seasonal dynamics of the canopy and the seasonal dynamics of the sensitive and resistant pathogen population. We consider the resistant strain to have emerged, when its survival during 100 consecutive growing seasons in the absence of new mutations is 95% or higher. The emergence time is then defined as the number of consecutive growing seasons since the start of a treatment until the resistant strain emerges. The results suggest that the mean emergence time of a resistant strain initially decreases and then stabilises with increasing dose-rate of a high-risk fungicide.

Protect U.S.—Engaging researchers, county faculty, and K-12 teachers in invasive species education

A. C. HODGES (1), S. T. Ratcliffe (2), M. A. Draper (3), S. D. Stocks (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) University of Illinois, Urbana, IL, U.S.A.; (3) USDA NIFA, Washington, DC, U.S.A.
Phytopathology 102:S4.54

Protect U.S., the community invasive species network **(**www.protectingusnow.org) educates small farm producers, homeowners, the general public, and K-12 audiences about invasive species issues. The network is a collaborative partnership between the National Plant Diagnostic Network (NPDN), Regional Integrated Pest Management (IPM) Centers, United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), National Institute of Food and Agriculture (NIFA), the National Plant Board, the Department of Homeland Security (DHS), Land Grant University Extension, and other organizations involved in invasive species issues. Protect U.S. delivers their educational content online in three different formats: scripted presentations, e-learning modules, and K-12 lesson plans. The scripted presentations are for educator use (e.g. professors, county extension agents, crop consultants, and First Detector educators). The e-learning modules (which are based on the scripted presentations) are designed for use by small farm producers, master gardeners, homeowners, and the general public. K-12 lesson plans are based on the National Science Education Standards (particularly the Life Science standards) and feature a scripted presentation for use by the teacher along with several grade appropriate activities from which to choose for the students (e.g. an experiential assignment, a report project, and a computer lab activity). These multiple delivery options and material content allow Protect U.S. to provide invasive species educational options to many diverse audiences. Project outcomes, including the number of times presentations have been downloaded, number of learners completing available e-learning modules, web statistics, and opportunities for content authorship will be presented.

WITHDRAWN

WITHDRAWN

Xylem hydraulic conductance in southern highbush blueberry cultivars with different levels of field resistance to bacterial leaf scorch

R. M. HOLLAND (1), H. Scherm (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 102:S4.54

Xylella fastidiosa causes bacterial leaf scorch, a new disease of southern highbush blueberry in the southeastern U.S. The bacterium occludes the xylem of affected plants, causing drought-like symptoms and eventually plant death. Here, we investigate host xylem efficiency in response to natural infection by *X. fastidiosa* in three cultivars differing in field resistance to the disease. Using a high-pressure flow meter, hydraulic conductance (K_h) was measured on field-collected stem sections from 5 to 6-year-old plants of cultivars FL 86-19 (highly susceptible), Star (intermediate), and Emerald (field-resistant). On asymptomatic plants, K_h values were greater for Emerald than for Star or FL 86-19 for all tissue types tested (lignified stems, partially lignified stems, green shoots, and petioles), with differences being most pronounced for lignified and partially lignified stems. This shows overall greater potential for water flow in the xylem system of Emerald. In the

moderately susceptible Star, disease severity class (asymptomatic, symptomatic) had no effect on K_h, indicating that this cultivar maintained xylem function despite the presence of disease. In contrast, on the highly susceptible FL 86-19, K_h was reduced in petioles of symptomatic plants. This effect was also evident when expressed in terms of leaf-specific hydraulic conductance. Loss of xylem function in the petiole may help explain the greater propensity for plant death in FL 86-19 compared with Star.

Validation of water quality fluctuation patterns in runoff water containment basins of eastern and central Virginia

C. HONG (1)
(1) Virginia Polytechnic Institute & State University, Virginia Beach, VA, U.S.A.
Phytopathology 102:S4.55

Water quality such as pH, conductivity impacts pathogen survival in recycling irrigation systems. We previously reported that nine major water quality parameters fluctuate in four seasonal and two diurnal patterns in a runoff containment basin. The objective of this study was to validate these fluctuation patterns in two additional basins in eastern and central Virginia, respectively. Water depth (m), temperature (oC), conductivity (µS/cm), pH, dissolved oxygen (mg/L), oxidation-reduction potential (mV), turbidity (NTU), chlorophyll a (µg/L), blue-green algae (cells/ml) were recorded with a multiprobe Sonde (YSI, Inc) at each location every 15 minutes from April 1 to November 8, 2012. These data were transmitted real-time to an office at the Virginia Tech's Hampton Roads Agricultural Research and Extension Center in Virginia Beach through telemetry systems and Verizon Satellites. These data confirmed all four seasonal and two diurnal patterns. Additionally, they indicated that (i) blue-green algae as measured by the YSI probe accounted only for a small portion of the total chlorophyll a in water; (ii) temperature appeared another factor affecting water quality dynamics; and (iii) turbidity was also affected by algal blooming cycles. The practical implications of these new data are discussed.

Long-term control of Pierce's disease in various grape genotypes with a benign strain of *Xylella fastidiosa*

D. L. HOPKINS (1)
(1) University of Florida, Apopka, FL, U.S.A.
Phytopathology 102:S4.55

Pierce's disease of grapevine is especially damaging in Florida, where it is endemic and prevents the production of susceptible cultivars. A benign strain of *Xylella fastidiosa* (EB92-1) has controlled Pierce's disease in *V. vinifera* 'Cabernet Sauvignon' for 14 years in the vineyard. In 2 long-term field trials in Central Florida, strain EB92-1 was evaluated for biological control of Pierce's disease in several grape genotypes, including *V. vinifera* 'Cabernet Franc', 'Chardonnay', 'Chenin Blanc', and 'Ruby Cabernet', as well as *V. aestivalis* 'Cynthiana' and a French-American interspecific hybrid 'Chambourcin'. After 8 years in trial 1, incidences of Pierce's disease in the untreated 'Cabernet Franc', 'Chambourcin', and 'Cynthiana' were 83%, 50%, and 33%, respectively, compared with 27%, 18%, and 9% in the treated. After 7 years in trial 2, strain EB92-1 had provided excellent control of Pierce's disease in 'Chardonnay' and 'Ruby Cabernet'. EB92-1 had controlled Pierce's disease in 'Chenin Blanc' through 5 years, but many of these vines were lost to a fungal dieback disease in years 6 and 7. *X. fastidiosa* strain EB92-1 provided biological control of Pierce's disease in all grape genotypes tested in these trials. Biological control should allow the sustainable production of Pierce's disease resistant grape cultivars in Florida and other areas where the disease is endemic.

Host range of *Xylella fastidiosa* strains that cause blueberry leaf scorch

D. HOPKINS (1), P. Harmon (2), P. Brannen (3)
(1) University of Florida, Apopka, FL, U.S.A.; (2) University of Florida, Gainesville, FL, U.S.A.; (3) University of Georgia, Athens, GA, U.S.A.
Phytopathology 102:S4.55

Blueberry leaf scorch, caused by *Xylella fastidiosa*, is widespread in Florida and Georgia, predominantly in southern highbush cultivars. To evaluate possible sources of the blueberry strains of *X. fastidiosa*, host ranges of strains from other hosts of origin were compared with the blueberry strain. *X. fastidiosa* subsp. *fastidiosa* strains originally isolated from grapevine, elderberry and lupine, as well as subsp. *multiplex* strains from almond, blackberry, mulberry, oak, and sycamore were inoculated into blueberry cultivars Star and FL86-19 by pin-pricking 2 lower internodes through a drop of bacterial suspension. *X. fastidiosa* strains from elderberry, lupine, almond, and blackberry produced blueberry leaf scorch symptoms after inoculation. *X. fastidiosa* strains from blueberry produced leaf scorch symptoms in almond and colonized elderberry without symptom production. In addition to their host of origin, all these strains, except the one from blackberry, produced symptoms in almond. Strains that caused blueberry leaf scorch were primarily recombinant *X. fastidiosa* subsp. *multiplex* strains. Blueberry leaf scorch strains could have originated from phony peach or plum leaf scorch strains which occur in the southeastern U.S.

Real-time PCR quantification of live bacteria in citrus and noncitrus hosts of citrus huanglongbing

H. HU (1), R. Brlansky (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.55

Citrus huanglongbing (HLB) is one of the most devastating citrus diseases worldwide. It is associated with a phloem-restricted bacterium '*Candidatus* Liberibacter asiaticus' and primarily transmitted by Asian citrus psyllid in Florida. Due to the uncultured bacterial pathogen, HLB early diagnosis relies on DNA-based methods like polymerase chain reactions (PCR) including real-time quantitative PCR (qPCR). Although estimating live bacterial population (LBP) is critical for HLB research, PCR has limitations on differentiating live and dead cells, thus tends to overestimate LBP in hosts. Propidium monoazide (PMA), a novel DNA-binding dye, has already been successfully used on many bacterial plant pathogens to effectively remove DNA from dead cells, but no applications on uncultured bacteria were reported. In this study, PMA-qPCR protocols were first optimized to work with plant and psyllid materials, respectively. Then, they were used to monitor LBP dynamics inside HLB positive citrus and non-citrus hosts monthly through an 18-month period. Different LBP developing patterns were observed, which could indicate different living micro-environments inside different hosts for HLB bacteria. This rapid qPCR method provides an accurate way to estimate LBP in HLB hosts, which in turn should benefit various researches like disease epidemiology and serves as a crucial component in HLB management.

TAL effector PthA4-mediated virulence and host gene induction in citrus canker

Y. HU (1), J. Zhang (2), F. F. White (2), N. Wang (3), J. B. Jones (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) Kansas State University, Manhattan, KS, U.S.A.; (3) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.55

Citrus canker type A, caused by *Xanthomonas citri* subsp. *citri* (Xcc), is an important disease of citrus in Asia, Africa and the Americas. Dissecting the virulence mechanisms of Xcc will aid in designing effective disease management strategies. One of the most important type III effector groups in the bacterium involved in Xcc pathogenicity is the PthA/AvrBs3 family of proteins known as transcription activator like (TAL) effectors. TAL effectors specifically bind promoters of plant genes via recognition by a central domain of tandem repeats. In strain Xac306, one of the four TAL effectors (PthA4) was important for virulence as assessed by pustule symptoms and bacterial populations in leaves. Microarray analysis was conducted at 6 h, 24 h and 120 h after infiltration of wild type Xac306 and Xac306 Δ*pthA4* mutant into sweet orange Valencia (*Citrus sinensis*). Among the probe sets that were upmost up-regulated by Xac306 in comparison with the mutant were Cit.37210.1.S1_at, which codes a protein belonging to lateral organ boundaries (LOB) domain family, and Cit.3027.1.S1_s_at, which isa member of the nodulin MtN3 gene family. The promoters of both genes contain predicted PthA4 binding elements. Their induction was validated by RT-PCR and promoter analysis studies. Functional tests are in progress to determine whether one or more of the target genes are the direct targets of PthA4 and required for pustule formation and enhanced bacterial leaf populations.

The role of MoHyr1 and MoyAP1 in tolerating reactive oxygen species generated during the *Magnaporthe*-barley interaction

K. HUANG (1), K. J. Czymmek (2), J. L. Caplan (3), J. A. Sweigard (4), N. M. Donofrio (1)
(1) University of Delaware, Newark, DE, U.S.A.; (2) Delaware Biotechnology Institute/University of Delaware, Newark, DE, U.S.A.; (3) Delaware Biotechnology Institute, Newark, DE, U.S.A.; (4) DuPont, Newark, DE, U.S.A.
Phytopathology 102:S4.55

Reactive Oxygen Species (ROS) generated during plant-pathogen interactions are antimicrobial compounds that can either directly kill the pathogens or slow the infection process. ROS can activate defense responses to ameliorate the amount of disease, and affect the expression of genes in ROS-generation and detoxification pathways. We observe an increase in ROS levels during a short post-inoculation period in barley. ROS halos are formed directly beneath appressorium of *Magnaporthe oryzae*, accompanied by cell wall appositions. A successful pathogen will likely have ROS regulating mechanisms to tolerate such inhospitable situations. MoHyr1 and MoAp1 have been found to be

involved in regulating oxidative stress in *M. oryzae*. *MoHYR1* has a glutathione peroxidase domain and in *Saccharomyces cerevisiae*, its homolog specifically detoxifies phospholipid peroxides by forming an inter-molecular disulfide bond with yAP1. We generated fungal mutants lacking this gene and noted their decreased ability to tolerate ROS *in vitro* and *in planta*. Moreover, deletion of this gene caused a virulence defect in *M. oryzae*. We further discovered that MoHyr1p and MoyAp1p appear to not interact with each other based on co-localization and yeast two hybrid assays, which is contrary to findings in *S. cerevisiae*. We also discovered that over-expressing MoyAp1p partially rescues the *Mohyr1* mutant phenotype. These results, along with several ROS generation pattern studies, will be presented.

Development of a real-time polymerase chain reaction assay to detect and quantify *Fusarium oxysporum* f. sp. *lycopersici* in soil

C. HUANG (1)
(1) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.56

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is an important disease of tomato. Pathogenicity and vegetative compatibility tests, although reliable, are laborious for identification and cannot quantify inoculum densities. The object of this study was to develop a rapid, sensitive, and quantitative real-time polymerase chain reaction (qPCR) assay for detecting and quantifying FOL in soil. To approach the object, an inexpensive and simple method for soil DNA extraction and purification was developed using bead-beating and silica-based methods. Compared to a commercial kit using similar approaches above, our protocol performed better to remove soil inhibitors in Florida's soils. A TaqMan probe and primers were developed using the DNA sequence of the virulence gene *SIX1*, which is only carried by FOL but not found in the other *formae speciales* and nonpathogenic isolates of *F. oxysporum*. The qPCR assay has successfully quantified DNA content of FOL in field soils. In addition, correlation analysis showed a significant positive correlation between disease severity and DNA content of FOL in artificially inoculated soils. The qPCR assay may be used to determine population densities of FOL in soil and to develop future threshold models to predict the likely extent of the disease.

Influence of dew removal methods and plant growth regulators on fungicide efficacy for the control of dollar spot in turf

Y. HUANG (1), P. Landschoot (1), J. Kaminski (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.56

Dollar spot, caused by the pathogen *Sclerotinia homoeocarpa* F.T. Bennett, is a disease of all turfgrass species and is considered the most economical important disease on golf courses. Canopy moisture (e.g., leaf wetness) plays an important role in the development of dollar spot. It is suggested that displacement of dew in the morning can reduce symptoms by interrupting prolonged periods of leaf wetness required for disease development. In addition to the use of cultural practices, fungicides are often applied throughout the season to provide additional suppression. The effect of canopy moisture at the time of fungicide application, however, is not well understood. The objectives of this field study were to elucidate the influence of dew removal methods and plant growth regulators on the length of dollar spot control with fungicides. In 2011, a field study was initiated at the Joseph Valentine Turfgrass Research Center on a mature 'Penneagle' creeping bentgrass (*Agrostis stolonifera* L.) maintained as a golf course fairway. The study setup was a 2 x 3 x 4 factorial arranged as a randomized complete split-plot design with four replications. The main factors included three dew removal strategies (none, rolled and mowed) prior to the application of fungicides (none, chlorothalonil, propiconazole and iprodione) and plant growth regulators (none and trinexapac-ethyl). On 17 August, dew removal treatments were initiated at 0800 and immediately prior to the application of fungicides and PGRs. While dew removal treatments and fungicides were applied once, PGR treatments were applied every 14-days for a total of 5 applications. Once active, dollar spot severity was assessed three times per week by counting the number of infection centers (IC) per plot. Dollar spot IC and area under the disease progress curve data were analyzed using the PROC MIXED procedure in SAS. Trace levels of dollar spot were present when treatments were initiated on 17 Aug. Disease pressure continued to increase and by 23 Aug all fungicides provided a significantly fewer IC's than the untreated control. On all remaining rating dates, all fungicide-treated plots (regardless of treatment) had significantly less dollar spot when compared to the untreated control. On 5 of 14 rating dates, a fungicide x PGR interaction was observed. On these dates, plots treated with trinexapac-ethyl had equal or fewer dollar spot IC when compared across their respective fungicide treatment. In plots receiving no fungicides, trinexapac-ethyl reduced dollar spot IC on all five dates where the interaction was significant. Results from this study indicate that the presence or absence of dew at the time of fungicide application has little influence on the duration of fungicide efficacy. In situations where fungicide use is restricted, however, the application of trinexapac-ethyl may result in a reduction in dollar spot severity. Additional trials will be initiated in 2012 to determine potential seasonal variation in dollar spot suppression relates to dew removal.

Temperature adaptation of *Ralstonia solanacearum* strains correlates with disease incidence

A. I. HUERTA (1), A. Milling (1), C. Allen (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.56

Bacterial wilt, caused by members of the *Ralstonia solanacearum* species complex, is an economically important vascular disease affecting many crops. Strains of this heterogenous group of soilborne bacteria have adapted to diverse environments by unknown mechanisms. Human activity has disseminated *R. solanacearum* strains, increasing their global agricultural impact, but intermingling of strains has not been observed in the field. We hypothesized that tropical strains enjoy a competitive advantage over temperate ones in tropical environments and that temperate strains can outcompete tropical strains in temperate conditions. *R. solanacearum* strains GMI1000 (tropical), UW551 (temperate Race 3 biovar 2) and K60 (U.S. warm-temperate) are phylogenetically distant but can all wilt tomato. While GMI1000 does not survive in the cooler regions where UW551 originated and causes disease, UW551 and K60 can cause disease at both temperate (24°C day/19° night) and tropical (28°) temperatures in a controlled environment. In 1:1 competition assays on tomato plants, subtropical strain K60 was significantly more competitive than GMI1000 and UW551 in tomato rhizospheres and stems at 28°C. Tropical strain GMI1000 also outcompeted UW551 at 28°C. As expected, at 24°/19°C, temperate strain UW551 outcompeted both GMI1000 and K60 in the rhizosphere, but showed no competitive advantage over GMI1000 in stems, whereas K60 did. K60's high competitive ability *in planta* may result from its production of a diffusible factor that inhibited growth of GMI1000 and UW551 on agar plates. These results suggest that temperature adaptation could explain why R3bv2 strains rarely cause disease in tropical lowlands.

Overexpression of *ShCYP51B* and *ShatrD* in *Sclerotinia homoeocarpa* field isolates exhibiting practical field resistance to propiconazole

J. HULVEY (1), J. T. Popko (1), H. Sang (1), G. Jung (1)
(1) University of Massachusetts, Amherst, MA, U.S.A.
Phytopathology 102:S4.56

We sought to investigate mechanisms governing propiconazole sensitivity of *Sclerotinia homoeocarpa* field isolates collected during a two-year field efficacy study in New England. The *CYP51* gene in *S. homoeocarpa* (*ShCYP51B*) and ~1000 bp upstream were sequenced in a panel of isolates displaying a greater than fifty-fold range propiconazole sensitivity. One single nucleotide polymorphism (SNP) was found in the upstream region and was shared by two of four insensitive isolates. Further, analysis of *ShCYP51B* relative gene expression yielded no significant differences in constitutive gene expression, but following one-hour exposure to propiconazole, insensitive isolates displayed two-fold greater relative expression. To search for additional genes involved in propiconazole sensitivity, we mined RNA-seq transcriptome data and uncovered a gene homolog of *BcatrD* (*ShatrD*), an ABC transporter known to efflux DMI fungicides. This gene showed much higher overexpression in the insensitive isolates, but no SNPs were detected within the ~1000 bp region upstream of the gene. We further screened relative expression of *ShCYP51B* and *ShatrD* in isolates that exhibited practical field resistance to propiconazole from two sites, and showed population and genotype specific gene expression patterns for the two genes. Linear regression among 24 isolates revealed a stronger relationship between propiconazole sensitivity and relative gene expression of *ShatrD* than *ShCYP51B*. In summary, our results point to efflux as a major factor in reduced DMI sensitivity of *S. homoeocarpa* genotypes in New England, which may have implications for current and future emergence of multi-drug resistance phenotypes of this important turfgrass pathogen.

Molecular characterization of a natural intramolecular recombinant begomovirus with close relatives in southwestern Arabia

A. M. IDRIS (1), M. Al-Saleh (2), I. Al-Shahwan (2), J. K. Brown (3)
(1) KAUST, Thuwal, Saudi Arabia; (2) King Saud University, Riyadh, Saudi Arabia; (3) The University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.56

Tomato leaf curl Sudan virus (ToLCSDV) is a single stranded DNA begomovirus of tomato so far reported only from The Nile Basin. Infection by ToLCSDV results in downward leaf curl, plant yellowing and stunting, and

contributes significantly to yield reduction. These symptoms resemble those caused by *Tomato yellow leaf curl virus*, a begomovirus originating from the Middle East that has recently become widespread. Most recently, tomato plants exhibiting leaf curling and yellowing, and overall stunting were observed in Gezira, Sudan. Total DNA isolated from symptomatic leaves was subjected to rolling circle amplification. The apparent full-length genomic component was cloned and the sequence was determined in both directions. Comparison of the complete genome sequence of 2766 nucleotides (nt) revealed that it shared 89-96% nt identity with ToLCSDV strains previously reported in Yemen and Sudan, thereby confirming the presence of a new strain of ToLCSDV. To fulfill Koch's postulates, we have constructed a greater-than-genome-length ToLCSDV in a binary vector. The constructs were transformed into *Agrobacterium tumefaciens* and agro-inoculated to *Nicotiana benthamiana* plant. The results indicated that the cloned begomovirus genome was infectious based on symptom development 7-10 days post-inoculation that were like those observed in field-infected plants. Recombination analyses revealed that the new strain is a recombinant between two previously reported ToLCSDV strains.

Emerging plant pathogens in Russia

A. IGNATOV (1), V. Dubovoy (1), N. Zhemchuzhina (1), S. Abramova (1), A. Makarov (1)
(1) Russian Research Institute of Phytopathology, Moscow, Russia
Phytopathology 102:S4.57

Russia had a population of plant pathogenic microorganisms adapted to its unique climate, today it is threatened by many pathogens that were recently introduced with imported plants. Under circumstances of climate changes, the pathogens found favorable conditions for establishment in local eco-niches. Most of them are bacteria that are already affecting Russian agriculture. A number of plant pathogens increased their presence on crop plants across different regions of Russia, including bacteria *X. arboricola*, *X. gardneri*, *X. hortorum*, *Pseudomonas syringae*, *Dickeya dianthicola*, *D. solani*, *Clavibacter michiganensis* subsp. *michiganensis*, toxicogenic *Alternaria* spp. and *Fusarium* spp. We have sequenced a number of key genes for MLST analysis in the isolates of bacteria and fungi that have been recently recognized in Russia as emerging plant pathogens. Correlating their phylogeny based on MLST and geographic location of isolation, we are reconstructing their routes of further spread. Comparing allelic differences, we also studied their recent evolution in regard to their adaptation mechanisms in order to identify targets for their detection and diagnostics. This work was a part of genetic study of microbes stored at the State Collection of Plant Pathogenic Microorganisms in RRI of Phytopathology. This work was supported by ISTC projects #2685 and 3431.

Utility of grafting to manage Verticillium wilt of tomato and extension education of grafting in North Carolina

M. IOTT (1), J. G. Driver (1), F. J. Louws (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.57

In the state of North Carolina, soil-borne pathogens are a major problem in vegetable production. One of the most devastating pathogens for Western NC (WNC) tomato growers is Verticillium wilt (VW) caused by *Verticillium dahliae* race 2. Host resistance has been widely deployed to manage race 1, but host resistance to manage race 2 has not been discovered. Currently, fumigation is the only control method available to conventional growers, and there is no control method for organic growers. With the increasing regulations for fumigation and the growing number of organic growers, alternative or complementary management practices are sought. Using the tube grafting method, rootstocks were grafted to Mountain Fresh (scion) and achieved statistically similar yield and plant vigor compared to standard conventional management practices in WNC fields with a history of VW pressure. Tissue samples were collected from rootstocks and at incremental distances up the scion to assess levels of tolerance/resistance to *V. dahliae* race 2. Grafted research was complemented with an extension training program to enable growers and other professionals to graft tomatoes. Core clientele included conventional and organic growers through hands-on training in multiple workshops to increase the state-wide capacity to graft tomatoes and adoption of this technology. Field evaluations and assessments of pathogen colonization provide a basis to formulate recommendations to growers.

Flutriafol, a new fungicide for managing root rot of cotton, caused by *Phymatotrichopsis omnivora*, in Texas

T. Isakeit (1), R. R. Minzenmayer (2), G. D. Morgan (3), D. R. Drake (4), D. A. Mott (3), D. D. Fromme (5), W. L. Multer (6), M. P. Jungman (7), A. Abrameit (8), R. L. NICHOLS (9)
(1) Texas A&M University, College Station, TX, U.S.A.; (2) Texas AgriLife Extension Service, Ballinger, TX, U.S.A.; (3) Texas AgriLife Extension Service, College Station, TX, U.S.A.; (4) Texas AgriLife Extension Service, San Angelo, TX, U.S.A.; (5) Texas AgriLife Extension Service, Corpus Christi, TX, U.S.A.; (6) Texas AgriLife Extension Service, Garden City, TX, U.S.A.; (7) Texas AgriLife Extension Service, Hillsboro, TX, U.S.A.; (8) Texas AgriLife Extension Service, Thrall, TX, U.S.A.; (9) Cotton Incorporated, Cary, NC, U.S.A.
Phytopathology 102:S4.57

Phymatotrichopsis root rot (PRR, also known as Texas root rot), caused by the fungus *Phymatotrichopsis omnivora*, is a serious disease in many of the cotton production areas of Texas and other southwestern states, causing annual losses of $29 million dollars in Texas and limiting where cotton can be grown. In 2005, we started screening fungicides for control of PRR. In 2008, we found that flutriafol (Topguard, Cheminova, Inc.) was effective when applied via drip irrigation. Subsequently, we determined that the fungicide, when applied to the soil at planting, could control the disease later in the season (approximately at flowering), when the pathogen became active. Based on our data over two years, a section 18 exemption for Topguard use in Texas for PRR control was granted in February, 2012. As specified on the section 18 label, the fungicide is applied at 1-2 pints/A in a T-band spray in the planting furrow. Flutriafol at these rates shows good-to-excellent potential for management of PRR, but additional experiments are needed to optimize effectiveness.

Disruption of *Fvcp1*, a cyclophilin-encoding gene in *Fusarium virguliforme*

K. T. ISLAM (1), J. P. Bond (1), A. M. Fakhoury (1)
(1) Department of Plant, Soil and Agriculture Systems, Southern Illinois University, Carbondale, IL, U.S.A.
Phytopathology 102:S4.57

Fusarium virguliforme is a soil-borne pathogen that causes Sudden Death Syndrome (SDS), an important disease of soybean resulting in significant losses in yields every year. Despite the importance of SDS, little is known about the fungal genes involved in the development of the disease. From our sequencing-based transcript analysis of *F. virguliforme* genes showing high levels of expression *in planta*, we have identified *Fvcp1*, a *F. virguliforme* cyclophilin encoding gene, as a candidate for gene disruption. Cyclophilins are peptidyl-prolyl cis-trans isomerases that are conserved among eukaryotes and are the cellular target of the immunosuppressive drug cyclosporin A (CsA). In addition, cyclophilins act as virulence determinants in several phytopathogenic fungi. They have also been implicated in a wide variety of cellular processes, including the response to environmental stresses, cell cycle control, the regulation of calcium signaling, and the control of transcriptional repression. In this study, we targeted the *Fvcp1* gene and disrupted it in *F. virguliforme*. Characterization of the resulting *Fv*Δ*cp1* mutant revealed that this gene is involved in the development of SDS.

Inhibition of *Ophiognomonia clavigignenti-juglandacearum* in vitro by fungi associated with butternut, Japanese walnut, and hybrid butternut

J. JACOBS (1), K. Woeste (2), M. Ostry (3), C. Michler (2)
(1) Hardwood Tree Improvement and Regeneration Center, Purdue University, Department of Forestry and Natural Resources, West Lafayette, IN, U.S.A.; (2) USDA Forest Service, Hardwood Tree Improvement and Regeneration Center, Purdue University, Department Forestry and Natural Resources, West Lafayette, IN, U.S.A.; (3) USDA Forest Service, Northern Research Station, St. Paul, MN, U.S.A.
Phytopathology 102:S4.57

Ophiognomonia clavigignenti-juglandacearum (OCJ) has decimated butternut (*J. cinerea*) throughout its native range. Many *J. cinerea* trees have been found canker-free in areas where OCJ is present but these trees can be infected with artificial inoculation. Japanese walnut (*J. ailantifolia*) is considered resistant as is the hybrid with butternut (*J.* × *bixbyi*). In this study we document the culturable endophytic and epiphytic fungal community found in *J. cinerea*, *J.* × *bixbyi*, and *J. ailantifolia* stem tissue on two sites in the Midwest. In addition to assessing the mycobiota of these three species we conducted an in vitro challenge assay between selected isolates and OCJ. While *Phoma* spp. was the dominant fungal genus on the Rosemount, MN site it was not the dominant genus in the collection from West Lafayette, IN. Varied levels of antagonism occurred between study fungi and a single isolate of OCJ. Granulated hyphae with extensive inhibition of OCJ growth on 1% malt extract agar were observed when challenged with many isolates. Results indicate these three *Juglans* species harbor similar assemblages of fungi when growing on the same site. Comparison between the two sites indicates that the fungal community may be locally derived. Additionally, many of these fungi are able to inhibit growth of OCJ in vitro. If this inhibition is functional in-planta it may partially explain the heterogeneous distribution of cankers on individuals and within stands.

Long-term and area-wide influences of atoxigenic strain biocontrol technology for aflatoxin contamination

R. JAIME (1), M. Foley (2), L. Antilla (2), P. J. Cotty (3)
(1) University of Arizona, Tucson, AZ, U.S.A.; (2) Arizona Cotton Research and Protection Council, Phoenix, AZ, U.S.A.; (3) USDA-ARS, University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.58

Use of atoxigenic strains of *Aspergillus flavus* to manage aflatoxin contamination in crops is a proven commercially used technology in Arizona, where it has been used for over a decade. The ultimate goal of the biocontrol is to modify *A. flavus* population structure in order to reduce the aflatoxin-producing potential and thus crop aflatoxin content. Long-term and area-wide influences of applications improve both efficacy and value of applications. During 2009 to 2011 soil from fields with history of biocontrol treatment were analyzed for incidences of both the biocontrol AF36 and the highly toxigenic strain S. Incidences of the biocontrol in areas regularly treated were significantly higher than in areas where use had been discontinued. In areas with high frequencies of applications, even fields where the biocontrol was never applied had relatively higher incidences of AF36. One year after a single application the biocontrol composes ~70% of the *A. flavus* community in the soil. The proportion of the biocontrol declines with time, fitting an exponential decay model. Persistence of the biocontrol differed between areas, with a steeper decline in the Yuma Valley reaching the natural levels of the biocontrol (~3%) by the third year, compared to the Mohawk Valley, where over 15% persisted even after four years. The results suggest establishing area-wide aflatoxin management programs would be beneficial. However they require periodic re-application of the biocontrol.

Gene content or gene expression: Which determines the difference in the host specificity and virulence of strains of *Xanthomonas citri* subsp. *citri*?

N. JALAN (1), D. Kumar (2), N. Wang (3)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) Rutgers University, Piscataway, NJ, U.S.A.; (3) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.58

Xanthomonas citri subsp. *citri* (Xcc) causes citrus canker, which has significant impact on citrus production. The citrus canker pathogen is distinguished into different groups primarily by host range including A and A^w. The A type, or the Asiatic form is the most virulent and affects the widest range of hosts, including *Citrus* spp. and many closely related rutaceous plants whereas A^w strain has restricted host ranges including Key or Mexican lime (*Citrus aurantifolia*). The A^w strain can elicit a strong hypersensitive response (HR) on grapefruit. To understand the genetic determinants of the difference in the host specificity and virulence of strains of Xcc, comparative genomic and transcriptome analyses were conducted. The genome of $XccA^w$ 12879 was completely sequenced using 454-pyrosequening, Illumina (Solexa) sequencing and Opgen optical mapping. The finished genome (chromosome and two plasmids) of $XccA^w$ was annotated, curated and compared with already published XccA genome. Whole genome comparison disclosed several genome rearrangements and insertion/deletion regions indicating genome plasticity. An all against all BLASTp of the complete proteomes revealed unique genes in $XccA^w$ and A. Comparative genomic analysis showed various changes in genes encoding effectors, cell wall-degrading enzymes, and lipopolysaccharides. Furthermore, RNA-Seq was used for expression profiling of transcriptomes of $XccA^w$ and XccA subjected to different conditions. The transcriptome was evaluated using Illumina sequencing and quantitative analysis revealed differences in expression levels of various genes. The contribution of gene content and gene expression to difference in virulence and host specificity of different Xcc strains will be discussed in this study.

Evaluating the degree and rates of evolutionary change in *Pseudomonas syringae* pv. *tomato*, and their impacts on forensic investigations

M. M. JAMES (1), U. Melcher (1), J. Fletcher (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.58

Attributing an agroterrorism event or crime to a perpetrator may require a forensic investigation to trace a suspect pathogen to its source or to compare microbial signatures or fingerprints found at the scene to those found on associated evidentiary material. Methods commonly used to produce microbial fingerprints include multiple-locus variable number tandem repeat (VNTR) analysis (MLVA) and multilocus sequence typing (MLST). However, the use of these typing systems in forensics investigations involving plant pathogens may be problematic. Long lag periods between the time that a pathogen is introduced and the discovery of the ensuing disease may provide ample time for the pathogen to undergo evolutionary change within regions of the genome targeted by the assays; thus, making it difficult to match a field isolate with its original source. In this work, the occurrence and rates of mutation of VNTR loci and housekeeping genes within the genome of a model phytopathogen, the bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*), exposed to optimal or sub-optimal growth conditions, while being serially sub-cultured for 1 year, was assessed using MLVA and MLST typing systems. No mutations were detected in the tested VNTR and core genome regions by either assay. The results indicate that these targeted regions within the *Pst* genome are sufficiently stable for forensic use and that mutations do not impact MLVA and MLST typing results for this pathogen.

Interaction of *Nicotiana benthamiana* PSBO1 with AltMV TGB3 correlates with chloroplast vesiculation and veinal necrosis caused by TGB3 overexpression

C. Jang (1), J. Nam (1), M. Li (1), Y. Kim (1), S. Yu (1), H. Kim (1), J. Beom (2), J. Hammond (3), H. LIM (1)
(1) Chungnam National University, Daejeon, South Korea; (2) Chungcheongnam-do Agricultural Research and Extension Services, Chrysanthemum Experiment Station, Yesan, South Korea; (3) USDA-ARS FNPRU, Beltsville, MD, U.S.A.
Phytopathology 102:S4.58

We previously compared the Triple Gene Block 3 (TGB3) movement-associated protein of *Alternanthera mosaic virus* (AltMV) with that of PVX, demonstrating differential localization of AltMV TGB3 to the chloroplast, and the induction of veinal necrosis in *Nicotiana benthamiana* when AltMV TGB3 was over-expressed. Under dark conditions over-expression of TGB3 induced lethal chloroplast damage. *In vitro* Yeast-two-hybrid assay and Bimolecular Fluorescence Complementation (BiFC) *in vivo* showed that *Arabidopsis thaliana* photosynthetic oxygen-evolving protein PSBO1 interacts strongly with TGB3; *N. benthamiana* PSBO1 also interacts with TGB3 in BiFC. These results confirm the important role of TGB3 in cell-to-cell movement and virus:host plant interactions at the chloroplast. We therefore examined residues involved in TGB3 interactions with the photosystem II protein PSBO1, and with symptom development. Multiple N- and C-terminal TGB3 deletion mutants were examined for interaction with *N. benthamiana* PSBO1 by BiFC; N-terminal TGB3 deletion mutants had significantly weaker interactions with PSBO1 than wild-type or C-terminal deleted TGB3. To further determine the effects of TGB3:PSBO1 interactions on symptom expression, 12 natural AltMV TGB3 sequence variants are being examined using a TGB3 expression vector in *N. benthamiana*; the strength of PSBO1 interactions with these TGB3 variant is being assessed by BiFC, and PSBO1 binding affinity determined by GST pull-down assay.

Resident bacteria of plums and their potential for controlling brown rot after harvest

W. J. JANISIEWICZ (1), W. M. Jurick (2), I. Vico (2), K. A. Peter (2), J. S. Buyer (3)
(1) USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV, U.S.A.; (2) USDA-ARS, Food Quality Laboratory, Beltsville, MD, U.S.A.; (3) USDA- ARS, Sustainable Agricultural System Laboratory, Beltsville, MD, U.S.A.
Phytopathology 102:S4.58

Fruit microflora has been the richest source of antagonists against fruit decays and the active ingredient in all currently available commercial biocontrol products. A comprehensive evaluation of plum bacteria for biocontrol activity against *Monilinia fructicola*, causing brown rot of stone fruit, would allow us to determine their biocontrol potential. We characterized resident culturable bacterial microflora of plum fruit from early development until maturity. The most dominant genera were *Curtobacterium* (19.88%), *Pseudomonas* (15.06 %), *Microbacterium* (13.86%), and *Clavibacter* (12.65%). These genera occurred at all four isolation times and accounted for 61.45% of all isolates. *Microbacterium* and *Curtobacterium* dominated at the early stage of fruit development while *Pseudomonas* and *Clavibacter* were dominant at the end of the season. Less prevalent genera were *Enterobacter* (5.42%), *Chrysomonas* (4.82%), and *Pantoea* (4.22%). Most frequently isolated species were *Microbacterium lacticum*, *Clavibacter michiganensis*, *Curtobacterium flaccumfaciens*, *Enterobacter intermedius*, and *Chrysomonas luteola*. The seasonal succession of genera was observed in both MANOVA and frequency analysis. Primary and secondary screening of plum bacteria for control of brown rot on wounded fruit resulted in selection of several antagonists from which *Pantonea agglomerans*, *Chryseomonas luteola* and *Citrobacter freundii* were the most effective.

Effect of chlorine dioxide on disinfecting fungi in wheat seeds under various relative humidity conditions

Y. JEON (1), H. Lee (1), Y. Lee (1), S. Lee (1), S. Yu (2)
(1) National Academy of Agricultural Science, Suwon, South Korea; (2) Chungnam National University, Suwon, South Korea
Phytopathology 102:S4.59

We examined the effectiveness of chlorine dioxide (ClO_2) treatments on inactivating fungi in wheat (*Triticum aestivum* L. cv. Olgeurumil) seeds naturally infected with *Penicillium expansum*, *Aspergillus niger*, *Rhizopus oryzae* and *Fusarium* sp. *P. expansum* was the most abundant fungus with the infection rate of 83%. The germination rate of the dry seeds was 57% and seed moisture content (SMC) was 9.72%. Dry seeds were treated with stabilized ClO_2 which the concentration gradually increased to 23 ?/? after 24 hrs at 20?. The ClO_2-treatment was most effective to disinfecting *P. expansum* after 4hr, although *A. niger*, *R. oryzae* and *Fusarium* sp. were still detected. In treated seeds, the germination rate was little influenced up to 4 hrs, however, that of 20-hr and 24-hr treated seeds decreased to 33% and 29%, respectively. In order to investigate the influence of relative humidity (RH) on disinfection of *P. expansum*, the dry seeds were treated with ClO_2 for 10 hrs under various RH of 30~80±2%. After 12 days of incubation, the infection rate of *P. expansum* decreased dependently on RH: 47% under 30% RH, 44% under 50% RH, 1% under 70% RH and zero under 80% RH. As a result, ClO_2 was effective on disinfecting *P. expansum* in wheat seeds when treated for more than 4 hrs and upper 70% RH. These observations will be useful when developing an effective strategy to enhance the seed health prior to the long-term storage in seed bank.

WITHDRAWN

Natural variation and evolution of the avirulence genes in *Magnaporthe oryzae*

Y. JIA (1), Z. Zhang (2), J. Xing (3), Y. Wang (2), J. Correll (4), R. Cartwright (4)
(1) USDA-ARS DBNRRC, Stuttgart, AR, U.S.A.; (2) Zhejiang Academy of Agricultural Sciences, Hang Zhou, Peoples Republic of China; (3) National Hybrid Rice Research and Development Center, Changsha, Peoples Republic of China; (4) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 102:S4.59

The avirulence genes in *Magnaporthe oryzae* are important determinants for the corresponding resistance genes in rice. In the present study, we analyzed DNA sequence variation of the five avirulence genes, *AVR-Pita1*, *AVR-Pik*, *AVR-Piz(t)*, *AVR-Pia* and *AVR-Pii* in field blast isolates in order to understand the effectiveness of the resistance genes, *Pi-ta*, *Pi-k*, *Pi-z*, *Pi-a* and *Pi-i*. Genomic DNA of 350 blast isolates collected from the southern US from 1970-2009 were used for PCR amplification to examine the existence of *AVR-Pita1*, *AVR-Pik*, *AVR-Piz(t)* and *AVR-Pia* using gene specific PCR markers. Results of PCR products revealed that 230 isolates of *M. oryzae* carry *AVR-Pita1*, 200 isolates carry *AVR-Piz(t)*, 40 isolates carry *AVR-Pik*, 10 isolates carry *AVR-Pia*, and 1 isolates carries *AVR-Pii*. PCR products were sequenced, and DNA sequence variation was analyzed by $DNA_{sp\ 4.5}$ and $T_{CS1.21}$. It was found that *AVR-Pita1* was highly unstable, and a total of 40 AVR-Pita1 variants were identified in avirulent isolates whereas another 4 *AVR* genes were relatively stable with few minor sequence changes. Point mutation, insertion, deletion of nucleotides, and transposon insertions resulting in altered AVR proteins were found in virulent isolates. These findings suggest that *M. oryzae* utilizes a sophisticated and multifaceted mechanism to "defeat" host resistance genes. The impact of these findings for breeding for improved blast resistance using major resistance genes will be presented.

Magnetic capture hybridization and real-time PCR detection of *Agrobacterium vitis* in grapevines

K. L. JOHNSON (1), S. Kaewan (1), D. Zheng (1), T. Burr (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.59

Agrobacterium vitis, the causal agent of crown gall of grapes, can have severe economic effects when introduced to vineyards. To prevent introduction of *A. vitis* via grapevine cuttings, clean planting material is required. Currently there are no standard tests to identify *A. vitis* in grapevines, and the methods used can be time consuming and inconclusive. We have developed a magnetic capture hybridization (MCH) real-time PCR protocol for the specific detection of tumorigenic *A. vitis* on grapevines. Real-time PCR primers specific for tumorigenic strains of *A. vitis* were developed using the *virD2* gene sequence. While real-time PCR is ideal for rapid detection, it can be inhibited by compounds present in plant tissue. We therefore incorporated the use of magnetic capture hybridization to enrich target nucleic acid and allow the removal of PCR inhibitors. The MCH real-time PCR assay was 1000 fold more sensitive than direct real-time PCR, with a detection threshold of 10^1 CFU/mL compared to 10^4 CFU/mL. The assay was able to detect *A. vitis* in grapevine cuttings taken from infected plants and did not detect bacteria on cuttings taken from clean tissue, indicating the viability of this method as an assay for pathogen detection on grapevine.

Early root infection and damage in citrus huanglongbing disease development

E. JOHNSON (1), D. B. Bright (1), J. H. Graham (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.59

Huanglongbing (HLB) is a systemic disease of citrus caused by the phloem-inhabiting bacterium '*Candidatus* Liberibacter asiaticus' (Las). Disease in grove trees is initially identified by foliar symptoms, most commonly blotchy mottle. Detection of Las in leaf tissue by qPCR early in disease development is usually limited to symptomatic leaves and proximal young leaves. Over multiple years, disease symptoms spread to the rest of the canopy. Although Las has been detected in root tissue, the decline of roots has been assumed to happen later in disease development when photosynthate production and transport have been significantly diminished in the tree canopy. The negative exponential relationship observed between disease severity and yield decline show larger than expected yield loss when symptoms are limited to one or a few branches. Observations of initial spread of Las from the bud-inoculation site in the trunk of 1-yr-old potted trees have revealed that Las is frequently detectable in roots months before detection of Las in leaves and foliar symptom development. Recently identified HLB infected grove trees have from 32-46% reduced root density compared to apparently healthy trees. These results suggest that early infection of roots by Las leads to root decline before the appearance of foliar symptoms and is likely the cause of larger than expected yield reduction on trees with limited foliar symptoms.

Steam disinfection of *Phytophthora ramorum* from research field soils at the National Ornamentals Research Site at Dominican University of California

S. A. Johnson-Brousseau (1), K. L. KOSTA (2), G. Copeland (1), M. Henkes (1), R. Bulluck (3), S. Ghosh (1)
(1) Dominican University of California, San Rafael, CA, U.S.A.; (2) California Department of Food and Agriculture, Sacramento, CA, U.S.A.; (3) USDA APHIS PPQ CPHST, Raleigh, NC, U.S.A.
Phytopathology 102:S4.59

Steam is a proven method of sterilization and has been used to disinfect pathogenic organisms. At the National Ornamentals Research Site at Dominican University of California, steam is employed to disinfect research plants and field soil infested with *Phytophthora ramorum*. To assess effectiveness of different temperatures on *P. ramorum* mortality, we carried out steam experiments in eight areas previously used for *P. ramorum*-infested soils research. Steam was delivered using the Thomas method. Onset temperature probes connected to weatherproof data loggers were used to generate temperature gradient profiles pre-steam, during steam delivery, and in post-steam cooling and to monitor heat transfer across soil depths of 5-45 cm. Three field areas were chosen to bury, at variable soil depths, teabag sachets containing soil mixed with viable *P. ramorum* sand inoculum. All pre-

steam and post-steam soil samples were assessed for *P. ramorum* by dilution plating and leaf bait assays. Post-steam soils were held for 30 days at 4°C, and diagnostic assays were repeated. Whereas pre-steam sachets had an average of 29 cfu/cm^3 of *P. ramorum*, all 28 sachets post-steam were negative for *P. ramorum*. Baiting results similarly demonstrated that soil was *P. ramorum* positive pre-steam and negative post-steam. The steam experiments confirmed that target temperatures of 50°C and higher for 30 minutes were effective at eradicating *P. ramorum* in high clay content field soils.

Steam disinfection of *Phytophthora ramorum* from field soil in a U.S. federally quarantined nursery

S. A. Johnson-Brousseau (1), K. L. KOSTA (2), J. Hernandez (3), S. Rooney-Latham (2), R. Arias (4), T. Pelican (5), T. L. Widmer (6), R. Bulluck (7), S. Ghosh (1)
(1) Dominican University of California, San Rafael, CA, U.S.A.; (2) California Department of Food and Agriculture, Sacramento, CA, U.S.A.; (3) Morris Wholesale Nursery, Riverbank, CA, U.S.A.; (4) California Department of Food and Agriculture, Riverside, CA, U.S.A.; (5) Stanislaus County Agricultural Commissioner's Office, Modesto, CA, U.S.A.; (6) USDA-ARS FDWSRU, Fort Detrick, CA, U.S.A.; (7) USDA APHIS PPQ CPHST, Raleigh, NC, U.S.A.
Phytopathology 102:S4.60

Effective disinfection of field soil infested with *Phytophthora ramorum* was demonstrated at the National Ornamentals Research Site at Dominican University of California (NORS-DUC). As a benefit to the California nursery industry, steam treatment was applied to a nursery with *P. ramorum*-infested field soil under quarantine. Steaming was performed using methods developed at NORS-DUC, targeting 50°C for 30 minutes down to 30 cm in a sandy loam soil-type. County regulatory officials collected soil samples post-treatment and samples were processed at the California Department of Food and Agriculture (CDFA) Plant Pest Diagnostics Center using approved APHIS guidelines for *P. ramorum* regulatory samples. In parallel, independent samples pre- and post-steam were collected across the quarantined area and processed at the NORS-DUC laboratory. All post-steam samples processed at NORS were *P. ramorum* negative and in agreement with CDFA results that confirmed the regulatory samples were *P. ramorum* negative. Other *Phytophthora* species previously present in the nursery beds were also eliminated. To restore beneficial soil microorganisms, a soil amendment was applied after post-steam sampling. Soil remediation of *P. ramorum*-infested soil is a top nursery industry priority. The current findings support steam as a viable mitigation option in nursery field settings for this pathogen of quarantine significance and provides a solution to soil treatment in environmentally-sensitive areas where current fumigation methods are prohibited.

Oomycete and bacterial pathogens in New York surface irrigation water: Survey results and ultraviolet treatment

L. A. JONES (1), R. W. Worobo (1), C. D. Smart (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.60

Approximately 60% of the water used to irrigate crops in the United States is obtained from surface waters, which can be contaminated with human and plant pathogens. Currently, there are no suitable water treatment options available for growers to treat contaminated water primarily due to the varying pH and turbidity of most surface water. A survey of pathogens in surface water reservoirs was conducted throughout New York during the growing seasons of 2010 and 2011 to determine the presence of pertinent bacterial and oomycete pathogens. Monthly water samples were collected from each site. In addition to water samples, a pathogen bait trap was used to survey for oomycete plant pathogens. Potential pathogens were cultured on semi-selective media from filtered water samples or baits and identified by DNA sequence analysis. Generic *Escherichia coli* and *Salmonella* spp. were found frequently with variable cfu/ml, *Cryptosporidium parvum* was found in one pond, potential *Pseudomonas* and *Xanthomonas* pathogens have been cultured and are awaiting further characterization, *Clavibacter michiganensis* subsp. *michiganensis* was found in a creek, at least 10 species of *Phytophthora* and 15 species of *Pythium* have been identified from survey samples. Testing has begun with an ultraviolet treatment system to determine if this technology can be used to treat surface quality water infested with pathogens, preliminary experiments have shown promising results.

Statewide survey of grapevine leafroll-associated viruses and management of its vector, grape mealybug, in Virginia

T. J. JONES (1), N. A. Rayapati (2), M. Nita (1)
(1) Virginia Tech, AHS AREC, Winchester, VA, U.S.A.; (2) Washington State University, IAREC, Prosser, WA, U.S.A.
Phytopathology 102:S4.60

Presence of *Grapevine leafroll-associated virus-2* (GLRaV-2), GLRaV-3, and *Grapevine fleck virus* (GfkV) was surveyed in VA during the 2009-11 seasons. These viruses can cause significant crop loss and affect wine quality by reducing sugar accumulation and compromising skin color. Petiole samples were collected from random vines from various commercial vineyards. Using a one-tube, one-step RT-PCR and PCR method, we tested over 1,300 vine samples (39 different wine grape varieties) from 136 vineyards. Testing results showed 7.3%, 24.6%, and 0.5% of sampled vines were positive for GLRaV-2, GLRaV-3, and GfkV, respectively. Moreover, 61% of sampled vineyards had at least one positive sample. The high incidence of infected vines warranted investigation into management of mealybugs, the vectors of GLRaV-3. Two field experiments were conducted at different locations: a contact insecticide (pyrethroid) treatment was tested at one experimental site, and two systemic insecticides (neonicotinoid) treatments were tested at the other. A significant increase in mealybug populations (P<0.05) was observed at the location using the contact insecticide while a significant decrease in vector populations over time (P<0.05) was observed at the location using the systemic materials. With the sudden awareness of viral diseases in Virginia, our review of management strategies will help developing necessary tools to prevent further spread of disease and establishment of vectors.

Management of cherry leaf spot disease in flowering cherry in mid-Tennessee

J. O. JOSHUA (1)
(1) Tennessee State University, College of Agriculture, McMinnville, TN, U.S.A.
Phytopathology 102:S4.60

Cherry Leaf spot (CLS) caused by *Blumeriella jaapii* affects flowering cherry and most *Prunus* species. This study was initiated in response to grower's complaints on ineffective fungicide applications in Tennessee nursery fields. Winter survival of the pathogen and the timing of infection establishment in relation to mid-Tennessee weather were evaluated. Survival of *B. jaapii* in previously infested leaf debris and on dormant buds were evaluated as the source of primary inoculum. Winter survival on previously infected dormant trees of six flowering cherry cultivars was evaluated in a controlled environment protected from airborne spore. Results showed that clearly that both leaf debris and dormant buds from previous infection were important sources of initial infection in mid-Tennessee. Ascospores that were morphologically similar to those of *B. jaapii* were trapped on sticky slides placed in a field of cherry trees starting March through June with a peak in May. Both ascospores and conidiospores were trapped before infection was observed in the field suggesting that initial infection started earlier than disease symptoms were observed. All plants in controlled environment developed typical leaf spot symptoms with leaf yellowing shot holes. These results indicated that primary inoculum was available when first leaves emerged. Thus, in order to improve disease management, fungicide applications should be initiated early during petal drop so as to protect new leaves and plant propagation should be strictly from disease free plants.

Status of streptomycin-resistant *Erwinia amylovora* in Illinois apple orchards

A. G. JURGENS (1), M. Babadoost (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.60

Fire blight, caused by the bacterium *Erwinia amylovora*, is a serious threat to apple production in Illinois and worldwide. Streptomycin has been the most effective antibiotic for control of fire blight, but streptomycin-resistant isolates have been reported in many regions, including the Midwest. Following wide-spread occurrence of fire blight in Illinois during 2008-2009, state-wide surveys were conducted to determine if streptomycin-resistance is present. In 2010, infected shoots from 24 orchards and in 2011, blossoms and infected shoots from 35 orchards were collected. Samples were ground in 0.5x PBS buffer, serially diluted, and plated onto Luria-Bertani (LB) medium amended with 50 mg/L cycloheximide. *E. amylovora* identity was confirmed with specific primers and pathogenicity was determined by inoculating immature pear fruit. Isolates were evaluated on LB medium amended with 50 mg/L streptomycin (Agrimycin 17). Controls were streptomycin-resistant and -susceptible *E. amylovora* isolates. None of the 246 Illinois isolates was resistant to streptomycin. Ten isolates were grown in LB broth containing 0, 0.5, 1, 2, 3, 4, and 5 mg/L streptomycin for 18 h. Bacterial cell density was measured with a spectrophotometer at OD 600 and compared to un-amended cultures. At 1 mg/L, cell densities of all ten isolates were significantly lower than the resistant control. Streptomycin-resistant *E. amylovora* has not yet been detected in Illinois.

Evaluation of *Aegilops tauschii* as a source of adult plant resistance to leaf rust of wheat

B. KALIA (1), D. L. Wilson (1), R. L. Bowden (2), B. S. Gill (1)
(1) Kansas State University, Manhattan, KS, U.S.A.; (2) USDA-ARS, Manhattan, KS, U.S.A.
Phytopathology 102:S4.61

Leaf rust caused by *Puccinia triticina* f. sp. *tritici* is an important foliar disease of wheat. Several race-specific resistance genes expressed at seedling stage have been identified and deployed in wheat breeding programs to control leaf rust but they often are defeated by new virulent races. Another effective approach is to use resistance genes which confer partial, race non-specific resistance expressed at adult plant stage, also called adult plant resistance (APR). *Aegilops tauschii*, the D genome donor of hexaploid wheat, has been a rich source of seedling resistance genes to leaf rust, but has not been explored for APR. To identify potentially new APR genes, 286 accessions from WGGRC, susceptible at seedling stage, were evaluated with leaf rust composite culture LR-COMP at adult plant stage under field conditions for four years. 50 accessions with low to moderate levels of disease severity were tested at seedling stage in the greenhouse with four races of leaf rust and 24 were susceptible to all races. Most resistant accessions came from Afghanistan (42%) and Iran (21%) and a few each from Turkey (13%), Georgia (8%), Armenia (4%), Azerbaijan (4%), Uzbekistan (4%) and Southern Russia (4%). 11 accessions showed disease severity ranging from 5 to 35% and came from countries of Iran (5), Afghanistan (4), Georgia (1) and Turkey (1). Newly identified APR genes are an important source for breeding wheat for durable resistance. Transfer of APR from *Ae. tauschii* to wheat has been initiated for 5 of the accessions.

Effect of inoculation method, inoculum concentration, and plant growth stage on development of wheat bacterial leaf streak

Y. R. KANDEL (1), K. D. Glover (1), L. E. Osborne (2)
(1) South Dakota State University, Brookings, SD, U.S.A.; (2) Pioneer Hi-Bred International, Brookings, SD, U.S.A.
Phytopathology 102:S4.61

Bacterial leaf streak (*Xanthomonas campestris* pv. *translucens*) has recently emerged as a serious disease of wheat in the mid-western U.S. Information is scarce regarding factors that influence disease development. This study was conducted under controlled environmental conditions to determine the effect of inoculation method, bacterial concentration, and plant age on disease development in moderately resistant and susceptible genotypes. Five inoculation methods were examined. Six inoculum concentrations were tested on plants from 12 to 49-days old. After inoculation, plants were immediately placed beneath a misting system to facilitate infection. Disease severity was assessed as percentage leaf area diseased at 14 days after inoculation. Results showed that inoculation method, inoculum concentration, and plant age had significant effects on disease development on both resistant and susceptible genotypes. All plants inoculated via leaf-infiltration and spray-inoculation methods showed distinct symptoms. Plants showed susceptibility to the pathogen at all growth stages; however, symptoms on 12-day old seedlings were most severe. No symptoms were observed with the lowest inoculum concentration (3×10^4 cfu/ml). Disease severity increased significantly with an increase in inoculum concentration from 3×10^5 to 3×10^9 cfu/ml. Findings of the study can be applied to further research leading to the development of routine germplasm screening techniques.

Susceptibility of red potato cultivars (*Solanum tuberosum* L.) to *Meloidogyne incognita*, *M. javanica*, and *M. konaensis*

B. Kandouh (1), B. SIPES (1)
(1) University of Hawaii at Manoa, Honolulu, HI, U.S.A.
Phytopathology 102:S4.61

Susceptibility of the red skinned potato cultivars Desiree, Mountain Rose, Pink Pearl and Red Thumb to the root-knot nematodes *Meloidogyne incognita*, *M. javanica* and *M. konaensis* was evaluated. Each cultivar was challenged with 10,000 eggs of each *Meloidogyne* spp. under greenhouse conditions 10 days after planting. Each cultivar nematode combination was replicated four times. Plants were harvested 60 days after inoculation. Tuber weight (TW) was recorded and nematodes were extracted from the tubers and roots. A reproductive factor (Rf) was calculated. *M. incognita* reduced TW in all cultivars except Mountain Rose ($P<0.01$). TW increased with *M. incognita* infection in Mountain Rose compared to the uninoculated control. However, *M. javanica* reduced TW of Mountain Rose. Both *M. javanica* and *M. konaensis* increased TW in Pink Pearl compared to the uninoculated control and *M. incognita*. Pink Pearl was the best host, supporting the highest Rf across all *Meloidogyne* spp. *M. incognita* had the highest Rf among the nematodes in all cultivars but Mountain Rose. *M. konaensis* had the lowest Rf among the 3 nematode species across all cultivars. Mountain Rose was a particularly good host for *M. javanica*. Desiree, Pink Pearl and Red Thumb were susceptible-intolerant cultivars to *M. incognita*. Pink Pearl was a susceptible-tolerant cultivar to *M. javanica* and *M. konaensis*. Mountain Rose was a susceptible-intolerant cultivar to *M. javanica* but a resistant-tolerant cultivar to *M. incognita* and *M. konaensis*. This study provides important information on red skinned potatoes for breeders to develop new cultivars and for growers in choosing cultivars for fields infested with *Meloidogyne*.

WITHDRAWN

WITHDRAWN

Survey of viruses present in wine grapes in Idaho

E. KANUYA (1), L. A. Clayton (2), R. A. Naidu (3), A. V. Karasev (1)
(1) University of Idaho, Moscow, ID, U.S.A.; (2) University of Idaho, Lewiston, ID, U.S.A.; (3) Washington State University, Prosser, WA, U.S.A.
Phytopathology 102:S4.61

Idaho has a growing viticulture industry, with nearly 1,600 acres of wine grapes in the state. Until recently, very limited information was available on the prevalence of wine grape viruses in the state. In 2009-2011, a survey of virus presence in Idaho vineyards was conducted, spanning both Snake and Clearwater River valleys. Samples were collected at the end of the growing season, from late September to early November, when leafroll symptoms were expressed in red cultivars. Both symptomatic and/or asymptomatic leaf samples were collected from Cabernet Sauvignon, Cabernet Franc, Merlot, Syrah, Lemberger, Barbera, Pinot Noir, and Petit Verdot cultivars, while only random, asymptomatic leaf samples were collected from white cultivars Chardonnay, Riesling, Pinot Gris, Sauvignon Blanc, and Semillon. Total nucleic acids were extracted from leaf petioles collected, and subjected to RT-PCR tests with specific primers. All RT-PCR bands from initially identified positive samples were cloned into a plasmid vector and sequenced. *Grapevine leafroll-associated viruses* (GLRaV) *-1*, *-3*, *-4*, and *-5* from the leafroll virus

complex, and *Rupestris stem pitting associated virus* (RSPaV) and *Grapevine viruses A* and *B* (GVA and GVB) from the rugose wood virus complex were found in Idaho vineyards. Prevalence and distribution of viruses differed between individual vineyards and specific cultivars, suggesting significant role played by growers in choosing nursery material, and methods to control vector populations.

Antigenic structure of *Potato virus Y*

A. KARASEV (1), O. V. Nikolaeva (1), D. J. Roop (1), S. Galvino-Costa (2), A. Figueira (2), S. M. Gray (3)
(1) University of Idaho, Moscow, ID, U.S.A.; (2) Federal University of Lavras, Lavras, Brazil; (3) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.62

Potato virus Y (PVY) is an important viral pathogen of potato responsible for reducing tuber yield and quality across the globe. The PVY^{N} and PVY^{NTN} strains, the latter of which induces potato tuber necrotic ringspot disease, are regulated for international potato trade, and have been routinely detected using monoclonal antibodies (MAbs) that discriminate between PVY^{N} and PVY^{O} serotypes. Distinct binding sites were characterized in the capsid protein of PVY for the four main PVY^{N}-specific MAbs, 1F5, Bioreba-N, SASA-N, and Neogen-N, available commercially. These binding domains were mapped through a combination of TAS-ELISA testing of MAbs on multiple reference isolates of PVY, sequence analysis, heterologous expression of capsid protein fragments, and synthetic peptide binding experiments. Three MAbs were found to have linear, continuous epitopes mapped within the first N-terminal 30 amino acids of the capsid protein, and one to have a conformational epitope mapped near residue 98. The data obtained suggested that testing with more than one PVY^{N} serotype-specific MAb could assure a reliable serological identification of a PVY^{N} or PVY^{NTN} isolate.

Naturally occurring avirulent strains of *Burkholderia glumae* isolated from rice fields fail to express multiple virulence genes

H. S. KARKI (1), J. Ham (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.62

Burkholderia glumae causes bacterial panicle blight of rice, one of the major rice diseases in the southern United States including Louisiana and Arkansas. The LuxI/LuxR type TofI/TofR-mediated quorum sensing (QS) mechanism plays an important role in controlling the known virulence-related phenotypes including toxoflavin and lipase production, flagella-mediated motility, and catalase activity. Previously, we reported that some of natural *B. glumae* strains failed to cause disease in rice. Here we report that those natural avirulent *B. glumae* strains are deficient in multiple virulence factors and their associated genes are not expressed. We tested production of major virulence factors, toxoflavin and lipase, and flagella-dependent motility as well as the presence of the functional TofI/TofR-mediated QS system. We found that all the natural avirulent *B. glumae* strains lack toxoflavin production and flagella-dependent motility. In addition, most of them showed less lipase activity and impaired QS. Reverse transcription-PCR (RT-PCR) was used to examine the expression several genes for virulence related phenotypes such as *tofI* and *tofR* (QS), *toxA*, *toxR* and *toxJ* (toxoflavin), *lipA* (lipase), and *qsmR* and *fliA* (flagellar biogenesis). Our RT-PCR results indicated that all the avirulent *B. glumae* strains failed to express *toxR* and *fliA*. Similarly *tofI* and *lipA* were also not expressed in most of the avirulent *B. glumae* strains.

Risk analysis for *Verticillium nonalfalfae* isolate VnAa40, causal agent of Verticillium wilt of *Ailanthus altissima*

M. T. KASSON (1), D. D. Davis (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.62

Unprecedented mortality of the invasive *Ailanthus altissima* by an indigenous *Verticillium nonalfalfae* is currently widespread in south-central Pennsylvania where >13,000 trees are dead or dying following inoculation of 65 canopy trees. Because *Verticillium* spp. cause wilt diseases of many plant species, 64 species were stem-inoculated with *V. nonalfalfae* isolate VnAa40. Seventeen species exhibited wilt and vascular discoloration following inoculation: Amur corktree, autumn olive, black locust, catalpa, corkwood, crossvine, elderberry, Japanese maple, Norway maple, poison-ivy, redbay, redbud, sassafras, staghorn sumac, striped maple, and tree-of-paradise. However, mortality varied among species with only *Ailanthus*, elderberry, poison-ivy, striped maple, and sumac exhibiting >50% mortality. Furthermore, natural spread of this fungus within diseased *Ailanthus* stands was observed only for *Ailanthus*, devil's walkingstick, and striped maple. Vascular discoloration, but without wilt or mortality, was observed in 16 inoculated species. Although artificial inoculations provide an evaluation of potential damage to non-target hosts, the low incidence of disease and mortality of these non-target hosts among inoculated *Ailanthus* offer support that VnAa40 may be host-adapted. Pending the outcome of molecular studies, *V. nonalfalfae* should be considered as a potential biocontrol for *Ailanthus*.

Evaluation, validation, and economic analysis of IPM technology in tomato through farmer participatory approach

N. KAUSHIK (1)
(1) TERI, New Delhi, India
Phytopathology 102:S4.62

Tomato IPM module was evaluated in farmer participatory approach in Western Uttar Pradesh during 2008-09 and large scale validation trials were conducted in 2010. The components of IPM technology consisted with soil treatment by de oiled Neem cake @ 250 kg/ ha, seed treatment with *Trichoderma viridae* + *Pseudomonas florescence* @ 5g/kg each, installation of yellow sticky traps @ 30/ ha, installation of pheromone traps for monitoring and mass trapping of *Helicoverpa armigera* @ 20 /ha, hand collection and destruction of fruit borer infected fruits and larvae, 2 sprays of NPV-H at 40 and 50 DAT, 3 sprays of Neem formulation at 15, 25 and 45 DAT, one spray of *Bacillus thuringiensis* var. *kurstaki* at 65 DAT and two spray of *Beauveria bassiana* at 30 and 55 DAT with the support of 1-2 sprays of safe chemical pesticide was reduced tomato insect pest population, minimize yield losses and increase yield. Adoption of IPM resulted reduction of chemical pesticides spray to 1-2 from 13-14 in control farm and with higher average yield 33000 kg /ha in IPM and 18500 kg/ha in control co farm.

New antagonistic strains of nonpathogenic *Rhizobium vitis* to control grapevine crown gall

A. KAWAGUCHI (1), K. Inoue (1)
(1) Research Institute for Agriculture, Okayama Prefectural Technology Center for Agriculture, Forestry and Fisheries, Akaiwa, Japan
Phytopathology 102:S4.62

Graft unions of nursery stock of grapevine (*Vitis vinifera* L.) collected in Japan yielded nonpathogenic strains of *Rhizobium*. On the basis of classic diagnostic tests, a sequence analysis, and a previously reported multiplex PCR method, the nonpathogenic strains were identified as *Rhizobium vitis*. Stems of grapevine seedlings were inoculated with both a cell suspension of seven mixed strains of *R. vitis* (Ti) as a pathogen and one of a new strain or *R. vitis* strain VAR03-1, one of the biological control agents against crown gall previously reported, as competitors to assay the suppression of tumor formation caused by the pathogen. In a test with a 1:1 cell ratio of pathogen:nonpathogen, all new strains of nonpathogenic *R. vitis* reduced the tumor incidence. In particular, one of the new strains named "ARK-1" was most effective in inhibiting tumor information on grapevine and appears to be a promising new agent to control grapevine crown gall.

Distribution of genotypes of *Phytopthora infestans* isolated from potato and tomato in Canada in 2011

L. M. Kawchuk (1), R. D. PETERS (2), K. I. Al-Mughrabi (3), K. Conn (4), K. F. Dobinson (4), F. Daayf (5), H. W. Platt (2), B. W. Beaton (6), C. J. Banks (6), A. MacPhail (2)
(1) Agriculture and Agri-Food Canada, Lethbridge, AB, Canada; (2) Agriculture and Agri-Food Canada, Charlottetown, PE, Canada; (3) New Brunswick Department of Agriculture and Aquaculture, Wicklow, NB, Canada; (4) Agriculture and Agri-Food Canada, London, ON, Canada; (5) University of Manitoba, Winnipeg, MB, Canada; (6) Prince Edward Island Department of Agriculture, Charlottetown, PE, Canada
Phytopathology 102:S4.62

Late blight, caused by *Phytophthora infestans*, is a devastating disease of potatoes and tomatoes that occurs worldwide and causes significant crop losses annually. In recent years, late blight has been very severe in Canada on both potatoes and tomatoes. Since new strains of *P. infestans* began to appear in Canada in the mid 1990s, disease control has become even more difficult. A survey conducted in 2010 identified a geographic divide among genotypes in Canada, with US-8 dominating in eastern Canada and the novel genotypes US-23 and US-24 dominating in western Canada. Results from a national survey in 2011 indicated that although the US-8 genotype of *P. infestans* still dominated pathogen populations in Prince Edward Island, novel US-23 and US-24 genotypes became entrenched in other parts of eastern Canada. The US-22 genotype predominated in populations of the pathogen isolated from tomato in Ontario. Migration via infected tomato transplants or infected potato seed were identified as mechanisms for long-distance transport of pathogen genotypes. In some situations, both A1 and A2 mating types of the pathogen were found in the same production region. This is of concern because it increases the potential for sexual mating within pathogen populations leading to the rapid generation of new strains and the production of oospores which can potentially survive in soils outside of host tissues.

Screening citrus and its relatives in Aurantioideae for tolerance to huanglongbing

M. KEREMANE (1), C. Ramadugu (2), E. Stover (3), S. E. Halbert (4), Y. Duan (3), R. F. Lee (5)
(1) USDA-ARS, Citrus Germplasm Repository, Riverside, CA, U.S.A.; (2) University of California, Riverside, CA, U.S.A.; (3) USDA-ARS USHRL, Fort Pierce, FL, U.S.A.; (4) FDACS Division of Plant Industry, Gainesville, FL, U.S.A.; (5) National Clonal Germplasm Repository for Citrus & Dates, USDA-ARS, Riverside, CA, U.S.A.
Phytopathology 102:S4.63

Huanglongbing (HLB), a devastating disease of citrus, has been endemic in several Asian and African countries for about a century. Since its first report from the Western hemisphere in 2004, it has become a serious problem to the citrus industries in Brazil and Florida and both the disease and its psyllid vector, *Diaphorina citri* have spread to other citrus growing regions. A field trial was established in Fort Pierce, Florida where HLB has become endemic to assess the HLB tolerance level of different cultivars of citrus and citrus relatives. Seedlings of a total of 96 cultivars (8 replications each) belonging to 18 genera of the subfamily Aurantioideae and family Rutaceae were evaluated. Leaf samples were collected at 6 month intervals in spring and fall seasons over a 3-year period, and tested for the presence of HLB associated bacterium, '*Candidatus* Liberibacter asiaticus' (LAS) by real time PCR. While most cultivars were found to be susceptible to HLB, the bacterium (LAS) was not detectable in about 20 cultivars for at least up to three years. These include many trifoliate and trifoliate hybrids, some species of *Bergera*, *Casimiroa*, *Clausena*, *Eremocitrus*, *Glycosmis*, *Microcitrus*, *Murraya*, *Naringi*, and *Zanthoxylum*. In the genus *Citrus*, only two species showed either no or very low levels of LAS. Information on varietal tolerance of citrus and its relatives to HLB is very important for management of the disease.

Evidence for heterokaryon formation and nuclear disproportion in *Sclerotinia homoeocarpa* using fungicide sensitivity phenotypes and genotypic markers

D. KESSLER (1), J. Hulvey (1), G. Jung (1)
(1) University of Massachusetts-Amherst, Amherst, MA, U.S.A.
Phytopathology 102:S4.63

Sclerotinia homoeocarpa, the causal agent of dollar spot in turf, is a sterile fungal pathogen that can exhibit resistance to multiple fungicides. Compatible homokaryotic strains of this fungus are known to form heterokaryons, and we demonstrate that single-drug resistant field strains can form heterokaryons with multi-drug resistance. Homokaryons, referred to as "parent isolates", each with single-drug resistance, were co-cultured, and hyphal tips from the junction of the parent isolates were sub-cultured onto media amended with propiconazole (PP), thiophanate-methyl (TM), both fungicides (PP+TM), or non-amended. The heterokaryons grew on either PP or TM amendments, but were unable to grow on media amended with both fungicides. Heterokaryons grew when swapped between TM to PP media, or vice versa, as long as concentrations were fungistatic instead of fungicidal. To better understand this phenomenon, isolates were genotyped using genomic microsatellite markers. Shifts in genotype caused by fungicide selection on the TM-amended medium were confirmed by sequencing a known TM resistance point mutation in the beta-tubulin gene. These data suggest that *S. homoeocarpa* can harbor heterogeneous nuclei from homokaryons with different fungicide resistances profiles. The occurrence of nuclear disproportional responses to fungicide selection pressures allows adaptability of *S. homoeocarpa* to multiple fungicide pressures, likely leading to further problems for disease management issues in the field stemming from multi-drug resistance.

Managing *Rhizoctonia solani* on sugar beet with fungicides

M. KHAN (1)
(1) North Dakota State University/University of Minnesota, Fargo, ND, U.S.A.
Phytopathology 102:S4.63

Rhizoctonia solani causes damping off, and crown and root rot of sugar beet (*Beta vulgaris* L.). These diseases are the most important problems for growers in Minnesota and North Dakota. Most commercial sugar beet varieties are susceptible to or have partial resistance to *R. solani*. Fungicides that provide protection from *R. solani* are needed for effective disease management. Penthiopyrad was used as a seed treatment at three rates alone or followed by a band application of azoxystrobin. Azoxystrobin was also applied in-furrow; in-furrow followed by a band application; and as a band application. Stand counts were taken during the growing season and just prior to harvest. Roots were harvested and evaluated for recoverable sucrose. Penthiopyrad as seed treatments always resulted in significantly greater recoverable sucrose than the non-treated control but stand counts were not always significantly higher. Penthiopyrad at all rates followed by azoxystrobin resulted in significantly higher stand counts and greater recoverable sucrose compared to the non-treated control. Azoxystrobin resulted in significantly greater stand counts and higher recoverable sucrose compared to the control. The use of penthiopyrad as a seed treatment however, will facilitate faster planting, and when followed by azoxystrobin will serve as a fungicide resistance management strategy while providing effective disease control.

Inhibitors and inducers of the type III secretion system of *Erwinia amylovora*

D. KHOKHANI (1), Q. Zeng (2), X. Chen (3), C. Yang (1)
(1) Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, U.S.A.; (2) Department of Plant Pathology, Michigan State University, East Lansing, MI, U.S.A.; (3) School of Pharmaceutical & Life Sciences, Changzhou University, Jiangsu, Peoples Republic of China
Phytopathology 102:S4.63

The type III secretion system (T3SS) is a major virulence factor in many plant, and animal bacterial pathogens. The T3SS translocates effector proteins directly into the cytosol of host cells. These effectors manipulate host cellular activities for the benefit of the pathogen. The aim of this research is to identify small molecule phenolic compounds that affect the T3SS of the plant pathogen *Erwinia amylovora* 273 (Ea273). Along with known plant phenolic compounds, a library of phenolic derivatives was synthesized and their effect on T3SS expression was examined by a green fluorescent reporter assay in conjunction with flow cytometry. Several novel compounds were identified that either induced or inhibited the T3SS of Ea273. Further studies on the regulatory mechanism indicated that these phenolic compounds affect T3SS expression primarily through HrpS-HrpL, and moderately through the $RsmA_{Ea}$-$rsmB_{Ea}$-HrpL pathways. Moreover, some of the inhibitory compounds were also found to reduce the hypersensitive response (HR) in tobacco. To rule out the possibility that these compounds suppressed the plant defense response leading to a reduction in HR, *Agrobacterium tumefaciens* GV3101 strain carrying the *hopQ1* effector gene of *P. syringae* DC 3000 strain were infiltrated into tobacco leaves, allowing transient gene expression of *hopQ1* in tobacco plants. Compounds were then infiltrated at different time points. Our results showed that HR development in the transgenic tobacco plants was not affected in the presence of these compounds. Taken together, these results suggest that the compounds specifically target T3SS expression. With increasing numbers of bacterial strains resistant to available antibiotics, targeting bacterial virulence processes may provide an alternative approach for developing novel antimicrobial therapies.

Characterization of *Cucumber mosaic virus* (CMV) isolated from passion fruits in Korea

M. KIM (1), H. Kwak (1), H. Young (2), S. Lee (3), J. Kim (1), K. Kim (4), B. Cha (5), H. Choi (1)
(1) National Academy of Agricultural Science, RDA, Suwon, South Korea; (2) Jeju Agricultural Research and Extension Services, Jeju, South Korea; (3) Kyungpook National University, Daegu, South Korea; (4) Seoul National University, Seoul, South Korea; (5) Chungbuk National University, Cheongju, South Korea
Phytopathology 102:S4.63

Passion fruits are a tropical or subtropical vine plant of the family *Passifloraceae*. Passion fruits are grown in small scale from Jeju-island in Korea. Jeju-Island is the only subtropical region of the country. Symptoms of mosaic, vein clearing and mottle were observed on leave of passion fruits. This causal virus was as *Cucumber mosaic virus* (CMV) based upon biological, serological, and molecular characteristics. In host range studies, the CMV isolate produced local lesions on *Datura stramonium*, *Chenopodium amaranticolor*, *Ch. quinoa*, whereas this isolate produced systemic infection on *Nicotiana tabacum*, *N. glutinosa*, *Capsicum annuum*, *Lycopersicon esculenturn*, *Gomphrena globosa*, *Cucurbita pepo*, *Cucurbita moschata*. Complete nucleotide sequences of the each RNA segment had more than 90% sequence identity to those of CMV in subgroup I. Phylogenetic analysis of the five ORF gene revealed that CMV isolate were belonged to subgroup I. To our knowledge, this is the first report of CMV in Subgroup I infection in passion fruit in Korea.

Phylogenetic relationships among northern hemisphere *Armillaria* species based on the *tef*-1α locus

M. KIM (1), J. E. Stewart (2), Y. Ota (3), J. W. Hanna (4), A. L. Ross-Davis (4), N. B. Klopfenstein (4)
(1) Kookmin University, Seoul, South Korea; (2) USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR, U.S.A.; (3) Forestry and Forest Products Research Institute, Tsukuba, Japan; (4) USDA-FS, Rocky Mountain Research Station, Moscow, ID, U.S.A.
Phytopathology 102:S4.63

Armillaria possesses several intriguing characteristics that have inspired wide interest in understanding phylogenetic relationships within and among species of this genus. Previous DNA sequence-based analyses of *Armillaria* phylogeny provided limited information for phylogenetic studies among widely

divergent taxa, but these sequences were not reliable for separating closely related North American species, such as *A. gallica*, *A. calvescens*, *A. cepistipes*, and *A. sinapina*, or other closely related Eurasian species. Recent studies showed that translation elongation factor 1-α (*tef*-1α) appears quite useful for phylogenetic analysis of *Armillaria* spp. from diverse global regions. Based on the *tef*-1α-based phylogeny from this study, *Armillaria* spp. from the northern hemisphere generally appear to be comprised within four major clades: 1) *A. solidipes* clade (North American *A. solidipes* and *A. gemina*, European *A. borealis* groups, Eurasian *A. ostoyae*, *A. cepistipes*, and *A. sinapina*); 2) *A. gallica* clade (Japanese Nag E, North American *A. gallica* groups, Japanese *A. gallica*, European *A. gallica*, *A. calvescens*, North American *A. cepistipes*, North American Biological Species X, North American *A. nabsnona*, and Japanese *A. nabsnona*); 3) *A. mellea* clade (North American *A. mellea* groups, European *A. mellea*, and Japanese *A. mellea*); and 4) exannulate *Armillaria* clade (Eurasian *A. ectypa*, North American *A. tabescens*, and Eurasian *A. socialis*/*tabescens* groups).

Production of phytotoxin solanapyrones and generation of solanapyrone-deficient mutants in *Ascochyta rabiei*
W. KIM (1), W. Chen (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.64

Ascochyta rabiei, the fungus that causes Ascochyta blight of chickpea, produces phytotoxin solanapyrones. Generating mutants deficient in the toxin biosynthesis would provide information on the role of the toxins during infection processes. Partial genomic sequences of the solanapyrone biosynthesis gene cluster in *A. rabiei* were identified in this study, based on the homologous *sol* gene cluster (*sol*1 - *sol*6) in *Alternaria solani*. The *sol*5 and *sol*4 genes which encode solanapyrone synthase and a fungal specific transcription factor, respectively, were targeted to generate toxin-deficient mutants. *sol*5 mutants produced no solanapyrone and accumulated prosolanapyrone II, the precursor of solanapyrone, whereas *sol*4 mutants did not produce solanapyrone or prosolanapyrone II. Both the mutants showed normal growth patterns and conidiation in PDA or V8 agar media. Virulence of the toxin-deficient mutants was examined using two-week old chickpea seedlings by means of spray inoculation and both mutants were not significantly different from the wild-type progenitor isolate in infecting chickpea. These findings indicate that *sol*4 gene is a potential positive regulator for solanapyrone biosynthesis, and that solanapyrone is not essential for the chickpea seedling infection. Additional pathogenicity tests using different inoculation techniques and growth stages of chickpea are ongoing and the results will be presented.

Diversity of *Phytophthora* and *Pythium* in Pennsylvania forest streams
S. KIM (1), T. J. Hall (2), E. Nikolaeva (3), L. H. Lawson (1), S. Kang (3)
(1) Pennsylvania Department of Agriculture, Harrisburg, PA, U.S.A.; (2) Pennsylvania Bureau of Forestry, Middletown, PA, U.S.A.; (3) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.64

We cultured and identified *Phytophthora* and *Pythium* isolates in PA forest streams in 2006-2011. Twenty-one streams were selected for bait monitoring of *P. ramorum* using mature, healthy leaves of *Rhododendron maximum* L. populations. All stream bait locations had species common to PA -- intact mixed hardwood (*Quercus*, *Carya*, *Betula*, *Acer*) and conifer (*Pinus*, *Tsuga*) -- with a substantial understory of susceptible foliar hosts consisting of *R. maximum*, *Kalmia latifolia* L. and *Vaccinum* spp. At each location, two nylon mesh bait bags were left in stream for two weeks; three separate bait periods were implemented during the spring and autumn when stream flows were consistent and stream temperatures remained between 15 C and 22 C. A total of 278 samples were analyzed using PPQ PCR protocols for *P. ramorum* detection, all of which came negative. Cultures isolated on PARP were subcultured to V8-200 media and to hempseed water for long-term storage (390 isolates in total). All samples were PCR and morphology negative for *P. ramorum*. Species identify was determined for 48 isolates collected in 2006 isolates using their ITS sequences, which revealed the presence of several *Phytophthora* (*citricola*, *gonapodyides*, and an unidentified species) and *Pythium* (*undulatum*, _04, _10, _11, _12, and _15) species. These ITS sequences were deposited to the *Phytophthora* Database (http://www.Phytophthoradb.org).

Predictive model of eradicative and management strategies for pale cyst nematode
G. R. KNUDSEN (1), L. C. Dandurand (1)
(1) University of Idaho, Moscow, ID, U.S.A.
Phytopathology 102:S4.64

Intensive efforts are underway to eradicate the pale cyst nematode (PCN), *Globodera pallida*, from Idaho potato fields. To date, in the USA this invasive plant pathogen has been found only in Idaho. The fumigant methyl bromide has been effective but soon may no longer be available. Non-chemical methods (microbial biocontrol agents, trap crops, hatching factor) have promise for eradication or management of PCN, but quarantine considerations preclude comprehensive field testing of these methods. We developed a systems dynamics computer simulation model for the PCN disease cycle, used it to identify susceptible points in the nematode's life cycle and epidemiology, and then estimated effects of the different eradication or management strategies focused on those points. The model was implemented using the systems dynamics software package VENSIM. Sensitivity analysis of simulation results was used to estimate levels of control achievable with the different control methods, individually or in combination. Model results suggest that an optimal eradicative outcome would be achieved by using combinations of control strategies, e.g., trap crops or chemical hatching factor to stimulate emergence of J2 larvae that survive chemical fumigation, in combination with nematophagous fungi that are able to colonize cysts containing unhatched eggs. This quantitative approach will provide a useful tool to optimize strategies to eradicate or manage this important plant pest.

Factors involved in Indiana bitter rot outbreaks
S. KOENIG (1), G. W. Sundin (2), J. Beckerman (1)
(1) Purdue University, West Lafayette, IN, U.S.A.; (2) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.64

Bitter rot, caused by *Colletotrichum acutatum* and *C. gloeosporioides*, is a significant late-season disease of commercial apples. We identified four factors implicated in the Indiana bitter rot outbreaks of 2010 and 2011: cultivar susceptibility, weather conditions, fungicide spray schedules and fungicide resistance. Summer temperatures and rainfall exceeded the 30-year average by at least 1 degree Celsius and 2.5 cm, respectively, in both years. Review of spray records found that growers with losses to bitter rot did not use captan at the maximum-labeled rate and extended applications to the longest labeled interval. Isolates were obtained from fruit collected from three Indiana apple orchards to test whether fungicide resistance played a role in this outbreak. Thirty-five isolates from each orchard were screened for resistance to the fungicides kresoxim-methyl and thiophanate-methyl. Preliminary data suggest that 20% of the isolates were resistant at 0.2 ppm kresoxim-methyl, and 30% of all isolates were shifted in resistance. In 2 of the 3 orchards, 100% of the isolates were resistant to thiophanate-methyl; in the third, only 3% tested as resistant. These findings suggest that growers should modify their bitter rot management practices during seasons that are unusually hot and wet by using the maximum rate and shortest interval when applying captan while also recognizing the risk of resistance to strobilurin fungicides.

RNA-Seq analysis of '*Candidatus* Liberibacter asiaticus' gene expression in the two distinct habitats citrus and psyllid
S. Kogenaru (1), V. Aritua (1), N. WANG (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.64

Huanglongbing (HLB) or citrus greening disease is a destructive disease of citrus in the United States, which is associated with '*Candidatus* Liberibacter asiaticus'. This phloem-limited fastidious pathogen is transmitted by the Asian citrus psyllid, *Diaphorina citri*, and appears to be an intracellular pathogen that maintains an intimate association with the psyllid or the plant throughout its life cycle. The molecular basis of the interaction of this pathogen with its hosts is not well understood. We hypothesized that during infection, '*Ca*. L. asiaticus' differentially expresses the genes critical for its survival and/or pathogenicity in either host. In our previous study, quantitative reverse transcription PCR (QRT-PCR) was utilized to compare the gene expression of 362 genes of '*Ca*. L. asiaticus' *in planta* and in psyllid with samples collected from greenhouse. In the present work, we further expanded our study by investigating the gene expression of the pathogen collected from greenhouse and citrus grove using RNA-Seq. Our analysis revealed that one quarter of the '*Ca*. L. asiaticus' genes (~250 of 1109 genes) were differential expressed in these two habitats. The implication of the gene expression pattern to the pathogen adaption of '*Ca*. L. asiaticus' to its distinct hosts, is presented.

***Dickeya* spp.—Emerging pathogen of potato in Russia**
K. KORNEV (1), A. Ignatov (1), A. Karlov (2), G. Karlov (2), F. Dzhalilov (2), E. Pekhtereva (1), D. Luster (3)
(1) Russian Research Institute of Phytopathology, Moscow, Russia; (2) Russian State Agrarian University-MSKhA, Moscow, Russia; (3) USDA-ARS, Foreign Disease, Weed Science Research Unit, Fort Detrick, MD, U.S.A.
Phytopathology 102:S4.64

Several regions of Russia were assayed for potato bacterial diseases at 2009-2011. Bacteria causing black leg and soft rot diseases belonged to three

genera: *Pectobacterium* (75% of all isolates), *Serratia* (~10%), and *Dickeya* (~15%). Strains of *Dickeya* spp. isolated at Lipetsk, Novgorod, Moscow, Voronezh, and Bryansk regions were tested for biochemical traits, virulence and genetic properties, including MLST for 8 genes. All, but the strains from Voronezh region, belonged to *D. dianthicola*, and were similar to strains of *Erwinia chryzanthemi* isolated at 1990-2000 at South of Russia (Krasnodar and Stavropol regions) from corn and potato plants. A few strains from Voronezh region were identified as *D. "solani"* group. All the *Dickeya* strains were virulent for potato, tomato, tobacco and iris plants, and could be identified by real-time PCR with ADE1/ADE2 primers with original ADE3 fluorescent Taqman® probe. The work was partly supported by ISTC projects #3431 and #2685.

Analysis of *Iris yellow spot virus* N-gene sequences from the United States, 2003-2011

V. KOUNDAL (1), R. Iftikhar (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.
Phytopathology 102:S4.65

Iris yellow spot virus (IYSV; family *Bunyaviridae*, genus *Tospovirus*) is becoming an increasingly important constraint to the production of bulb and seed onions (*Allium cepa* L.) in many onion-growing regions of the world. To better understand the population structure and variability over time, IYSV isolates were collected during summer 2011 from Colorado, Idaho, New Mexico, New York and Washington. The full length N-gene was cloned and sequenced. The N-gene sequences of these five isolates had 99.6-100% identity with one another. Sequences from the 2011 isolates were aligned with those reported prior to 2011 from various parts of the US. In a phylogenetic tree consisting of all the N-gene sequences reported between 2003-2011 from the US, the 2011 Idaho isolate grouped with the previously reported California isolate from 2005, while the remaining 2011 isolates grouped with one another forming one cluster. Phylogenetic analyses also showed clustering of all the five 2011 isolates with those reported between 2003-2006, while isolates from 2008 and 2010 grouped with one another in a separate clade. *In silico* analysis detected recombination in one of the 2011 isolates out of the 27 sequences reported between 2003 to 2011, suggesting evidence of some genetic divergence and evolution in the IYSV N-gene sequences over this time period.

Effect of SqVYV-resistant pollenizers on development and spread of watermelon vine decline in seedless watermelon

C. S. KOUSIK (1), S. Adkins (2), C. G. Webster (2), W. Turechek (2), P. D. Roberts (3)
(1) USDA-ARS, Charleston, SC, U.S.A.; (2) U.S. Horticultural Research Laboratory, USDA-ARS, Fort Pierce, FL, U.S.A.; (3) University of Florida, Immokalee, FL, U.S.A.
Phytopathology 102:S4.65

Watermelon vine decline (WVD) caused by the whitefly-transmitted *Squash vein yellowing virus* (SqVYV) is a major limiting factor in watermelon (*Citrullus lanatus*) production in south Florida over the past few years. The disease causes sudden decline of the vines and affects the internal fruit quality. WVD was estimated to have caused >$60 million in losses in 2004. We have developed several sources of SqVYV-resistant germplasm from U.S. plant introductions (PI) to WVD. Resistant germplasm were used as pollenizers in seedless watermelon (triploid) production, thus creating a mixed genotype situation of resistant pollenizers and susceptible triploid plants. Field trials were conducted in Immokalee, FL during spring and fall season of 2010, and 2011. In 2011, an integrated approach using a combination of insecticides and resistant pollenizers was evaluated. No significant interaction between pollenizers and insecticide treatments were observed in 2011. In all the four seasons plots with the SqVYV resistant line USVL291 (diploid, seeded) as the pollenizer had significantly fewer number of susceptible seedless (Tri-X 313) fruit with WVD symptoms compared to seedless (Tri-X 313) fruit in plots with a susceptible pollenizer 'Mickey Lee'(diploid). Whitefly counts were not significantly different among the resistant or susceptible pollenizer plots. Developing commercial pollenizers with resistance to SqVYV can be an additional tool for managing WVD in Florida.

Predisposition to Phytophthora root rot varies among rhododendron genotypes subjected to flooding stress

S. KREBS (1), P. Bonello (2)
(1) The Holden Arboretum, Kirtland, OH, U.S.A.; (2) The Ohio State University, Columbus, OH, U.S.A.
Phytopathology 102:S4.65

Root rot caused by the invasive fungal pathogen *Phytophthora cinnamomi* is a major source of mortality for *Rhododendron* and other popular ornamental genera. A breeding program is underway to produce resistant rhododendrons that can be grown more successfully and sustainably under a broad range of production and landscape conditions. Current hybridization is based on *R. hyperythrum*, a species from Taiwan that exhibits more root rot resistance under flooding conditions than other resistant genotypes. In a replicated field trial where rhododendrons were subjected to repeated flooding throughout one growing season, three resistant cultivars included as benchmarks showed advanced disease symptoms, with necrosis occurring in the crown tissue (average disease rating = 4.1 on a scale of 1-5 where 5 is a dead plant). In contrast, symptoms in *R. hyperythrum* were limited to fine and coarse roots, resulting in a significantly lower disease rating (2.7). Eight genetically diverse F_1 hybrids – crosses between *R. hyperythrum* and cold hardy, susceptible cultivars – averaged a root rot rating of 3.3 and were significantly less diseased following flooding stress than the resistant benchmarks. Presence of *P. cinnamomi* in symptomatic tissue was confirmed by isolate morphology and sequence data. Compared to other resistant genotypes, *R. hyperythrum* and its F_1 hybrids are less predisposed to root rot in poorly drained soils, a common and often fatal feature of the home landscape.

Construction of a *Cucumber mosaic virus* stably expressing eGFP in *Nicotiana benthamiana* following transmission by the aphid vector *Myzus persicae*

B. KRENZ (1), X. Lu (1), J. R. Thompson (1), K. L. Perry (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.65

Cucumber mosaic virus (CMV) is a positive-sense RNA virus with three genomic RNAs. An infectious cDNA clone of RNA 2 of CMV was modified to express enhanced green fluorescent protein (eGFP), the ORF of eGFP being fused to the 3'-end of a truncated 2b protein gene. eGFP expression was observed in the cells of infected *Nicotiana benthamiana* leaves when transcripts of the modified RNA 2 were co-inoculated with RNAs 1 and 3. eGFP expression was sustained in systemically infected leaves, and the resulting virus progeny (CMV:GFP) were transmitted by both mechanical inoculation and by the aphid *Myzus persicae*. The RNA2 construct and eGFP expression proved to be stable following five generations of passage (mechanical and aphid) over approximately an eight week period. During the initial period beginning 2 days post inoculation (dpi) by the aphid vector, eGFP expression could be monitored in an expanding lesion from the site of inoculation. Upon reaching major veins, eGFP was observed to progress upward in the vein, followed by expression in upper expanding leaves at 4 to 6 dpi. The availability of an eGFP-tagged CMV provides a powerful tool to monitor virus movement to distal parts of a plant, and for studying tissue specificity, virus-host and virus-vector interactions.

Wavelet analysis as a statistical tool for spatial and temporal analysis of epidemics

A. B. KRISS (1), T. R. Gottwald (2), P. A. Paul (3), L. V. Madden (3)
(1) USDA-ARS, Fort Pierce, FL, U.S.A.; (2) USDA, Fort Pierce, FL, U.S.A.; (3) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.65

Wavelet analysis is a method to analyze variations in scale and position of spatial and temporal signals, such as disease or environmental data. It is similar to spectral analysis, in that the frequency at which a signal occurs can be determined, but wavelet analysis also allows one to identify exact locations within a signal where the frequency occurs. This is due to the inherent difference between sine waves and wavelets as sine waves extend to infinity, whereas wavelets have limited duration. A spatial wavelet analysis was conducted on a large citrus planting in Florida where over 260,000 trees were assessed for incidence of Huanglongbing (HLB) over 5 sampling times. In addition to identifying spatial scales and locations within the planting at which trees are diseased, wavelet analysis can also de-noise (or smooth) such large datasets. In a temporal setting, wavelet analysis was utilized to determine at which points in time the Oceanic Niño Index (ONI) was related to Fusarium head blight (FHB) in Ohio. A previous spectral analysis showed the ONI and FHB were coherent at periods around 5 years, but the results from wavelet analysis showed that within the entire 46 years, there were specific clusters of years when the observed relationship was more/less pronounced, indicating a potentially evolving temporal relationship within the overall process.

Climate patterns as predictors of *Stagonospora nodorum* glume blotch in Ohio

A. B. KRISS (1), P. A. Paul (2), L. V. Madden (2)
(1) USDA-ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL, U.S.A.; (2) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.65

Stagonospora nodorum glume blotch (SGB) is one of the most economically important fungal diseases of wheat in Ohio and many other areas. However,

the severity of SGB is variable across years. To investigate the possible effect of global climate variability on SGB, we used cross-spectral analysis, which is a tool used to partition the variance of time-series data into temporal scales or periods. The time-series investigated were the Oceanic Nino Index (ONI), North Atlantic Oscillation (NAO), and SGB rating in Ohio from 1965 to 2011. Cross-spectral analysis had been utilized previously to investigate the effect of climate patterns on Fusarium head blight (FHB) of wheat in Ohio. SGB and ONI were coherent at periods of around 4 to 5.5 years, while SGB and NAO were coherent at slightly shorter periods of 3 to 4.5 years. The phase shifts indicated that SGB and the ONI had a negative relationship, while SGB and the NAO had a positive relationship. This work, in addition to previous work on FHB, indicate that the use of climate patterns in future predictive models may increase our ability to determine which growing seasons sporadic plant diseases are more likely to be severe.

Canker development in Buddha Hand (*Citrus medica* var. *sarcodactylis*)
N. KUMAR (1), R. C. Ebel (2), P. D. Roberts (2)
(1) University of Florida, Fort Myers, FL, U.S.A.; (2) University of Florida, Immokalee, FL, U.S.A.
Phytopathology 102:S4.66

Citrus canker is an economically important disease and causes enormous production losses throughout world. In the present investigation, canker development was studied in Buddha Hand (*Citrus medica* var. *sarcodactylis*), which is an ornamental tree. Basal matured leaves (fully expanded) were inoculated using a tuberculin syringe (1 cc) without needle. *Xanthomonas citri* pv. *citri* (*Xcc*) inoculum (1.1 x 10^6 CFU/cm^2) was slowly infiltrated in to the abaxial leaf surface on both sides of the mid vein to produce a zone of water soaked tissue. Control leaves were mock inoculated with sterile nutrient broth. The first symptom of canker development was water soaking, which appeared 3 dai (days after inoculation) and coincided with 1.9 x 10^8 CFU/cm^2. The epidermis became thickened and raised at 5 dai and never ruptured. The most striking feature of canker development was observed at 6 dai constituting small circular isolated patches of necrosis within the zone of inoculum infiltration. The necrotic areas increased at 8 dai and thereafter. The highest *Xcc* population was 1.9 x 10^{11} CFU/cm^2 at 8 dai. The *Xcc* population declined by 100-fold between 8 to 12 dai and coincided with rapid necrosis of host cells. The levels of lipid peroxidation and H_2O_2 production were high at later stages of canker development. It seems Buddha Hand (*Citrus medica* var. *sarcodactylis*) represents a delayed type of hypersensitive response, which induced cell death at 6 dai. Furthermore, the epidermis of the infected zone remained intact to prevent spread of bacterium by wind or rain. Buddha Hand may serves as a promising citrus species to produce canker resistant transgenic plants and in traditional breeding programs.

First report of citrus huanglongbing in Texas
M. KUNTA (1), M. Sétamou (2), M. Skaria (2), J. E. Rascoe (3), W. Li (3), M. K. Nakhla (3), J. V. da Graça (2)
(1) Texas A&M University-Kingsville, Weslaco, TX, U.S.A.; (2) Texas A&M University, Kingsville Citrus Center, Weslaco, TX, U.S.A.; (3) USDA APHIS, Beltsville, MD, U.S.A.
Phytopathology 102:S4.66

Huanglongbing (HLB), also known as citrus greening, is a destructive citrus disease associated with three alpha-proteobacteria species of '*Candidatus* Liberibacter'. The first report of HLB in the USA was from Florida in 2005 and '*Ca.* L. asiaticus' (CLas) is the only species currently found in USA. On January 13, 2012, a Valencia sweet orange leaf sample from a commercial orchard in San Juan, TX gave a quantitative real-time PCR (qPCR) threshold cycle value of 23.41 and a positive PCR amplification product in conventional PCR (cPCR) using primers based on 16S rRNA and beta-operon genes. The cPCR products were sequenced and were 100% homologous to '*Ca.* L. asiaticus'. The sample tested negative for '*Ca.* L. americanus' and '*Ca.* L. africanus'. Leaf blotchy mottle, twig die-back, yellow shoot, veinal chlorosis, lopsided and greening fruits were observed on CLas-positive trees, which immediately triggered an intensive survey of the disease in the area. CLas has only been detected in 25 Valencia trees (1%) and 10 Rio Red grapefruit trees (0.5%) in two adjacent orchards located on either side of a paved road, in the 2 months post initial disease confirmation. Infected trees have been removed and intensive psyllid control and surveys are continuing.

Proteomic analysis of *Salmonella enterica* plant colonization: Insights into the metabolic requirements of human pathogens on plants
G. KWAN (1), J. D. Barak (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.66

Salmonella enterica is the leading bacterial cause of food-borne illness due to consumption of fresh produce. Despite the advances in human pathogen research on plants in recent years, our understanding of the general biology of enteric human pathogens *in planta* is still in its infancy. We hypothesize that understanding the essential networks that contribute to fitness in association with plants will identify targets for intervention strategies against human pathogens. To better characterize the metabolic requirements of *S. enterica* during growth *in planta*, we have identified 306 of the most abundant *S. enterica* proteins expressed during alfalfa seedling colonization at 24 h post-inoculation by LC-MS/MS. The high abundance of these proteins suggests that their metabolic, signaling, regulatory, or unknown functions are important for *S. enterica* colonization of the plant environment. The necessity of specific *S. enterica* pathways active during growth *in planta*, as identified by the detected high abundance of pathway proteins, will be assessed computationally through metabolic modeling and verified with *in planta* experiments using targeted pathway mutants. Understanding the metabolic and signaling networks that contribute to the fitness of *S. enterica* on plants will identify factors limiting *S. enterica* proliferation on plants and reveal general mechanisms of host colonization by plant-associated bacteria.

Biorational alternatives to control the soilborne plant pathogen *Rhizoctonia solani*
D. K. LAKSHMAN (1), K. Chauhan (2)
(1) USDA-ARS, FNPRU & SASL, Beltsville, MD, U.S.A.; (2) USDA-ARS, IIBBL, Beltsville, MD, U.S.A.
Phytopathology 102:S4.66

Rhizoctonia solani is a ubiquitous soilborne fungal pathogen causing pre- and post-emergence damping off, root rots and aerial blights of economically important crops, forest trees, ornamentals and turfgrasses, as well decay of postharvest fruits and vegetables. Soilborne pathogens have traditionally been controlled using chemical pesticides, some of which are inconsistent in efficacies and toxic to the environment. Also, prolonged use of fungicides can result in development of pathogenic resistance. As a result, eco-friendly management using safer chemicals is being sought to control plant diseases. Plants can be considered as renewable reservoirs of secondary metabolites, some of which are antimicrobial (i.e., biorationals) in nature. Some of these chemicals are less toxic to animals and plants, more systemic, easily biodegradable, and stimulate host plant metabolism. Since PEs and modified plant chemicals are considered "Generally Recognized as Safe" (GRAS) chemicals, we have tested thirty PEs and synthetic derivatives of plant chemicals using poisoned food and filter disk techniques against broad host range isolates of *R. solani* (AG 4). While several PEs were confirmed to be fungicidal or fungistatic in the in vitro bioassays, we also demonstrated that some PEs are effective soil amendments for the control of pre- and post-emergence damping off of cucumber seedlings caused by *R. solani* in the greenhouse.

Management of foliar nematodes, *Aphelenchoides fragariae*, in ornamentals
J. A. LAMONDIA (1)
(1) Connecticut Agricultural Experiment Station, Windsor, CT, U.S.A.
Phytopathology 102:S4.66

Greenhouse experiments were conducted to determine the efficacy of Avid (abamectin), Azatin (azadirachtin), Kontos (spirotetramat), MeloCon (*Paecilomyces lilacinus* Strain 251), Neemix 4 (azadirachtin), NI-108X (biological), Pylon (chlorfenapyr), Safari 20SG (dinotefuran), and Proclaim (emamectin benzoate) against foliar nematode infection. Treatments were applied to foliage of four plants each of *Anemone sylvestris* 'Serenade' and 'Pamina' until runoff. Kontos was also applied as a soil drench. Kontos and Pylon foliar sprays were re-applied after 4 weeks. *Aphelenchoides fragariae* from naturally infected *Salvia greggii* leaves were inoculated 2 days after treatment to pre-wet foliage (18,000 viable nematodes per plant) using a hand-held sprayer. Anemone leaves were sampled and nematodes recovered after 4, 8 or 12 weeks. Data were analyzed by Kruskal-Wallis one-way Analysis of Variance. Numbers of *A. fragariae* extracted from Serenade were low and there were no differences between treatments. The lowest nematode numbers recovered from Pamila were observed in the Pylon, Neemix, Kontos drench and Avid treatments with 26, 149, 337, and 352 *A. fragariae* per plant, respectively (P = 0.03). The NI 108X biological and untreated controls had the highest populations, with 20,911 and 8,085 *A. fragariae* per plant, respectively. No treatments were free of nematodes after 2 to 3 months; for practical management of *A. fragariae*, multiple applications of efficacious materials may be required in combination with sanitation.

Evaluating efficacy of black rot control caused by *Xanthomonas campestris* pv. *campestris* in greenhouse transplant production
H. W. LANGE (1), C. D. Smart (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.66

Black rot caused by the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*) is often a serious disease in New York State crucifer fields. This pathogen is seed borne and even rigorous seed testing can not guarantee every seed is free from bacteria. Moreover, greenhouse transplant operations conditions with dense plant populations and overhead watering are ideal for the spread of *Xcc*. Asymptomatic transplants can initiate field infections which are very difficult to control. We have investigated the efficacy of chemical and biorational products to control spread of black rot in cabbage seedlings during transplant production. Following treatment, *Xcc* was quantified from seedlings using plated leaf washes and real-time PCR which enabled us to determine that several treatments in the greenhouse were effective at suppressing *Xcc* in an environment favoring disease spread. Continued treatments after transplant to the field extended black rot control through harvest.

Longer-term potato cropping system effects on soilborne diseases and tuber yield

R. P. LARKIN (1), J. M. Halloran (1)
(1) USDA-ARS, New England Plant, Soil, and Water Laboratory, Orono, ME, U.S.A.
Phytopathology 102:S4.67

In field trials established in 2004, different 3-yr potato cropping systems focused on specific crop management goals of soil conservation (SC), soil improvement (SI), and disease-suppression (DS) were evaluated for their effects on soilborne diseases and tuber yield. These systems were compared to a typical 2-yr standard rotation (SQ) and a non-rotation control (PP), as well as under both rainfed and irrigated conditions. Previously, we reported on the effects after one full rotation cycle. Now, after 8 years and multiple seasons following two full rotation cycles, the DS system, which utilized *Brassica* and other disease-suppressive rotation crops, maintained lower soilborne disease levels than all other rotations as well as high yields, whereas relative disease levels were higher and yield lower in the SQ system than previously observed. The SI system, characterized by yearly compost amendments, and irrigation, both resulted in higher yields, but also greater levels of black scurf and common scab. After several years of treatment, irrigated plots resulted in significant effects on disease and yield even in years when no irrigation was applied. Overall results indicate that over time, there were greater differences among the systems, with SQ demonstrating increased diseases and reduced yields relative to all the 3-yr systems. The DS system continues to perform well regarding maintenance of low disease levels and relatively high yields.

Occurrence of spot-form net blotch of barley in the Mon-Dak area of the United States

R. T. LARTEY (1), R. G. Evans (1), T. Caesar-TonThat (1), A. J. Caesar (1)
(1) USDA-ARS, Sidney, MT, U.S.A.
Phytopathology 102:S4.67

Pyrenophora teres causes net blotch of barley, a common foliar disease around the world. Two forms, net form net blotch (NFNB) caused by *P. teres* f. *teres* and spot form net blotch (SFNB) caused by *P. teres* f. *maculata* are recognized. In the Northern Great Plains including the Mon-Dak area (Western North Dakota and Eastern Montana), NFNB is prevalent. SFNB was first reported in Western Montana in 1983 and more recently in Eastern North Dakota in 2010 but not in the Mon-Dak area. Observation of unusual spot lesions on barley in fields at Williston ND, Nesson Valley ND and Sidney MT area in 2011 led to examination for occurrence of SFNB in the area. Diseased leaves from various barley cultivars showing spot symptoms were collected from the three locations. These were subjected to conidia examination and PCR using *P. teres* actin specific and ITS primers. Amplicons from the positive PCR results were purified and sequenced. Conidial morphology and sequence alignment with control *P. teres* f. *teres* and *P. teres* f. *maculata* cultures indicated the presence of SFNB incidence at all three locations. Pathogenicity tests were conducted by extracting conidia from diseased leaves from the three locations and inoculating barley plants. The infected plants were maintained in the greenhouse and observed for SFNB symptoms. Leaves showing spot-form lesions were harvested, examined by PCR and conidia characteristics as previously described. The results confirmed the observed incidence of SFNB. This is the first detection of SFNB in the Mon-Dak area.

A toxin-antitoxin system encoded by the *Xylella fastidiosa* chromosome regulates growth

M. LEE (1), E. E. Rogers (1), D. C. Stenger (1)
(1) USDA-ARS, Parlier, CA, U.S.A.
Phytopathology 102:S4.67

Bacterial toxin-antitoxin (TA) systems consist of a stable toxin and a cognate labile antitoxin. When encoded by a plasmid, TA systems confer stable plasmid inheritance. When encoded by the chromosome, TA systems may regulate growth in response to environmental stress. The chromosome of *Xylella fastidiosa* (Xf) strain Temecula includes a TA system operon encoding homologs of DinJ antitoxin and RelE toxin. In vitro assays indicated that purified RelE toxin was a potent ribonuclease active only after removal of bound DinJ antitoxin. These modes of action are similar to those of other TA system components. Knockout mutants of *dinJ* and *relE* were constructed in Xf strain Temecula. The *dinJ* mutant exhibited reduced biofilm formation and reduced planktonic growth compared to wild type. The *relE* mutant exhibited increased planktonic growth relative to wild type but biofilm formation was unaffected. Complementation of each mutant with the respective wild type gene restored growth phenotypes. These results indicate that 1) the *dinJ/relE* TA system can inhibit Xf growth if RelE toxin activity is not blocked by bound DinJ antitoxin and 2) under non-stressful conditions, the *dinJ/relE* TA system partially inhibits growth, probably due to low levels of unbound RelE toxin. Whether or not the *dinJ/relE* TA system limits Xf growth in response to environmental stress or affects virulence remains to be determined.

WITHDRAWN

Requirement for a gene encoding a predicted acyl carrier protein for full virulence of the fire blight pathogen *Erwinia amylovora*

S. A. Lee (1), B. Lehman (2), H. K. Ngugi (3), T. MCNELLIS (2)
(1) Cornell University, Ithaca, NY, U.S.A.; (2) The Pennsylvania State University, University Park, PA, U.S.A.; (3) Biglerville, PA, U.S.A.
Phytopathology 102:S4.67

The gram-negative bacterium *Erwinia amylovora* is the causal agent of fire blight, the most destructive bacterial disease of apple and pear trees. During the course of a genetic screen for virulence-defective *E. amylovora* mutants, we identified a mutant with reduced virulence that had a Tn*5* insertion in the *acp* gene, which encodes a predicted acyl carrier protein. The *acp70*::Tn*5* mutant exhibited a quantitative reduction in disease severity in apple trees and reduced growth in hawthorn flowers compared to the wild-type. The *acp70*::Tn*5* virulence defect in apple trees and hawthorn flowers was complemented using a plasmid-borne copy of the *acp* gene, indicating that *acp* is required for full virulence of *E. amylovora*. Bacterial acyl carrier proteins donate acyl groups during biosynthesis of fatty acids, bacterial toxins, phospholipids, and N-acyl-homoserine lactone autoinducer signaling molecules. Compared to the wild-type, *acp70*::Tn*5* was defective in activation of an N-acyl-homoserine lactone biosensor and also had some alterations in its lipid profile. Mutation of the predicted Acp 4'-phosphopantetheine prosthetic group attachment site abolished *acp*-dependent autoinducer production. Our results suggest that *acp* encodes an acyl carrier protein involved in N-acyl-homoserine lactone biosynthesis and possibly the production of other fatty acids. In addition, these findings suggest that *acp*-dependent N-acyl-homoserine lactone production might play a role in *E. amylovora* virulence.

Use of young indicator plants for biological indexing: Application to citrus certification programs

R. F. LEE (1), K. Manjunath (2), C. Ramadugu (3)
(1) National Clonal Germplasm Repository for Citrus & Dates, USDA-ARS, Riverside, CA, U.S.A.; (2) USDA-ARS, Riverside, CA, U.S.A.; (3) University of California, Riverside, CA, U.S.A.
Phytopathology 102:S4.67

Citrus certification programs involve testing of a large number of plant samples for various pathogens. Standard protocols for biological indexing for presence of graft transmissible pathogens of citrus as required in citrus certification programs call for the use of large seedlings/budlings which are

six to 10 months old. The space available for biological indexing under the correct temperature conditions for symptom development is often a limiting factor for getting new accessions released from quarantine status and into the certification program. We are conducting trials to determine the value of using young plants, 2-3 months after being sown, in small conetainers as indicator plants. The results are being compared with data obtained using the standard protocol with traditional 8-10 month old indicator plants. Different sized conetainers and various potting media are being compared to assess performance. Preliminary results indicate that symptoms are often expressed earlier in young plants, and the system is more space efficient. A cost-effective and quick method of biological indexing will broaden the capability of a certification program.

Sanitation and disease modeling can help powdery mildew control in organic viticulture

S. LEGLER (1), T. Caffi (1), V. Rossi (1)
(1) Università Cattolica del Sacro Cuore, Piacenza, Italy
Phytopathology 102:S4.68

Erysiphe necator, the causal agent of grapevine powdery mildew, overwinters as chasmothecia in many grapevine growing regions. In the following spring, chasmothecia discharge ascospores that cause primary infections and trigger powdery mildew epidemics. In organic viticulture, only sulphur and biocontrol agents (BCAs) are available to control this disease, and the efficacy of these products must be maximized to obtain acceptable control. The possibility of including sanitation (via application of a BCA against the overwintering chasmothecia) in powdery mildew control was evaluated over a 4-year period in experimental vineyards. A biocontrol product "AQ10" that contained *Ampelomyces quisqualis* and that was applied twice (before and after harvest, i.e., during the formation and maturation of the chasmothecia), halved disease severity on bunches until the pea-sized berries stage in the following season. When sanitation with *A. quisqualis* was coupled with early season (i.e., between bud break and fruit set) sulphur applications scheduled according to the output of a mathematical model predicting ascospore infection based on weather data, disease severity on bunches was reduced by 98% (vs. an 80% reduction with sulphur sprays alone). In conclusion, the use of sanitation with *A. quisqualis* and a weather-driven model for ascospore infection can increase the efficacy of sulphur for control of powdery mildew in organic viticulture.

A unique genomic region of the *Cochliobolus sativus* pathotype 2 isolates carries genes for high virulence on barley cv. Bowman

Y. LENG (1), S. Zhong (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.68

Cochliobolus sativus (Anamorph: *Bipolaris sorokiniana*) is the causal agent of several important diseases, including spot blotch, common root rot, and black point, in cereal crops. Three pathotypes (0, 1, and 2) with differential virulence patterns on three barley genotypes (Bowman, ND5883 and NDB112) were identified. The pathotype 2 isolates showed high virulence on barley cv. Bowman but exhibited low virulence on the other two barley genotypes. Our previous studies indicated that the high virulence of the *C. sativus* pathotype 2 isolate ND90Pr was controlled by a single locus *VHv1* and genes for nonribosomal peptide synthetases (NRPSs) and/or polyketide synthases (PKSs) were involved in the biosynthesis of the unknown virulence factor. In this study, we identified a genomic region (approximately 137 kb in size) unique to ND90Pr based on genome sequence analysis in comparison with non-pathotype 2 isolates of *C. sativus*. This unique region contains an AFLP marker co-segregating with *VHv1* and thus is the location of the gene(s) conferring high virulence on Bowman. Of 43 predicted genes in this region, 17 had predicted annotations including two genes encoding for nonribosomal peptide synthetases (NRPSs). One of the genes for NRPSs was knocked out and the knockout mutants were significantly reduced in virulence on cv. Bowman, suggesting that it is involved in high virulence of the pathogen. Molecular and functional characterization of the genes related to virulence in this region will be presented.

Sensitivity of *Monilinia fructicola* to sterol demethylation inhibitors and analysis of *CYP51* promoter insertions in Michigan populations

K. E. LESNIAK (1), N. L. Rothwell (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.68

American brown rot (ABR; *Monilinia fructicola*) is a serious disease problem affecting stone fruit. The most effective class of fungicides for ABR management is the sterol demethylation inhibitors (DMIs); however, resistance has been reported in the southeastern US, NY, NJ, and OH. Recent publications suggest a PCR-based survey to detect a novel 376-bp insert termed 'MONA', upstream of the cytochrome demethylase gene (*CYP51*), can also detect DMI-resistant isolates. In the southeastern US, DMI resistance has been attributed to overexpression of *CYP51*, facilitated by increased transcription due to the presence of MONA. Since 2008, we have screened 1,043 and 422 *M. fructicola* isolates from 79 commercial orchards for DMI resistance using RG and PCR methods, respectively. This survey yielded only four DMI-resistant isolates; however, MONA was detected in 65% of isolates from southwest MI. Amplification and sequencing upstream of *CYP51* resulted in detection of DNA insertions in a wide range of isolates typed by DMI phenotype and the presence of MONA or other unique sequences. Further investigation of these inserts, in relation to their potential role in DMI phenotype and *CYP51* overexpression, was examined using real-time PCR. Comparative genetic analyses of the populations of *M. fructicola* in MI orchards was performed using genetic fingerprinting. These results suggest that DMI-sensitive strains containing MONA are invading MI stone fruit orchards from the south.

Distribution and detection of *Botrytis* species of blackberry and strawberry in the Southeast United States

X. LI (1), D. Fernandez-Ortuno (1), A. Grabke (1), G. Schnabel (1)
(1) Clemson University, Clemson, SC, U.S.A.
Phytopathology 102:S4.68

Botrytis spp. cause blossom blight and fruit rot on many crops, including strawberry and blackberry. In 2011, 400 isolates from blackberry and strawberry fields were collected from North and South Carolina and single-spore colonies were obtained and characterized. Two distinct species, *B. cinerea* Pers. and another newly described *Botrytis* species – *Botrytis caroliniana* X.P. Li & G. Schnabel, were identified based on examination of the coding G3PDH, HSP60, RPB2 and NEP1 genes and based on morphological characters. A PCR method was developed to distinguish *B. cinerea* from *B. caroliniana* but cultural characteristics can also be used for rapid distinction. Without exception, *B. cinerea* isolates sporulated on potato dextrose agar and kings medium B, while *B. caroliniana* isolates did not. The new species is pathogenic on blackberry, strawberry, broad beans and tomatoes, which is the second *Botrytis* species that has a broad host range besides *Botrytis cinerea*. Isolates from both species were subjected to cyprodinil, fenhexamid, fludioxonil, pyraclostrobin, boscalid, thiophanate-methyl and iprodione sensitivity evaluations using germination assays and several different fungicide resistance phenotypes were discovered.

Effects of piperidine alkaloids from the red imported fire ant on cucumber damping-off caused by *Pythium ultimum* in the greenhouse

S. Li (1), X. JIN (2), J. Chen (2)
(1) Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Baoding, Hebei Province, Peoples Republic of China; (2) USDA-ARS MSA, Stoneville, MS, U.S.A.
Phytopathology 102:S4.68

Pythium ultimum Trow is a plant pathogen that causes significant yield losses to many economically important crops. Chemical seed treatment has been used for disease control. In searching for alternatives, the venom alkaloids from the red imported fire ant were employed to control cucumber damping-off caused by *P. ultimum* in the greenhouse as seed and drench treatments. Seed soaking treatments did not provide control effects. Drenching treatment significantly improved the seedling emergence and seedling height of cucumber in the greenhouse. This is the first report using piperidine alkaloids from the red imported fire ant to control plant disease caused by *P. ultimum* in greenhouse. These findings may lead to the development of a new group of fungicides.

A new soybean rust resistance gene identified in PI 567102B

S. LI (1), J. Ray (1), J. Smith (1), R. Frederick (2)
(1) USDA-ARS, Crop Genetics Research Unit, Stoneville, MS, U.S.A.; (2) USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD, U.S.A.
Phytopathology 102:S4.68

Soybean rust (SBR) is one of the most destructive diseases of soybean. It is caused by *Phakopsora pachyrhizi* Syd. and P. Syd. Durable resistance to *P. pachyrhizi* is the most effective long-term strategy to control SBR. The objective of this study was to investigate the genetics of resistance to *P. pachyrhizi* in soybean accession PI 567102B. This accession was previously identified as resistant to SBR in Paraguay and to *P. pachyrhizi* isolates from seven states (Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas) in the USA. Analysis of two independent populations, one in which F_2 phenotypes were inferred from F_2-derived F_3 ($F_{2:3}$) families and the other in which F_2 plants had phenotypes measured directly, showed that the resistance in PI 567102B was controlled by a single dominant gene.

Two different isolates (MS06-1 and LA04-1) at different locations (Stoneville, MS and Ft. Detrick, MD) were used to independently assay the two populations. Linkage analysis of both populations indicated that the resistance locus was located on chromosome 18, but at a different location than either *Rpp1* or *Rpp4*, which were previously mapped to this chromosome. Therefore, the SBR resistance in PI 567102B appeared to be conditioned by a previously unreported locus, with an underlying single dominant gene inferred. We propose this gene to be designated *Rpp6*. Incorporating *Rpp6* into improved soybean cultivars may have wide benefits as PI 567102B has been shown to provide resistance to *P. pachyrhizi* isolates from Paraguay and the US.

Study on the culturable microbes from different organs of *Populus euphratica* and *P. pruinosa*

Q. LI (1), C. S. Gu (1), J. Z. Li (2), Q. J. Li (1)
(1) China Agricultural University, Beijing, Peoples Republic of China; (2) Tarim University, Alar, Peoples Republic of China
Phytopathology 102:S4.69

Populus euphratica and *P. pruinosa*, distributed at arid desert region and saline land with the characteristics of strong resistance and tolerance, are rare and important species for tree planting in afforestation. The culturable microbes from different organs of both species were studied by using washing method and plating tests, all the isolates were identified by morphology and molecular biology. The results showed that the quantity of microbes associated with seeds of *P. euphratica* was significantly higher than that of *P. pruinosa*. Comparing with *P. pruinosa*, the isolate rates of culturable fungi associated with buds, petioles, and branches of *P. euphratica* were lower, indicated significant differences in that of petioles, upper and central branches. For the both kinds of tree species, the isolate rates of culturable fungi associated with lower branches were up to 100 %. Seven fungal and two bacterial strains were isolated from seeds, buds, petioles and branches of both species, which were identified as *Alternaria* spp. (2 strains), *Aspergillus* spp. (3 strains), *Nigrospora* sp., *Epicoccum* sp. and *Stenotrophomonas* spp. (2 strains). It was first report that *Nigrospora* and *Epicoccum* were endophytic fungi, *Alternaria*, *Aspergillus* and *Stenotrophomonas* were seed associated fungi and bacteria of *P. euphratica* and *P. pruinosa*. The bioassay of above culturable microbes to seed germination and seedling growth of two species needs to be detected. The fungicides disinfection effect on seeds is being carried out.

Using green fluorescent protein to determine the effects of relative humidity on infection, fungal colonization, and conidiation of *Magnaporthe orzyae* on perennial ryegrass turf

Y. LI (1), W. Uddin (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.69

Gray leaf spot, caused by *Magnaporthe oryzae* B. Couch, is a devastating disease of perennial ryegrass (*Lolium perenne* L.) turf. The effects of relative humidity (R.H.) on *M. oryzae*-perennial ryegrass interaction and disease development were evaluated at various levels of R.H. ranging from 85% to 100% at 28°C. Green Fluorescent Protein (GFP)-labeled *M. oryzae* strain was employed to detect the early stage infection and assess the fungal colonization rate. The results showed that 100% R.H. was the most favorable for infection, and no infection by *M. oryzae* occurred at the R.H of 88% or lower. R.H. at 88% did not favor germination of the conidia on the host surface, but was sufficient for the conidia to retain the viability. Significant colonization of ryegrass tissue by *M. oryzae* mycelia occurred at 96% R.H or higher; however, there was only limited colonization at 92% R.H. Daily confocal microscopic observation and haemocytometer quantification indicated that the highest rate of conidiation occurred at 100% R.H. 9-10 days after inoculation. Few or no conidiation was observed at 96% R.H or below. Results of these studies will provide insight to understanding the epidemic development of gray leaf spot pathosystem.

A haloacid dehalogenase family phosphatase is required for extracellular polysaccharide production and virulence in *Xanthomonas citri* subsp. *citri*

J. LI (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.69

Haloacid dehalogenase (HAD) family phosphatases are widespread in prokaryotes and of various biological functions. In animal pathogenic bacteria, one of the important biological functions of HAD family phosphatases is its requirement for optimal invasion and intracellular survival in host tissues, which play important roles in virulence. However, the role of HAD family phosphatases in pathogenicity remains unknown in plant bacterial pathogens. In our previous work, the putative HAD like phosphatases encoding gene locus XAC0482 was identified as a biofilm formation related gene and named *bdp1* in the citrus pathogen *Xanthomonas citri* subsp. *citri* (*Xac*). Here we characterized the enzymatic activity of Bdp1 and renamed it as PspX for phosphoserine phosphatase activity. Furthermore, PspX mutants lacking phosphoserine phosphatase activity were nonmucoid on agar plates, and showed dramatically reduced multiplication and abolished virulence in susceptible host leaves. The PspX mutant also exhibited evident reduction in extracellular polysaccharide (EPS) production, cell motility, biofilm formation and stress tolerance. Genetic complementation assays showed that the affected phenotypes of the PspX mutant could be complemented to wild-type levels by the intact *pspX* gene. We propose that PspX dephosphorylates one or more proteins involved in EPS production, which in turn affect biofilm formation, cell motility, stress tolerance, and virulence on host plants.

RNA interference-induced *Heterodera glycines* resistance in soybean

J. LI (1), T. C. Todd (1), T. R. Oakley (1), H. N. Trick (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 102:S4.69

The soybean cyst nematode (SCN), *Heterodera glycines*, is the most severe biotic stress limiting soybean production, accounting for $4 billion loss per year worldwide. Current SCN management approaches nematicides, crop rotation and resistant varieties all have serious limitations. To explore alternative methods of SCN control, we deployed an *in planta* transgenic approach for suppressing SCN populations by transferring RNA interference (RNAi) constructs containing inverted repeats of 25 different *Heterodera glycines* genes into soybean plants, separately. Transgenic roots were evaluated by PCR, RT-PCR, Southern blot, siRNA Northern blot and small RNA sequencing. *H. glycines* bioassay results performed on the composite plants indicated that 7 out of 25 RNAi constructs resulted in significant reduction for *H. glycines* eggs, while the other 18 RNAi constructs did not have significant effects. Composite plants expressing RNAi construct of *Y-025* and *R-001* gene showed consistent suppression of *H. glycines* HG type 2.7 and HG type 7 populations. Stacking two genes together in one construct did not lead to more significant reduction for *H. glycines* development. Our research results indicated that RNAi strategy may provide effective and durable SCN control.

Molecular, serological, and biological characterization of a novel carlavirus infecting potatoes in China

Y. LI (1), R. Zhang (1), H. Xiang (1), H. Abouelnasr (1), D. Li (1), J. Yu (1), J. H. McBeath (2), C. Han (1)
(1) China Agricultural University, Beijing, Peoples Republic of China; (2) University of Alaska-Fairbanks, Fairbanks, AK, U.S.A.
Phytopathology 102:S4.69

A new carlavirus, tentatively named *Potato virus H* (PVH), was found on potato plants displaying mild symptoms in Hohhot, Inner Mongolia Autonomous Region. PVH was confirmed by genome sequencing, serologically reactions, electron microscopy and host index assay. The PVH particles were filamentous and slightly curved, with a modal length of 570 nm. The complete RNA genomic sequences of two isolates of PVH were determined by Reverse transcription PCR (RT-PCR) and 5`Rapid amplification of cDNA ends (5`RACE) methods. Sequence analysis revealed that the PVH had a genomic organization typical of members of the genus *Carlavirus*, with a positive-sense single-stranded genome of 8410nt. It shared CP and replicase amino acid sequence identities of 17.9-56.9% with those of reported carlaviruses. Phylogenetic analyses based on the amino acid sequences of replicase and CP revealed that PVH formed a distinct branch, which is only distantly related to the carlaviruses. Western blot assays showed that PVH was not serological related to other potato viruses (PVS, PVM and PoLV). PVH systemically infected *Nicotiana glutinosa*, but not *Nicotiana tabacum*, *Nicotiana benthamiana* or *Chenopodium quinoa* as other potato carlaviruses did. All these results support the classification of PVH as a novel species in the genus *Carlavirus*.

The host actin cytoskeleton is required for *Barley stripe mosaic virus* TGB3 cell wall localization

M. Li (1), J. Nam (1), C. Jang (1), E. Seo (1), J. Song (1), A. O. Jackson (2), J. Hammond (3), H. LIM (1)
(1) Chungnam National University, Daejeon, South Korea; (2) University of California-Berkeley, Berkeley, CA, U.S.A.; (3) USDA-ARS FNPRU, Beltsville, MD, U.S.A.
Phytopathology 102:S4.69

Barley stripe mosaic virus (BSMV) spreads from cell to cell through the coordinated actions of three triple gene block (TGB) proteins (TGB1, TGB2, TGB3) arranged in overlapping open reading frames (ORFs). The host cytoskeleton is one of the main routes for plant viruses to move within or

between cells. BSMV infection-induced actin filament thickening was visualized in the cytoskeleton of agroinfiltrated *Nicotiana benthamiana* epidermal cells expressing DsRed:Talin. Coexpression of fluorescent protein fusions GFP:TGB2 or GFP:TGB3 with the actin marker DsRed:Talin revealed that a portion of TGB2 and TGB3 co-localized with actin filaments, and that TGB2 and TGB3 induced filament thickening. GFP:TGB1 did not co-localize with DsRed:Talin and had no effect on the appearance of actin filaments in the absence of TGB2 and/or TGB3; however, in the presence of these proteins, a portion of the TGB1 fluorescence overlapped with Talin and was visualized in association with thick actin filaments. Treatment of cells with an inhibitor of actin polymerization, Latrunculin B, decreased the paired fluorescent foci at the plasmodesmata that are normally observed in cells expressing DsRed:TGB3 alone, or coexpressing GFP:TGB1, TGB2 + TGB3, and also retarded BSMV cell to cell movement. In addition, we are using virus induced gene silencing (VIGS) of actin expression to study whether deficiency of actin filaments affects TGB3 movement.

The effect of volatile organic compounds produced by *Ceratocystis fimbriata* on the growth of soilborne *Rhizoctonia solani* and rice seed germination

Q. LI (1), Q. J. Li (1)
(1) China Agricultural University, Beijing, Peoples Republic of China
Phytopathology 102:S4.70

Ceratocystis fimbriata, which produces volatile organic compounds (VOCs) during the cultivation on medium, is a widely distributed fungus in field soil and seriously causes kinds of plant diseases. The bioassay of *C. fimbriata* on the growth of soil-borne fungi and germination of crop seed is a key point for verifying the multi-action of this VOCs-fungus in soil ecosystem. Ten components of the compounds have been identified by headspace GC-MS method. The effect of the VOCs on the growing of soil-borne *Rhizoctonia solani* and rice seed germination was investigated. By means of vertical dual test in vitro without physical contact or diffusion through the culture medium, it indicated that mycelium growth of *R. solani* was strongly inhibited by VOCs generated from *C. fimbriata*, with the inhibitory rate of 82 %. The fumigated mycelium appeared thin, malformed, and even lost the ability of sclerotinia formation. The inoculated *R. solani* on rice seeds was completely controlled seven days after fumigation by six-day-cultured *C. fimbriata*. Remarkably, germination of rice seeds was also influenced by VOCs generated from *C. fimbriata.* These preliminary results pointed out clearly that VOCs-*C. fimbirata* possess both inhibition on soil-borne *R. solani* and rice seed germination.

Characterization and detection of *Tomato necrotic stunt virus*, a novel potyvirus infecting greenhouse tomatoes in Mexico

R. LI (1), Z. Fei (2), K. Ling (1)
(1) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC, U.S.A.; (2) Boyce Thompson Institute for Plant Research, USDA-ARS, Robert W. Holley Center for Agriculture and Health, Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.70

Greenhouse tomato production has increased significantly in recent years in North America. The highly intensive production system has created some unique ecological conditions for disease epidemic, especially viruses. Using small RNA deep sequencing, we previously identified a new potyvirus, named *Tomato necrotic stunt virus* (TNSV), from a tomato sample collected in 2009 in a greenhouse near Mexico City, Mexico. The complete virus genome sequence was obtained and validated through Sanger-sequencing. In the present study we were interested in characterizing the molecular and biological properties of TNSV and in the development of detection technologies. TNSV had a genome with less than 60% overall identity in both nucleotide and amino acid sequences to other potyviruses. Typical symptoms on the infected tomato were chlorotic and necrotic leaves, and plant stunting with poor fruit production. In a host range study in a growth chamber, TNSV caused local lesions on *Chenopodium* spp. and a systemic infection on a number of solanaceous plants. Several molecular-based detection methods, including RT-PCR, real-time RT-PCR and loop-mediated isothermal amplification (LAMP), were developed. A preliminary screening on diseased tomato samples collected from several greenhouses in Canada and the U. S. in 2012 did not detect the presence of TNSV. However, with the detection methods developed, additional survey will help us to reach a better understanding on its distribution.

The role of sigma factors in regulating virulence gene expression in *Erwinia amylovora*

W. LI (1), V. Ancona (1), Y. Zhao (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.70

In bacteria, gene expression is mainly regulated at the transcription initiation level and its core RNA polymerase (RNAP) requires sigma factors for promoter recognition and initiation. In this study, we investigated the role of several sigma factors in regulating virulence gene expression in *Erwinia amylovora*, a necrogenic enterobacterium causing fire blight of apples and pears. Early studies have shown that hrp-type III secretion (T3SS) in *E. amylovora* is regulated by HrpS, a member of the s54 enhancer binding proteins, and the master regulator HrpL, which belongs to the ECF subfamily of s factors. Other sigma factors characterized here include RpoN, a nitrogen-limitation s54 factor, and RpoS, which belongs to the s70 family. Our results showed that mutations in hrpS, hrpL, and rpoN, but not rpoS, resulted in non-pathogenic phenotype in host plant and no hypersensitive response in non-host tobacco. Consistently, expression of T3SS genes including hrpL, dspE, hrpN and hrpA was significantly reduced in hrpS, hrpL, and rpoN mutants than that of wild type (WT) strain. Amylovoran production was much higher in these mutants than that of WT strain, indicating sigma factors may also play roles in regulating exopolysaccharide production. These results suggest that sigma factors in *E. amylovora* are important virulence regulators and sigma factor cascades may exist in its regulatory networks.

Effects of light density on resistance of bigleaf hydrangea to Cercospora leaf spot

Y. LI (1), M. Windham (2), R. Trigiano (2), A. Windham (2), S. Reed (3), J. Spiers (4), T. Rinehart (5)
(1) Connecticut Agricultural Experiment Station, New Haven, CT, U.S.A.; (2) University of Tennessee, Knoxville, TN, U.S.A.; (3) USDA-ARS, McMinnville, TN, U.S.A.; (4) USDA-ARS, Poplarville, MS, U.S.A.
Phytopathology 102:S4.70

Bigleaf hydrangea (*Hydrangea macrophylla* Thunb) is one of the most popular, highly valued deciduous shrubs for its abundant dark-green foliage and its large flower clusters. Cercospora leaf spot (*Cercospora hydrangeae* Ellis & Everh.) is a common disease that causes premature defoliation and reduced plant vigor in nurseries and landscapes. Experiments were conducted to investigate the effect of light density on resistance of six bigleaf hydrangea varieties ('Blue Deckle,' 'Fasan, Lilacina,' 'Miranda,' 'Pretty Maiden,' and 'Sister Theresa') to Cercospora leaf spot. Two-year-old potted hydrangea plants were transplanted in the field plot with a randomized complete block design four light density treatments (0, 30, 60, and 90% density shade cloth hoophouses). Disease severity of Cercospora leaf spot was assessed on the plants at 7- to 10- day intervals from June to October in 2008 and 2009. The areas under the disease progress curves (AUPDC) were used to analyze effects of light density and hydrangea variety on the disease development. A significant interaction was detected between light density and hydrangea variety on standard AUDPC. In general, all six hydrangea varieties were more susceptible to Cercospora leaf spot with increasing light density. However, differences in standard AUDPC among light density treatments were more significant for Miranda than for the other varieties. The higher shade density the treatment was, the less differences in the standard AUDPC among hydrangea varieties were. These results provide valuable information for screening bigleaf hydrangea for resistance to Cercospora leaf spot and for disease management.

Inhibitory effects of 2-aminoimidazole compounds on *Monilinia fructicola*

K. L. LIBERATOR (1), R. J. Worthington (1), C. Melander (1), D. F. Ritchie (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.70

Brown rot is one of the most important diseases of stone fruits and is caused by the fungus *Monilinia fructicola* in the southeastern United States. Populations of this pathogen have evolved resistance to several fungicides since the early 1970's starting with benomyl. More recently, DMI fungicide resistance and reduced sensitivity to QoI fungicides have been reported in several eastern U.S. peach orchards. Synthetic analogues of the marine natural product oroidin, called 2-aminoimidazole (2-AI) derivatives, were recently shown to inhibit and disperse fungal (eg. *Candida*) and bacterial biofilms and suppress antibiotic resistance in human pathogenic bacteria. Thus our objective was to evaluate the potential antifungal activity of 2-AI compounds on plant pathogenic fungi using *M. fructicola*. *In vitro* screens were utilized to assess the impact on spore germination and to calculate the concentration required to suppress mycelial growth by 50%. Additionally, analogues with differing chemical structures were investigated and results indicated the importance of a positively charged compound for inhibition of conidial germination. A 2-AI compound also similarly inhibited growth of *M. fructicola* isolates with decreased sensitivity to benomyl, DMI, and QoI fungicides, and fungicide sensitive isolates *in vitro*. A study utilizing electron microscopy was conducted to examine the effect of a 2-AI analogue on the ultrastructure and gross morphology of *M. fructicola* conidia.

The first report of the occurrence of begomoviruses in a cucurbit species in Brazil

M. F. LIMA (1), N. R. Madeira (1)
(1) Embrapa, Brasília, Brazil
Phytopathology 102:S4.71

Geminiviruses transmitted by the whitefly *Bemisia tabaci* (genus *Begomovirus*) have been reported on cucurbit species in many countries, usually causing severe economic losses. In early December, 2011, stuffing cucumber plants (*Cyclanthera pedata* L. Schrad.) exhibiting intense yellow mosaic, leaf malformation, blistering, reduction in the leaf size and stunting, with nearly 80% of incidence, were observed in the experimental field at Embrapa Vegetables, Brasília-DF, Brazil. Extracts from infected plants tested negative by dot-blot analysis with antisera against potyviruses (*Papaya ringspot virus-type W*-PRSV-W; *Zucchini yellow mosaic virus*-ZYMV; *Watermelon mosaic virus-2*-WMV-2), a tospovirus (*Zucchini lethal chlorosis virus*-ZLCV), and a cucumovirus (*Cucumber mosaic virus*-CMV). Total DNA was extracted from symptomatic plants and tested for the presence of geminiviruses by PCR using universal primers. Unexpectedly, all symptomatic samples tested positive, producing DNA fragments of ca. 1.2 kb. The total DNA was subjected to rolling cycle amplification (RCA) resulted on the production of high molecular weight DNA molecules. A preliminary direct sequencing of the RCA-products confirmed the presence of at least two geminivirus species infecting stuffing cucumber plants. Enzymatic digestion of RCA-products to identify unique sites to facilitate cloning full-length geminiviral genomes is underway. These results clearly indicate the need of performing surveys of geminivirus occurrence on cucurbit species in the country. This is the first report of geminiviruses infecting cucurbits in Brazil.
Financial support: Embrapa, CNPq.

Physiological and proteomic characterizations of '*Candidatus* Liberibacter' associated diseases in citrus and potato plants

H. LIN (1), C. C. Nwugo (2), Y. Duan (3)
(1) USDA-ARS PWA, Parlier, CA, U.S.A.; (2) USDA-ARS, Parlier, CA, U.S.A.; (3) USDA-ARS, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.71

'*Candidatus* Liberibacter' species (Lib) are fastidious gram-negative bacteria transmitted by psyllids and associated with citrus huanglongbing (HLB) and potato zebra chip (ZC) diseases. Here, proteomic analyses via 2-DE and mass spectrometry were employed to elucidate protein expression profiles in tissues of grapefruit, lemon and potato plants that were infected or uninfected with Lib. Inductively Coupled Plasma Spectroscopy was also used to identify the effect of Lib infection on nutrient status of the same plants. The analysis indicated that Lib infection altered protein expression profiles in leaves of grapefruit (200 spots) and lemon (70 spots). Protein profiles of aboveground (107 spots) and belowground (95 spots) tissue of potatoes also were differentially expressed. Interestingly, protein mass spectrometry analysis showed that chitinase, starch synthase and patatin are among a group of proteins that were significantly up-regulated in citrus and/or potato tissues in response to Lib infection. Furthermore, nutrient status analysis suggests that accumulation of Zn and Ca in citrus plants, as well as Fe, Cu, and particularly K, in potato plants is associated with Lib infection. In summary, results provide insights into potential host-specific response mechanisms associated with HLB and ZC.

Genotyping and population genetic analysis of '*Candidatus* Liberibacter solanacearum', bacterium associated with potato zebra chip disease

H. LIN (1), M. S. Islam (2), A. Wen (3), N. Gudmestad (3)
(1) USDA-ARS PWA, Parlier, CA, U.S.A.; (2) USDA-ARS, Parlier, CA, U.S.A.; (3) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.71

'*Candidatus* Liberibacter solanacearum' (CLso) is associated with Zebra Chip disorder of potatoes. In this study, a panel of eight simple sequence repeat (SSR) markers was developed and used to characterize CLso isolates obtained from ZC-affected potato plants grown in the United States and Mexico. Multilocus SSR markers effectively detected genetic variation among CLso isolates. Genotypic assignment analysis identified two major lineages of CLso in the North America populations. Structure analysis failed to identify haplotypic patterns associated with the geographical proximity in several regions, including Mexico, Colorado, Nebraska, Kansas, and California. Furthermore, genetic analyses in this study revealed that no host association was found among haplotypes of CLso isolates. This genetic marker typing system in combination with epidemiological data will advance our knowledge in understanding the sources and dynamics of the ZC disease and develop effective disease management.

A multiplex TaqMan real-time RT-PCR assay for detection of Asian prunus viruses, *Plum bark necrosis stem pitting associated virus*, and *Peach latent mosaic viroid*

L. LIN (1), R. Li (1), R. Mock (1), G. Kinard (1)
(1) USDA-ARS, Beltsville, MD, U.S.A.
Phytopathology 102:S4.71

Asian prunus viruses (APV 1, APV 2, APV 3), *Plum bark necrosis stem pitting-associated virus* (PBNSPaV), and *Peach latent mosaic viroid* (PLMVd) infect stone fruit trees, and may impact fruit yield and quality. A multiplex, single tube TaqMan real-time RT-PCR assay was developed for the simultaneous detection and identification of these pathogens. The protocol includes amplification and detection of a fluorogenic cytochrome oxidase gene (COX) as an internal control. The results of the multiplex TaqMan RT-PCR detection correlated with those from conventional RT-PCR, and the assay was 10 to 100 times more sensitive than conventional RT-PCR. The limits of detection were 10^{-7}, 10^{-6}, and 10^{-4} for APV 1, PLMVd, and PBNSPaV, respectively, in the multiplex format. The efficiency and accuracy of the assay was evaluated by testing samples from both our positive control collections and field samples. Several previously undocumented naturally occurring mixed infections were detected including two peach trees co-infected with three pathogens (APV 1, PBNSPaV, PLMVd), and one peach tree co-infected with one of the APVs (amplified fragment has highest percentage identity with APV 1) and PBNSPaV. Additional validation is needed to ensure the protocol detects all three APVs. This assay is simple, rapid and cost-effective and can be used by quarantine and certification programs where numerous stone fruit trees need to be tested for all three pathogens.

Genetic diversity and phylogenetic analysis revealed *Pepino mosaic virus* in North America has shifted to Chilean genotypes

K. LING (1), R. Li (1)
(1) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC, U.S.A.
Phytopathology 102:S4.71

In nearly a decade, *Pepino mosaic virus* (PepMV) has become endemic on greenhouse tomatoes in North America. Several distinct genotypes of PepMV have been identified, including EU, US1 (CH1) and CH2. Previous population genetic studies conducted in 2006-2007 revealed a predominant EU genotype in Canada and the U.S., with US1 and CH2 genotypes also detected in some localities. In early 2010, a more aggressive disease was observed in a greenhouse in British Columbia, Canada and CH2 was identified. Subsequently, another disease epidemic was reported in a greenhouse near Mexico City, Mexico and shown also to be CH2. The objective of this study was to assess the current status of genetic diversity of PepMV in North America. Surveys were conducted in 2011-2012 on several greenhouses. As expected, nearly 100% of symptomatic samples collected were infected by PepMV in ELISA or RT-PCR. To allow for an efficient assessment of genetic diversity of PepMV, a genotype-specific loop-mediated isothermal amplification (LAMP) technology was developed. The phylogenetic relationship of PepMV population was evaluated through nucleotide sequencing of selected genomic regions. Results showed that the predominant genotype of PepMV in North America has shifted to CH2. How this genotype was introduced was not determined. The fact that the US1 (CH1) and CH2 genotypes were originally identified in the U.S. from an imported commercial seed lot suggests the possibility of seed sources.

Identification of expressed resistance gene analog (RGA) from peanut (*Arachis hypogaea* L.) expressed sequence tags (ESTs) and development of RGA-SSR marker

Z. Liu (1), S. Feng (1), M. K. Pandey (1), A. Culbreath (1), B. GUO (2)
(1) University of Georgia, Department of Plant Pathology, Tifton, GA, U.S.A.; (2) USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA, U.S.A.
Phytopathology 102:S4.71

Cultivated peanut (*Arachis hypogaea* L.) is an important food and oil crop grown in more than 100 countries for providing edible oil and protein. A wide variety of pathogens including fungi, bacteria, viruses, and nematodes severely constrain peanut yield and quality. Therefore, it is very important to better understand the interaction between peanut and pathogens. Large number of peanut ESTs (225,264 ESTs by 11-11-2011) has been developed in recent years by peanut scientific community, offering resources and opportunities for gene identification and marker development. Based on the homology to typical disease resistance genes and the conservative domains, we identified 401 resistance gene analogs (RGAs), using a stringent BLAST search. These expressed RGAs are comprised of 75, 196, 89, 27, and 14 RGAs representing for NBS-LRR, protein kinase (PK), LRR-PK/TM, toxin reductase, and other domain encoding *R* genes, respectively. By using MISA

software, 33 SSRs were identified from 28 expressed RGAs. Primers were successfully designed for 29 RGA-tagged SSRs and used for polymorphic screening among parents of two mapping populations. One of these markers, RGA121, was clearly polymorphic and segregating in two mapping populations, which was mapped on linkage group 01 along with the putative *qtswv2* (Qin et al. 2012. TAG), reported to contribute to the resistance to TSWV.

QTL mapping reveals effector-triggered susceptibility underlying the barley-*Pyrenophora teres* f. *teres* interaction

Z. LIU (1), S. Chao (2), J. D. Faris (2), M. C. EDWARDS (2), T. L. Friesen (2)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) Cereal Crops Research Unit, Northern Crop Science Laboratory, USDA-ARS, Fargo, ND, U.S.A.
Phytopathology 102:S4.72

Barley net form net blotch (NFNB), caused by the necrotrophic fungus *Pyrenophora teres* f. *teres*, is a destructive foliar disease in barley growing regions worldwide. To investigate the genetic and molecular basis of the barley-*Pyrenophora teres* f. *teres* (*Ptt*) interaction, we developed a population of recombinant inbred lines (RILs) from the barley lines 'Hector' (susceptible) and 'NDB112' (resistant), and used the RIL population to map genomic regions associated with disease as well as sensitivity to fungal effectors. A total of 692 SNP and 77 SSR markers were generated in this population, leading to a well-defined linkage map for QTL detection. Disease evaluation in this population utilized 11 NFNB isolates that were collected from different countries, including Australia, Brazil, Canada, Japan, and the US. QTL mapping revealed that different barley genomic regions governed resistance/susceptibility to the various *Ptt* isolates used, suggesting that different effector-host gene interactions may be involved depending on the geographical region. However, several regions of the genome, including one on chromosome 6H, are regularly associated with disease QTL. Using protein extracts of intercellular wash fluids (IWF) from infected leaf tissues, we found that a major QTL on 6H co-localized with a region conditioning sensitivity to one or more fungal effectors present in the IWF. This work indicates that the barley-*Ptt* pathosystem may follow the effector-triggered susceptibility model that has been described in other necrotrophic fungal disease systems, especially in the Dothideomycete class of fungi.

Early disease development of cherry powdery mildew and population dynamics of *Podosphaera clandestina* in the orchard air in eastern Washington

Q. LIU (1), H. Yan (1), M. E. Nelson (1), G. G. Grove (1)
(1) Washington State University, Prosser, WA, U.S.A.
Phytopathology 102:S4.72

Powdery mildew of cherry, caused by *Podosphaera clandestina*, is the most important fungal disease affecting crop production in eastern Washington. In this study, incidence of early infections, disease development in trees with and without early infection, and pathogen population dynamics in the orchard air were determined. Percentage of infected leaf area (PILA) of 5 sampled leaves at each early infection locus (EIL) and for the first 5 fully expanded leaves of each terminal shoot (beginning from the shoot apex), 10 shoots per tree were recorded weekly within 40 trees in a cherry (cv. Bing) orchard from May 24 to June 16 in 2011. Conidia were sampled using two air samplers and their quantity was estimated with a qPCR assay. EIL were found in 9 of 40 trees, one per tree on foliage close to tree trunks or scaffold limbs. On June 2, average PILA was 19.2 for EIL, while only 0.18 on foliage from the outer portions of the tree canopy. During the evaluation period, average PILA and percentage of infected leaves increased from 0.16 to 2.4 and 10.2 to 81.1, respectively, for trees with EIL, while from 0.06 to 0.84 and 6.3 to 57.9, respectively, for trees without EIL. None or weak qPCR signals were detected in May, however, signals were detected daily from the beginning of June with gradually increasing strength. These results indicate that more vigorous management of EIL might be an effective practice in delaying early disease development and air sampling could help detect inoculum increases originating from early secondary infections.

Understanding the impact of *Pythium* species on floricultural crops in North Carolina

E. LOOKABAUGH (1), K. Ivors (1), M. Benson (1), B. Shew (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.72

Although *Pythium* is frequently diagnosed in floricultural crops, the practical significance of these findings often is unclear. Floricultural crops in NC have not been systematically sampled for the presence of *Pythium*, the predominant species have not been identified, and the extent of mefenoxam resistance is unknown. Herbaceous ornamentals exhibiting symptoms of Pythium root rot were collected from 18 greenhouses in 14 counties in NC. Roots were assayed on selective media for the presence of *Pythium*. Isolates were recovered from 24 host species. Isolates were screened for mefenoxam sensitivity on 5% clarified V8 agar amended with 100 ppm mefenoxam, dispensed in 48-well micro-titer plates. Colonization by 3 samples/isolate was scored after 24 to 48 h on a scale of 0 (no growth) to 5 (entire well colonized). Of 277 isolates, 54.2% were considered resistant to mefenoxam (mean score $\geq$ 4). Selected isolates were identified by sequencing of the ITS rDNA region. Twelve species were identified, with *P. myriotylum*, *P. aphanidermatum*, and *P. irregulare* comprising 85% of the 94 isolates sequenced thus far. *P. aphanidermatum* was recovered at 8 locations, *P. myriotylum* at 5, and *P. irregulare* at 7. Eight locations had >1 species present. All isolates (19) of *P. myriotylum* were sensitive to mefenoxam. We found both sensitive and resistant isolates of *P. aphanidermatum* and *P. irregulare*. Resistant and sensitive strains of the same species were found within the same greenhouse.

Evaluation of fungicides and mustard meal to manage black root rot of strawberry and analysis of *Pythium*, *Fusarium*, and *Rhizoctonia* on strawberry roots

F. Louws (1), J. SUN (1), H. Whittington (1), J. Driver (1), K. Peeden (1), B. Liu (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.72

Strawberry growers face increasing pressure to implement non-fumigation based approaches to manage soilborne pathogens. 2012 is the last year methyl bromide can be used as a pre-plant soil fumigant and new federal government measures confer greater regulatory impacts on fumigation application procedures. A multi-year fumigation experiment was conducted in eastern NC to determine the potential of fungicides or mustard meal to suppress selected fungal/oomycete black root rot (BRR) pathogens compared to fumigant standards. Fungicides were applied as dips prior to planting or drip applied at selected times based on knowledge of the complex and dynamics of the pathogen community. MeBr formulated with chloropicrin (50:50) and PicClor60 generated similar yields each year and were always significantly superior to non-fumigated controls as determined by plant growth and crop yield. In yr 1, selected fungicide dip/drip treatments generated modest yield benefits compared to controls and the fumigation standards. In yr 2, most fungicide-based programs performed similar to the controls and significantly poorer than the fumigants with one exception that is being repeated in yr 3 (2011-2012 cycle) for verification. Mustard meal and deactivated mustard meal applications had a modest impact on disease severity and yield. Field treatments were complemented by culture- and molecular-based methods to assess microbial communities of the soil and roots with an emphasis on *Pythium, Fusarium*, *Rhizoctonia* and *Trichoderma*. Field experiments complemented with microbial community analysis provide a framework to extend the science and practice of BRR management to growers, particularly those seeking to eliminate fumigant use.

Salicylic acid suppression of clubroot (*Plasmodiophora brassicae*) in *Arabidopsis thaliana* and *Brassica oleracea*

D. LOVELOCK (1), C. Donald (2), X. Conlan (1), D. Cahill (1)
(1) Deakin University, Geelong, Australia; (2) Department of Primary Industries, Ferntree Gully, Australia
Phytopathology 102:S4.72

The phytohormone, salicylic acid (SA), is required for a number of physiological processes within plants but primarily is a plant defence signalling molecule required for systemic acquired resistance (SAR). *Plasmodiophora brassicae*, causal agent of clubroot, is a soil borne obligate biotroph and is responsible for losses in Brassicaceae crops, including broccoli. We are investigating the response of a number of *Arabidopsis* ecotypes to infection by *P. brassicae* and following treatment with SA. Wild type Tul-0 and Tsu-0 was tolerant to an Australian isolate of *P. brassicae*, whilst Col-0 and Ler were susceptible. *Arabidopsis* mutants assessed for their response to the pathogen reveal that over-expressing SA mutants show restriction in gall formation. For these interactions SA is clearly a key molecule in regulating the outcome of the interaction. We are also examining SA-induced SAR in pre-plant and planting age (2 and 6 week old) broccoli seedlings, by pre-treatment of plants with SA. Broccoli plants were root drenched with SA at desired concentrations for 30 minutes before being transferred to pots containing *P. brassicae* inoculums at either 24 or 72 h post-treatment. A significant reduction in gall formation occurred in broccoli plants when treated with SA at concentrations between 2.5mM and 25mM in each treatment group. SA thus provides protection against clubroot in broccoli at the seedling stage.

The risk associated with irrigating ornamental nursery plants with water containing *Phytophthora*

A. LOYD (1), M. Benson (2), K. Ivors (3)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.; (3) North Carolina State University, Mills River, NC, U.S.A.
Phytopathology 102:S4.73

During the summer of 2011, disease-free plants were irrigated with *Phytophthora*-contaminated water to determine risk of plant infection. Two nurseries in Western North Carolina were selected because *Phytophthora* spp. had previously been detected in their irrigation water. Nursery I used water from an on-site retention basin, and nursery II used water from the French Broad River. Containerized *Rhododendron*, *Pieris*, and *Ilex* were used as 'trap' plants; water and plant roots were assayed for *Phytophthora* monthly for a total of 5 mo. Sixty-two isolates were recovered from the two water sources, while only eight isolates were recovered from the roots of plants. Isolates were identified by ITS sequence and included *P. cinnamomi*, *P. heveae*, *P. hungarica*, *P. hydropathica*, and members of the *P. pini-citricola* complex, plus six undescribed species. The most frequently recovered species from the river was *P.* taxon 'PgChlamydo', while, *P. hydropathica* was most frequently recovered in the retention basin. The only species recovered from roots and water was *P.* taxon 'PgChlamydo'. Slight root necrosis was observed on *Pieris* at nursery I, but root rot was not significant on the other plant species at either nursery. Although *Phytophthora* spp. were present in the irrigation water at about 20 and 75 propagules/liter at nursery I and II, respectively, the risk was low since foliar infection was nil and few root infections developed.

Differential expression of the pathogenesis-related protein 1 (*PR-1*) gene family in stem rust (*Puccinia graminis* f. sp. *tritici*)-wheat interactions

S. LU (1), T. L. Friesen (1), J. D. Faris (1)
(1) USDA-ARS, Cereal Crops Research Unit, Fargo, ND, U.S.A.
Phytopathology 102:S4.73

The group 1 pathogenesis-related (PR-1) proteins, known as hallmarks of defense pathways, are encoded by a multigene family in hexaploid wheat (*Triticum aestivum* L.) that includes at least 12 closely related *TaPr-1* genes responsive to infection by the necrotrophic pathogen *Stagonospora nodorum* (Sn). Here we report an expression analysis of the same set of *TaPr-1* genes in response to the biotrophic stem rust (SR) fungus *Puccinia graminis* f. sp. *tritici*. A SR-resistant cultivar (Bobwhite) and three susceptible lines (derived from Bobwhite) were grown and inoculated in the greenhouse under the same conditions. Reverse transcriptase PCR using *TaPr-1*-specific untranslated region-specified discrimination primers revealed that most Sn-inducible *TaPr-1* genes were also expressed in SR-wheat interactions, but the expression patterns differed significantly. Two *TaPr-1* genes that are up-regulated in both incompatible and compatible Sn-wheat interactions were found to be induced only in the SR-infected resistant line that showed a hypersensitive response (HR). In susceptible lines, several *TaPr-1* genes were expressed in un-inoculated plants, but not (or at a lower level) in inoculated plants that developed stem rust symptoms. These results suggested that PR-1 proteins play important roles in HR-based resistance and are controlled by independent defense pathways in necrotrophic vs. biotrophic pathogen-wheat interactions.

Factors affecting mycelium pigmentation and pathogenicity of *Sclerotinia sclerotiorum* on Valencia peanut

P. A. LUJAN (1), S. Sanogo (1), N. Puppala (2), J. Randall (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.; (2) New Mexico State University, Clovis, NM, U.S.A.
Phytopathology 102:S4.73

Sclerotinia sclerotiorum is reported as a fungal pathogen that typically produces white mycelium on growth medium. Recently, darkly-pigmented isolates (SD) of *S. sclerotiorum* were found on peanut in New Mexico and Texas. Studies were initiated to assess the effect of mycelium pigmentation on pathogenicity of *S. sclerotiorum*. Using melanin-inhibiting compounds, several non-pigmented mutants (SW) were generated. Melanin precursors, glyphosate, pH, and carbon sources were used to promote and suppress mycelium pigmentation in SD and SW isolates, and then the effect of mycelium pigmentation on pathogenicity on peanut was assessed. Both isolates remained non-pigmented on complete glucose and sucrose media. The SD isolate became darker on PDA amended with glyphosate than on non-amended PDA, while the SW isolate remained non-pigmented. The SD isolate became darker as temperature increased while the SW isolate was unaffected. Pigmentation was slightly affected by pH in the SD isolate but not in the SW isolate. Mycelium in the SD isolate was significantly lighter at pH 7 and 8 than at pH 5.6. On media amended with melanin precursors, the SW isolate became slightly darker than on non-amended PDA. Regardless of pigmentation, the SD isolate killed peanut plants within two weeks or caused lesions on leaflets within one week of inoculation. However, no such effects were observed with the SW isolate. These results suggest that mycelium pigmentation does not appear to affect pathogenicity of *S. sclerotiorum* on peanut.

Assessments of edge effect in intensity of HLB disease

W. LUO (1), T. Gottwald (2), M. S. Irey (3)
(1) USDA-ARS, Fort Pierce, FL, U.S.A.; (2) USDA, Fort Pierce, FL, U.S.A.; (3) Southern Gardens Citrus, US Sugar Corp., Clewiston, FL, U.S.A.
Phytopathology 102:S4.73

Better understanding of the vector-mediated patterns of Huanglongbing (HLB) spread is essential to inform and maximize disease management. From previous studies, edge effects are a significant characteristic of the HLB pathosystem and have been observed predominately in larger plantings. In this study, we investigated 1) the impact of different edges classes and orientations, 2) the quantitative influence of distance from edges, and 3) the temporal dynamics of each edge effect. Spatial analyses of edge effects were conducted on data from Southern Garden plantation in South Florida, where multiple assessments and mappings of HLB incidence were collected over a 2-yr period. Based on the geographical shape and orientation of the plantation, five different edge types were classified, including ponds, main roads (SN & WE), and internal plantation edges (voids) between blocks (SN & WE). With consideration given to the influence of variety and tree age as well, results clearly showed significant edge effect for ponds, SN and WE main road, and the estimated influence of distances from an edge, i.e., 120, 130 and 90m, respectively. These edge effects were consistent temporally as well across assessment dates. No obvious edge effects were found for internal plantation edges, which was probably due to their associated small void width. Placing more emphasis on management practices at plantation edges should result in improved HLB disease control.

WITHDRAWN

Repeating structure of internal transcribed spacer region 2 in *Peronosclerospora* spp. downy mildews

D. G. LUSTER (1), M. L. Carter (1), G. L. Peterson (1), M. B. McMahon (1)
(1) USDA-ARS, Fort Detrick, MD, U.S.A.
Phytopathology 102:S4.73

Peronosclerospora philippinensis and *P. sacchari* are closely related species of oomycetes causing downy mildew disease of sugarcane. Both species are also capable of causing significant disease loss in maize. Neither of these pathogens is endemic to the United States and introduction of either pathogen could be devastating to the sugarcane industry. *P. philippinensis* and *P. sacchari* are both listed as Select Agents by USDA- Animal and Plant Health Inspection Service, and diagnostic assays are needed to detect these pathogens in plant material and environmental samples. We present research that investigated the utility of the ribosomal RNA gene internal transcribed spacer 2 (ITS 2) region, a widely used marker for taxonomic identification, as the foundation for diagnostic development. Upon amplification, the ITS 2 regions of *P. philippinensis*, *P. sacchari* and closely related *Peronosclerospora* spp.

were found to be unique in structure, composed of a ladder of multiple amplified fragments in multiples of *ca.* 200bp, up to *ca.* 1.6Kbp. ITS sequences from *P. phillippinensis* and *P. sacchari* contained repeating elements of approximately 225 base pairs, resulting in a range in ITS 2 spacer region lengths from 225 (1 repeating element) to 1575 (7 elements) bp. The unique sequence element structure of this region demonstrates the potential of the ITS 2 spacer as a target in the design of specific primers for polymerase chain reaction assays and other diagnostic technologies.

WITHDRAWN

Sequence analysis of the capsid protein of a *Cherry leaf roll virus* isolate causing blackline disease of walnuts in California

N. Lynn (1), A. Rowhani (2), M. R. SUDARSHANA (1)
(1) USDA-ARS, Davis, CA, U.S.A.; (2) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.74

Blackline disease of walnuts (*Juglans regia* L.) is a major problem for walnut production in Northern California, particularly in the counties close to San Francisco Bay. The disease is a graft union disorder caused by hypersensitive response of rootstocks of *J. hindsii* parentage to systemic infection of English walnut scions by *Cherry leaf roll virus* (CLRV). To understand genetic relationship of the walnut strain of CLRV, we cloned and analyzed the viral genome spanning most of the coding regions of RNA-1 and RNA-2 of a virus isolate obtained from walnuts in California. The dsRNA fractions obtained from systemically infected *Chenopodium quinoa* plants inoculated with CLRV were converted into cDNA and subjected to deep sequencing by Illumina and the contigs were assembled. The draft sequence was corrected using sequence reads obtained from overlapping RT-PCR products spanning the virus genome. Analysis of the predicted amino acid sequence of the capsid protein indicated that the walnut isolate was 97% identical to a Rhubarb isolate from Germany and 86% identical to a cherry isolate of CLRV from Washington state in the US.

Population diversity of '*Candidatus* Liberibacter asiaticus' in southern China revealed by tandem repeat number variation in a hypervariable genomic locus

W. Ma (1), X. Wen (1), Y. Gu (1), X. DENG (1), J. Chen (2)
(1) South China Agricultural University, Guangzhou, Peoples Republic of China; (2) USDA-ARS PWA, Parlier, CA, U.S.A.
Phytopathology 102:S4.74

'*Candidatus* Liberibacter asiaticus' is a putative pathogen of citrus Huanglongbing (HLB, yellow shoot disease). HLB is highly destructive and has occurred in southern China for over a hundred years. Due to unsuccessful *in vitro* cultivation of the bacterium and lack of efficient markers to discriminate bacterial strains, population diversity of '*Ca*. L. asiaticus' in southern China remains poorly studied. A PCR primer set flanking a genomic region with variable tandem repeat numbers (TRNs) has been developed to differentiate '*Ca*. L. asiaticus' populations between Guangdong, China and Florida, U.S.A. In this study, TRN variations of '*Ca*. L. asiaticus' were further investigated with samples from Guangdong and seven other provinces (Fujian, Guangxi, Guizhou, Hainan, Jiangxi, Yunnan, and Zhejiang) in southern China. Among the 226 HLB samples studied, 177 (78.3%) yielded single PCR amplicons. Sequence analysis showed a continuous distribution of TRNs ranging from 2 to 24 among the 177 amplicons, with the mode at TRN=8 (39 samples, 22.0%). The wide TRN range suggests a high level of bacterial population diversity that matches well with the long history of HLB in China. On the other hand, over 60% of the samples had TRNs of 6, 7 or 8, suggesting selection of these genotypes. Interestingly, 49 out of the 226 (21.7%) samples showed multiple PCR amplicons. Results from sequence analyses suggested the presence of multiple genotypes/strains of '*Ca*. L. asiaticus' in a single citrus host.

Effect of two bacterial biocontrol agents on Macrophomina root rot and powdery mildew disease severity in flowering dogwood

L. MACKASMIEL (1)
(1) Tennessee State University, College of Agriculture, McMinnville, TN, U.S.A.
Phytopathology 102:S4.74

Previous studies have shown that foliar sprays or root drenching with two bacterial biocontrol agents (BCAs), B17A and B17B produced similar results in reducing powdery mildew severity and boosting plant growth in flowering dogwood. In vitro evaluation of B17A and B17B on root rot pathogen, *Macrophomina phaseolina* grown in potato dextrose agar, showed significant inhibition of *M. phaseolina* colony size. In vivo evaluation of the two BCAs on *M. phaseolina* in flowering dogwood seedlings exposed to powdery mildew from airborne inoculum, showed that the two bacterial BCAs reduced root rot severity and powdery mildew disease incidences and improved plant growth compared to non-treated controls. Effects of B17A and B17B were superior to those of fungicide treatments, fungal and yeast BCAs. Stem diameter and plant dry weights were significantly higher and plants maintained their green color longer than all other treatments. Results from this study shows that B17A and B17B have great potential not only in managing powdery mildew and boosting plant growth, but also in managing soil-borne Macrophomina root rot.

Predisposition factors affecting brown spot disease development in rice

N. F. MAGCULIA (1), A. H. Sparks (1)
(1) International Rice Research Institute, Los Baños Laguna, Philippines
Phytopathology 102:S4.74

Brown spot, causal agent *Cochliobolus miyabeanus* (*Bipolaris oryzae*) is a chronic disease of rice affecting millions of hectares of rice each season. However, much remains to be understood about the epidemiological processes involved in brown spot disease development. In this study, we wished to determine the effects of water regimes and soils, classified by brown spot incidence and severity, on the susceptibility of rice plants to brown spot. Inoculated and non-inoculated seeds of rice variety IR 24 were sown in potted soils representing three varying levels of brown spot disease intensity. At four weeks after emergence, each seed treatment by soil class combination was subjected to two water regimes expressed in fraction of transpirable soil water until maturity. Disease variables: tiller incidence (%); leaf incidence (%); and brown spot severity at leaf position three (%), were assessed at mid-tillering, booting, milk and ripening development stages. Significant effects ($P \leq 0.05$) were observed between soil classes, water regimes, and soil class by seed treatment by water regime. On the other hand, no significant seed treatment effect was observed, but, when seed treatment was coupled with either soil class or water regime, a significant combination effect was found. Our results show that soil classes, water regimes and their interaction with seed treatments contribute to brown spot disease development in rice.

Efficacy of chemical and biological treatments on the control of premature vine decline in California processing tomatoes

N. N. MAHARAJ (1), E. M. Miyao (2), J. H. Leveau (1), R. M. Davis (1)
(1) University of California-Davis, Davis, CA, U.S.A.; (2) University of California Cooperative Extension, Yolo County, Woodland, CA, U.S.A.
Phytopathology 102:S4.74

Premature vine decline of tomatoes is a phenomenon whereby vine health declines at the early fruit ripening stage. In 2011, two field trials were conducted in commercial fields in Yolo and Solano counties. Our objective was to evaluate the possible involvement of soilborne diseases in vine decline by the application of fungicides and biological materials through buried drip tape. Treatments included: Vapam (15 gal/A); Tenet (1134 g/A); Vapam + Tenet; Quadris (183 ml/A) + Ridomil Gold (473 ml/A, 3 applications); Vapam + Quadris + Ridomil Gold; Serenade Soil (1st application: 3785 ml/A, remaining applications: 1893 ml/A); Serenade Soil + Tenet; Serenade Soil + Quadris + Ridomil Gold; Vapam + Serenade Soil; SoilGard (2268 g/A); and composted chicken manure incorporated at 10 tons/A. Fungicides and

biological materials were applied four times at three week intervals starting at planting. At the first site, none of the treatments reduced the incidence of Fusarium Wilt and Fusarium Crown and Root Rot, which occurred in more than 30% of the plants, or Verticillium Wilt, which was identified in more than 20% of the plants. However, yields were significantly greater where the composted chicken manure was incorporated (45.4 tons/A) relative to the non-treated control (34.3 tons/A). At the second site, none of the treatments reduced the incidence of Verticillium Wilt (>65%) or Corky Root (>95%) or affected yields. The role of soilborne diseases in vine decline remains inconclusive since none of the treatments reduced disease.

Engineering plant defenses to broaden resistance in soybean to soybean cyst nematode

A. MALDONADO (1)
(1) USDA, Beltsville, MD, U.S.A.
Phytopathology 102:S4.75

Soybean (*Glycine max*) is a major commercial crop, cultivated in more than 78 million acres in the U.S., generating important revenues every year. Nevertheless, the yield is severely reduced by the effect of pathogens like the soybean cyst nematode (SCN), *Heterodera glycines*, that causes $500 to $900 million in soybean yield losses annually in the United States. However, there are no soybean cultivars available that are resistant to all SCN populations. Chemical control of pathogens has proven not only very polluting for the environment, but also economically inefficient; therefore efforts have been directed mainly to enhance plant genetic resistance as the means to enhance resistance of soybean to SCN. Through gene expression analysis using microarrays, Illumina RNA-Seq, and the literature, we identified candidate genes to over-express to determine if they provide some resistance to SCN. The genes were cloned into our in-house expression vector pRAP15 containing the gene for green fluorescent protein (eGFP). The gene constructs were inserted into *Agrobacterium rhizogenes* for transformation of the base of soybean plantlets to produce composite plants with transformed roots which were identified by the presence of eGFP. The roots were challenged with SCN and the number of the mature females were counted after 35 days after inoculation. Reduction in the number of the nematodes by some of the gene constructs indicated that those particular genes may be useful for engineering SCN resistance in soybean.

Goss's wilt: Can *Clavibacter michiganensis* subsp. *nebraskensis* infect corn through natural openings?

S. Mallowa (1), S. SCHEIDING (1), A. Ahamed (1), A. Robertson (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.75

Goss's wilt, caused by the Gram positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis* or Cmn, is a re-emerging disease that threatens corn production in the US Corn Belt. In 2008, for the first time after 25 years, Goss's wilt was reported in about 50% of Iowa's counties with significant yield losses reported by farmers. The main mode of Cmn infection is usually through wounds on the leaves, caused by strong winds or hail. However recent outbreaks in Iowa and Nebraska have sometimes been reported in the absence of obvious injury to the leaves. This has led to the hypothesis that epiphytic populations of Cmn could function as an inoculum reservoir for Goss's wilt, with the bacteria getting in through natural openings. Preliminary data from greenhouse studies has shown infection of corn seedlings can occur without injury at a low incidence of 20-30 percent compared to 90 percent when leaves are injured. Disease incidence appears to be a function of the growth stage of the corn seedling and inoculum concentrations. Greenhouse studies to determine when corn seedlings are most susceptible to infection and the minimum inoculum concentration needed to cause symptoms are currently on-going. This information would provide a clearer understanding of the role of epiphytic Cmn in Goss's wilt infection and assist in development of an improved management method for this economically important disease of corn.

Widespread distribution of Goss's bacterial leaf blight and wilt of corn and potential variation in virulence of *Clavibacter michiganensis* subsp. *nebraskensis* in Minnesota

D. K. MALVICK (1), R. D. Curland (1), C. A. Ishimaru (2)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) Department of Plant Pathology, University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 102:S4.75

Goss's wilt is a significant corn disease caused by *Clavibacter michiganensis* subsp. *nebraskensis* (CMN). Goss's wilt has recently been spreading and increasing in the Midwestern U.S. and was first confirmed in Minnesota (MN) in 2009. We initiated studies in 2011 to: (i) determine and confirm the distribution of Goss's wilt in MN, and (ii) to determine if isolates of CMN from MN vary in virulence to corn hybrids with different levels of resistance to Goss's wilt. Leaf samples with symptoms typical of Goss's wilt were collected across southern and central MN. Samples were diagnosed based on symptoms and signs, and Goss's wilt was confirmed by isolation of pure cultures of CMN from lesions. CMN isolates were verified with PCR and immunoassays. Goss's wilt was confirmed in 31 counties in southern and central MN and in the southern Red River Valley in MN, and it likely occurred in additional counties. Pathogenicity studies were conducted in a greenhouse with four isolates of CMN from MN and eight corn hybrids with resistance ratings ranging from susceptible to resistant. Preliminary results are consistent with the hypothesis that isolates vary in virulence on individual and among different hybrids. In summary, Goss's wilt is widespread in MN for the first time and there is an increased risk for this disease in most corn producing regions. Additional studies are in progress to determine the potential significance of variability in virulence among isolates of CMN.

Effect of planting date, fungicide timing, and varietal susceptibility levels on development of narrow brown leaf spot of rice

K. K. MANI (1), C. Hollier (1), D. E. Groth (2)
(1) Louisiana State University, Agcenter, Baton Rouge, LA, U.S.A.; (2) Louisiana State University, Agcenter, Crowley, LA, U.S.A.
Phytopathology 102:S4.75

Narrow brown leaf spot (NBLS) of rice is a fungal disease caused by *Cercospora janseana* L. Miyake. It produces long narrow lesions on leaves, netted lesions on sheaths, and discoloration of glumes and seeds of rice plants. Recent increases in incidence of high disease pressure have been reported and are a serious concern for growers and researchers. Studies have been conducted to determine the effect of planting date, fungicide timing and varietal susceptibility levels on development of NBLS disease at the Rice Research Station, Crowley. To accomplish the objectives, four varieties with varying levels of susceptibility; Cheniere, CL111, CL131 and CL151; were planted in mid-April and mid-May. Fungicide treatments of propiconazole (Tilt at 6 fl.oz /acre), plus an untreated check, were applied at panicle initiation, early boot or late boot stages. In-field weather stations were set up in the untreated variety plots to determine within-canopy conditions for disease development. Weekly observations were recorded on disease incidence and disease severity in lower, middle and upper canopy. Preliminary results have shown significant interactions among varieties, treatments and planting dates. Significantly lower yield loss was found at the mid-April planting date with a fungicide treatment at the early boot stage and at the second planting date at panicle initiation stage. Fungicide applications at panicle initiation in susceptible varieties and early boot and late boot stage in moderately susceptible variety (CL151) have resulted in less disease severity. Yield loss was found to be lower in the moderately susceptible variety (CL151) as compared to very susceptible varieties (CL131). Slow disease progression was observed in CL151 as compared CL131 and Cheniere.

The CRT1 family is required for plant immunity against *Phytophthora infestans*

P. M. MANOSALVA (1), H. Kang (1), D. F. Klessig (1)
(1) Boyce Thompson Institute for Plant Research, Ithaca, NY, U.S.A.
Phytopathology 102:S4.75

CRT1, an endosomal-localized ATPase, was identified in a genetic screen for mutants Compromised for Recognition of *Turnip crinkle virus*. The *CRT1* gene family was shown to be required for *R* gene-mediated resistance against bacteria and oomycete pathogens (Kang *et al.*, 2008 and 2010). We have assessed the role of *CRT1* family in non-host resistance against *Phytophthora infestans* using an *Arabidopsis* double knock out (dKO) mutant *crt1-2 crh1-1*, which lacks *CRT1* and its closest homolog. The *Arabidopsis* dKO is compromised in a set of pre-invasion defenses activated during the non-host interaction between *Arabidopsis* and *P. infestans* and is nearly as compromised for resistance as the prototypic non-host resistance mutant *pen2*. Interestingly, CRT1 was found to interact with the PAMP recognition receptor FLS2, as well as with their associated kinases BAK1 and BIK1. In addition the single *Arabidopsis* mutants *fls2*, *bak1*, and *bik1* as well as the triple mutants *dKOfls2*, dKObak1, and *dKObik1* were compromised in callose deposition after inoculations with *P. infestans*, indicating a role for these PTI components during non-host resistance. To determine the role of CRT1 during resistance against *P. infestans* in its solanaceous hosts, we assessed the effect of overexpression and silencing of CRT1's potato and tomato homologs on resistance to this oomycete pathogen in these two hosts using stable transgenics and using VIGS in *N. benthamiana*. Our results indicate that CRT1 is also required for host resistance against *P. infestans*. Together, these findings argue that CRT1 is an important component during plant immunity against this devastating oomycete pathogen.

Integrated measures approaches as a pest risk management strategy for plants for planting: The case of *Dracaena* plants from Costa Rica
C. MARASAS (1)
(1) USDA APHIS, Riverdale, MD, U.S.A.
Phytopathology 102:S4.76

The United States is a net importer of live plants. Costa Rica is one of the major countries from which live plants are imported into the United States, and *Dracaena* plants comprise a major proportion of live plant imports from Costa Rica. However, *Dracaena* plants from Costa Rica have been associated with high pest interception rates at ports of entry into the United States. Over the past years, the Animal and Plant Health Inspection Service of the United States and the National Phytosanitary Service of Costa Rica have collaborated in several efforts to reduce pest interception rates on *Dracaena* plants from Costa Rica. This has culminated in the development of a pest risk management strategy and regulations which require all *Dracaena* imported from Costa Rica into the United States to be produced, packed and shipped under a systems approach with several integrated measures. These regulatory initiatives are in line with current national and international phytosanitary policy, which increasingly emphasizes integrated measures approaches as strategies to manage the pest risks associated with plants for planting, instead of relying on single measures such as port of entry inspection or treatment alone.

Seed transmissibility of sugarcane white leaf phytoplasma
C. J. MAROON-LANGO (1), H. M. Brown (1), U. Pliansinchai (2)
(1) USDA APHIS PPQ PHP PGQP, Beltsville, MD, U.S.A.; (2) Mitr Phol Sugarcane Research Center Co., Ltd., Phukieo, Chaiyaphum, Thailand
Phytopathology 102:S4.76

Sugarcane is traditionally exchanged between countries in the form of vegetatively propagated cane setts. However, interest in importing sugarcane fuzz (true seed) has increased significantly due to increased desire to improve diversity in sugarcane breeding programs. Importing sugarcane fuzz into the United States involves quarantine, pathogen testing, and production of progeny fuzz in approved greenhouse facilities, which is nearly impossible due to the strict environmental and physiological requirements necessary for sugarcane to flower. Relaxation of such restrictions requires scientific data on seed transmissibility of exotic sugarcane pathogens, including phytoplasma, which unfortunately is lacking in the literature. To assess the seed transmissibility of white leaf phytoplasma in sugarcane, fuzz from a cross of sugarcane lines susceptible to white leaf phytoplasma was collected from sugarcane fields in Thailand that had the disease and evaluated under quarantine in Beltsville for the presence of the white leaf phytoplasma. DNA extracted from both untreated fuzz and fuzz treated with 30% commercial bleach (with 6.15% sodium hypochlorite) using the modified CTAB/DNeasy Plant Mini Kit Method (Qiagen) tested negative for phytoplasma by nested PCR using primer sets P1/P7 and P1A/16S-SR. A total of 536 three month-old sugarcane plants that germinated from bleach-treated fuzz tested negative for white leaf phytoplasma by nested PCR using the same primer sets.

Phylogeny of the genus *Phytophthora* estimated by multilocus analysis of mitochondrial genes and comparison with the nuclear phylogeny
F. N. MARTIN (1), J. E. Blair (2), M. D. Coffey (3)
(1) USDA-ARS, Salinas, CA, U.S.A.; (2) Franklin and Marshall College, Lancaster, PA, U.S.A.; (3) University of California, Riverside, CA, U.S.A.
Phytopathology 102:S4.76

A multilocus phylogeny of the genus *Phytophthora* was undertaken using the mitochondrial encoded cox2, nad9, rps10 and secY genes (2,373 bp). The same isolates used in the previous nuclear multilocus analysis of Blair et al. were included along with more recently described and provisional species (in particular from clades 6, 7 and 9) for a total of 106 accessions. The results obtained were nearly identical to the nuclear phylogeny. While some minor differences in the placement of some species within a clade was encountered, with a few exceptions these groupings were not supported by bootstrap values in either analysis. A combined analysis with both the nuclear and mitochondrial data (10 loci, 10,828 bp) generated a similar tree with improved bootstrap support for some nodes, although many of the basal nodes showing the relationship among the major clades are still unresolved. A greater level of sequence divergence was observed with the mitochondrial data as illustrated by the longer branch lengths compared to the nuclear data. Several species complexes were investigated using multiple isolates with the results supporting the need for further evaluation of potential new species descriptions of isolates closely affiliated with *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. meadii*, and *P. porri*.

Expanded analysis of *P. infestans* mitochondrial haplotypes and correlation with nuclear genotype
F. N. MARTIN (1), Y. Zhang (2), N. Grunwald (3), D. E. Cooke (4), M. D. Coffey (2)
(1) USDA-ARS, Salinas, CA, U.S.A.; (2) University of California, Riverside, CA, U.S.A.; (3) USDA-ARS, Corvallis, OR, U.S.A.; (4) The James Hutton Institute, Invergowrie, Dundee, Scotland
Phytopathology 102:S4.76

Mitochondrial haplotyping has been useful in tracking lineages of *P. infestans*. Historically, RFLP analysis defined five haplotypes but at a sequence level we observed much greater diversity. Analysis of five mitochondrial loci (3,750 to 5,775 bp) for 101 isolates from geographically diverse areas identified 36 haplotypes. These broadly matched the traditional type I and II haplotypes but exhibited additional subgrouping. Coalescent analysis generated two main clades reflective of haplotype I and II. Within type II, a and b subgroups were distinct but two main groups were resolved for type I; the first had isolates defined as Ia (with RFLPs) and was further divided into two groups broadly corresponding to geographic origin. The second clade had two groups of Ib isolates interspersed by isolates defined by RFLP as Ia. This confirms that restriction analysis is unsuitable for haplotype classification due to convergent evolution at restriction sites not reflecting evolutionary divergence across the mitochondrial genome. Isolates defined as Ic were distinct from the I and II lineages and, along with another novel lineage, formed outgroups. Haplotype evolution within clonal lineages of *P. infestans* was examined using nuclear SSR loci to define MLGs. PCA revealed groupings of isolates based on geographic origin and haplotype. Examples of conflicts between MLG and mtDNA haplotypes and correlations among haplotype, MLG and geographic distribution will be discussed.

Identification and characterization of tar spot on seashore paspalum in Georgia
A. D. MARTINEZ-ESPINOZA (1), O. A. Martinez-Uribe (2), D. Kim (3)
(1) University of Georgia, Griffin, GA, U.S.A.; (2) University of Georgia, Athens, GA, U.S.A.; (3) Georgia State University, Atlanta, GA, U.S.A.
Phytopathology 102:S4.76

Diseased seashore paspalum (*Paspalum vaginatum*) turfgrass plants were first observed at a research greenhouse at the University of Georgia-Griffin campus. Symptoms included defined, small, circular to slightly oval black spots or black spots with yellow tissue on the periphery. Some spots coalesced and formed short delineated strips. Symptoms closely resemble those of tar spot described for other turfgrasses. Light and scanning electron microscopy revealed that clypei are oval in shape with a slightly raised center. Clypei erupt through the epidermis of the plant tissue. Masses of ascospores ooze through small ruptures in the clypei. Asci were cylindrical and arranged in a palisade formation with vegetative tissue dispersed throughout the ascocarp. Asci size ranged from 7 to 8 µm width x 48 µm to 51 µm in length. Ascospores surface is smooth, ellipsoidal and many of them have a slightly conical end. Ascospore size ranged from 5.7 to 7 µm in width x 10 to 11 µm in length. Grass clippings were taken from the infected source and inoculated into healthy seashore cultivars. Healthy plants of cvs. SeaIsle 2000, Aloha, Seaisle Supreme and an experimental line were successfully infected with tar spot. Clypei, ascocarp, asci and ascopore morphology and measurements correspond to those described for *Phyllachora paspalicola* (Syn: *Phyllachora vaginata*). To our knowledge this is the first report of tar spot on seashore paspalum in Georgia.

Diversity of *Agrobacterium tumefaciens* found in California walnut-growing regions
M. Marutani-Hert (1), A. E. McClean (1), M. M. Maccree (2), L. E. Yakabe (2), D. A. KLUEPFEL (1)
(1) USDA-ARS, Davis, CA, U.S.A.; (2) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.76

Agrobacterium tumefaciens biovar 1 is the causal agent of crown gall disease and a serious pathogen of walnuts in California. To understand the genetic diversity of *A. tumefaciens*, about 240 isolates were collected from 13 walnut growing counties in California: Butte, Colusa, Contra Costa, Fresno, Kings, Lake, San Joaquin, Solano, Stanislaus, Sutter, Tulare, Yolo, and Yuba. All isolates were analyzed by repetitive sequence-based PCR (Rep-PCR) genomic fingerprinting using the BOX A1R primer. Rep-PCR analyses revealed unique fingerprints present in the collection. Unique members of this collection were identified at all spatial scales; i.e. for walnut growing regions, down to tree position in the orchard. The majority of strains, as defined by Rep-PCR patterns, were localized to a given county, but some strains with identical Rep-PCR profiles were found in 2 or more counties. Interestingly, Ti-plasmid presence did not contribute to Rep-PCR profiles. Sequence analysis of the *recA* gene revealed the presence of multiple genomovars. All *A. tumefaciens* isolates also were screened for their sensitivity to the agrocin 84 producing

strain K84. More than 65% of isolates from across the walnut growing regions of California were resistant to agrocin 84.

Analysis of allelic variation in the effector gene *Ave1* among *Verticillium* species

K. MARUTHACHALAM (1), B. P. Thomma (2), K. V. Subbarao (3)
(1) University of California, Salinas, CA, U.S.A.; (2) Wageningen University, Wageningen, Netherlands; (3) University of California-Davis, Plant Pathology, Davis, CA, U.S.A.
Phytopathology 102:S4.77

Verticillium species are soil-borne fungi that cause vascular wilt on a broad range of hosts resulting in billions of dollars' worth of crop losses annually. The species vary in pathogenicity and host range. Recently, the effector gene *Ave1* was characterized in *V. dahliae* race 1 strains, which activates the *Ve1* immune receptor in tomato. Allelic variation of *Ave1* was analyzed using gene-specific primers among ten *Verticillium* species. *Ave1* was amplified by three species of *Verticillium* including *V. dahliae* race 1, and this was further confirmed by Southern blot analyses. *Ave1* gene sequences were identical in *V. dahliae* and *V. nonalfalfae*, but *V. nubilum Ave1* alleles showed polymorphism. The polymorphism observed in the *V. nubilum Ave1* gene involved point mutations, which caused synonymous and non-synonymous modifications in the DNA sequence. We are currently investigating the effect of these changes in avirulence of *V. nubilum* on recognizing the *Ve1*-immune receptor in tomato and lettuce.

Posttranscriptional gene silencing of the gene encoding aldolase from soybean cyst nematode by transformed soybean roots

B. F. Matthews (1), R. Y. ABD EL KREEM (2)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) USDA, Beltsville, MD, U.S.A.
Phytopathology 102:S4.77

Plant parasitic nematodes cause approximately 157 billion US dollars in losses worldwide annually. The soybean cyst nematode (SCN), *Heterodera glycines* is responsible for an estimated one billion dollars in losses to the US farmer each year. A promising new approach for control of plant parasitic nematode control is gene silencing. We tested this approach by silencing the gene, *HgALD*, encoding fructose-1,6-diphosphate aldolase. This enzyme is important in the conversion of glucose into energy and may be especially important in actin-based motility during parasite invasion of its host. An RNAi construct targeted to silence *HgALD* was transformed into soybean roots of composite plants to examine its efficacy to reduce the development of females formed by SCN. The number of mature females on roots transformed with the RNAi construct designed to silence the *HgALD* gene was reduced by 58.15%. These results indicate that silencing the aldolase gene of SCN can greatly decrease the number of female SCN reaching maturity, and it is a promising step towards broadening resistance of plants against plant-parasitic nematodes.

Combining isolates to screen for novel sources of resistance to *Phytophthora sojae* in soybean

R. MATTHIESEN (1), N. Abeysekara (1), A. Robertson (1), S. Maroof (2)
(1) Iowa State University, Ames, IA, U.S.A.; (2) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 102:S4.77

Phytophthora root and stem rot, caused by *Phytophthora sojae*, is an economically important disease of soybean in the United States that is predominantly managed by utilizing resistant varieties with specific Rps genes. The pathogen is however, continually evolving, which compromises the sustainability of Rps genes. Traditionally, Rps genes are identified by inoculating the hypocotyl of soybean seedlings with a single isolate of *P. sojae* that is pathogenic on one or more Rps genes. There are very few isolates available that are pathogenic on all Rps genes, so multiple screenings with multiple isolates is necessary in order to characterize soybean lines. The objective of this study was to determine if isolates could be combined and used to screen soybean lines for novel sources of resistance to *P. sojae*. A fourteen soybean differential set was inoculated with three isolates of *P. sojae* individually or in combination. If less than 25 percent of inoculated seedlings were killed, the line was considered potentially resistant and was subjected to additional screening for confirmation. We found that using the isolates in combination was as effective at identifying Rps genes as inoculating each isolate individually. Advantages of combining isolates include reduced cost, ability to screen soybean lines with multiple isolates that are virulent on all known Rps genes at one time, and ease of identifying novel sources of resistance. This method is currently being used to screen soybean germplasm for novel sources of resistance.

Detection of *Helminthosporium solani* and *Colletotrichum coccodes* in organically grown asymptomatic and symptomatic potatoes

C. MATTUPALLI (1), R. K. Genger (1), A. O. Charkowski (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 102:S4.77

Silver scurf, caused by the fungus *Helminthosporium solani*, and black dot, caused by *Colletotrichum coccodes*, are cosmetic diseases of potatoes (*Solanum tuberosum*) affecting processing and fresh market trade. An observational study was undertaken to assess the importance of asymptomatic tubers in perpetuation of the pathogen. Ten asymptomatic tubers and fifteen symptomatic tubers from ten different varieties were collected from three different organic farms. PCR or incubation assays were performed on asymptomatic tubers to detect *H. solani* and *C. coccodes*. 75% and 94% of the asymptomatic tubers tested were positive for silver scurf and black dot respectively. It is thus misleading to look only at the visual symptoms on the tuber for assessing the disease level of silver scurf or black dot. None of the ten cultivars studied were resistant to either silver scurf or black dot. PCR and incubation assays were comparable and can thus be used to determine which fungi are present since both the lack of symptoms at harvest and the similarity of the symptoms caused by these two fungi make rating tubers at harvest unreliable. Both pathogens were present together on the same tuber in 69% of the tubers assessed. The presence of one pathogen did not affect the presence or absence of the other pathogen. The presence of *H. solani* reduced black dot severity, but the presence of *C. coccodes* did not affect the severity of silver scurf.

Replant disease control and soil system resilience to pathogen infestation in response to Brassicaceae seed meal amendment

M. MAZZOLA (1)
(1) USDA-ARS, Wenatchee, WA, U.S.A.
Phytopathology 102:S4.77

A Brassicaceae seed meal (SM) formulation was compared with pre-plant soil fumigation for the ability to control apple replant disease and to suppress pathogen/parasite re-infestation of organic orchard soils. Application of a *Brassica juncea/Sinapis alba* SM formulation provided disease control and enhanced tree growth in a manner that was comparable or superior to 1,3-dichloropropene/chloropicrin soil fumigation. At the STM orchard both fumigation and SM amendment effectively suppressed components of the causal pathogen complex during the initial growing season. However, at the end of the second growing season, relative to the no treatment control, Jonagold/G11 root infestation by *Pratylenchus penetrans* and infection by *Pythium* spp. were significantly elevated in fumigated soil but were suppressed in seed meal amended soils. Correspondingly, tree growth was superior in seed meal treated soil relative to the control and fumigated treatments. At the SR orchard, soil fumigation and seed meal amendment effectively controlled both *P. penetrans* and *Pythium* spp. and tree growth was equivalent between these treatments, but significantly greater than the no treatment control. Findings indicate that Brassicaceae SM amendment may modify soil biology in a manner that enhances system resilience, thereby suppressing re-infestation of orchard soils by parasitic nematodes and fungal/oomycete root pathogens; however, the response is likely to be orchard soil specific.

Transformation of soil microbial community structure in response to anaerobic soil disinfestation for soilborne disease control in strawberry

M. MAZZOLA (1), J. Muramoto (2), C. Shennan (2)
(1) USDA-ARS, Wenatchee, WA, U.S.A.; (2) University of California-Santa Cruz, Santa Cruz, CA, U.S.A.
Phytopathology 102:S4.77

Anaerobic soil disinfestation (ASD) has been used to control soil-borne pathogens and nematodes in various plant production systems including strawberries. Disease control is commonly attributed to the depletion of oxygen and the generation of toxic compounds, including organic acids and volatiles. However, recent evidence suggests that disease control, in part, may be dependent upon specific changes in soil biology that are induced by ASD. Although reported to control disease by depressing targeted microbial populations, ASD and ASD in conjunction with Brassicaceae seed meal or fish emulsion applications increased total bacterial, total fungi, and fluorescent pseudomonad densities in two test strawberry field soils. In certain instances, densities of potential pathogens including *Fusarium oxysporum* and *Pythium ultimum* were also elevated in response to ASD. ASD dramatically altered composition of the fungal community resident to these field soils, with the yeast *Galactomyces geotrichum* becoming dominant at both study sites. The fact that ASD elevates microbial densities but disease control is attained suggests that mechanisms other than oxygen depletion or generation of

organic acids, potentially including competitive interactions, may contribute to the observed plant response.

Managing root-knot nematode in tomato using resistant rootstocks
T. McAvoy (1), M. Paret (2), J. FREEMAN (1)
(1) Virginia Tech, Painter, VA, U.S.A.; (2) University of Florida, Quincy, FL, U.S.A.
Phytopathology 102:S4.78

Grafting of vegetables is being investigated as a management tool for soil-borne diseases and pests in the absence of the soil fumigant methyl bromide. Three hybrid tomato rootstocks were investigated for possible resistance to root-knot nematode (*Meloidogyne* spp.). These rootstocks were chosen because they have previously exhibited resistance to bacterial wilt (*Ralstonia solanacearum*). The root-knot susceptible tomato cultivar BHN 602 was grafted onto BHN 998, BHN 1054, or RST 04-106-T. These combinations as well as an un-grafted BHN 602 control were planted into a field that was naturally infested with root-knot nematode in Florida and inoculated with root-knot nematode in Virginia. In Florida, all grafted entries exhibited significantly lower root gall index (RGI) compared to the control and there were no differences between grafted entries. In Virginia, all grafted entries exhibited significantly lower RGI compared to the control but there were differences between grafted entries. Plants with rootstock RST 04-106-T exhibited least RGI, followed by BHN 998, which had significantly lower RGI than BHN 1054. At both locations, plants grafted to BHN 998 produced significantly greater marketable yield than the control. In Florida, plants grafted to RST 04-106-T produced yields similar to the control and in Virginia plants grafted to BHN 1054 yielded similar to the control. These data indicate that root-knot nematode can be managed with grafting and that resistant rootstocks can be used to manage a broad spectrum of soil-borne pests and pathogens.

Identification of a potential pyrophilous fungus following a forest fire in Bastrop County, TX
S. A. MCBRIDE (1), C. J. Richards (1), D. N. Appel (1), H. A. Pase (2)
(1) Texas A&M University, College Station, TX, U.S.A.; (2) Texas Forest Service, Lufkin, TX, U.S.A.
Phytopathology 102:S4.78

During early September, 2011, a fire devastated 55 square miles of loblolly pine forest near Bastrop, TX. Within weeks after the fire was extinguished, the trunks and limbs of some trees became covered with a white to cream colored fungal growth, eventually taking on an orange hue. The growth appeared extending from the base into the lower limbs and branches, sometimes in patterns spiraling up the trunks. Public concern regarding the nature of the fungus and whether it might be unsafe prompted us to attempt to carry out identification. The fungus was easily cultured on defined media from bark and branch samples, yielding cultures similar in color to that observed in the field. Morphological and molecular approaches were used for identification. DNA sequencing of a 580bp PCR fragment was obtained using the ITS region of the isolated fungus. Results from a GeneBank BLAST showed an 89% similarity with several fungi in the class of Pezizomycetes. Apothecia eventually appeared on the forest floor, which will provide additional information on the morphological characterization and subsequent identification to genus and species. Based on the features of these apothecia, the fungus is in the order Pezizales and family Pyronemataceae. There are numerous different fungi in this family observed growing on trees worldwide following fires, due to changes in bark chemistry and lack of competition. Efforts are underway to further identify the fungus to genus and species.

Identification of sources of crown gall resistance in the *Juglans* germplasm
A. E. McClean (1), D. A. KLUEPFEL (2), M. Aradyha (3), J. Moersfelder (3), W. P. Hackett (4), A. Dull (1), C. Marsden (5)
(1) USDA-ARS, University of California, Davis, CA, U.S.A.; (2) USDA-ARS, CPGRU, Davis, CA, U.S.A.; (3) USDA-ARS Germplasm Repository, Davis, CA, U.S.A.; (4) University of California-Davis, Davis, CA, U.S.A.; (5) USDA-ARS, Davis, CA, U.S.A.
Phytopathology 102:S4.78

Agrobacterium tumefaciens is a common soil-borne Gram-negative bacterium and causes crown gall (CG) of walnut which can severely stunt or kill young trees. In California walnut orchards, paradox hybrid rootstocks (*Juglans hindsii* x *Juglans regia*) are widely planted but are susceptible to CG. We previously screened more than 220 mother trees representing eight wild *Juglans* spp. for CG resistance. We identified Texas black walnut *Juglans microcarpa* mother trees as sources of open pollinated (OP) CG progeny. Thirty-seven percent of OP *J. microcarpa* seedlings screened in 2011 were putatively CG resistant. Fifty-three percent of rooted cuttings generated from putative CG resistant *J. microcarpa* OP seedlings in 2010 remained CG resistant after screening in 2011. Two directed crosses were made between a susceptible walnut, *J. regia* 'Serr' (pollen source) and *J. microcarpa* mother trees 31.07A and 31.09A previously shown to produce OP CG resistant progeny. Preliminary analysis of 11 progeny from the 31.07A x 'Serr' cross and 54 progeny from the 31.09A x 'Serr' cross shows a 3:1 and 2:1 segregation of CG susceptible to CG resistant seedlings respectively. The CG resistant seedlings will be cloned, retested and used in genetic mapping studies to characterize CG resistance.

Distribution and involvement of *Phytophthora cinnamomi* in white oak decline in northeastern U.S. forests
M. MCCONNELL (1), Y. Balci (1)
(1) University of Maryland, College Park, MD, U.S.A.
Phytopathology 102:S4.78

Sampling was conducted from June to October 2011 to test the hypothesis that the fine root pathogen *Phytophthora cinnamomi* is involved in white oak decline and to determine the distribution of this pathogen in Mid-Atlantic forests. In each stand, soil and root samples from healthy and declining white oaks (*Quercus alba*) were collected. Stands were located in Delaware, Maryland, Ohio, Pennsylvania, and West Virginia. Stands with varying site conditions were selected, including wet bottomlands, moist, fertile sites, and dry, hilly sites. *Phytophthora* was baited from soil samples using rhododendron leaves (*Rhododendron maximum*), and when tested negative, a second sample was baited with English oak (*Quercus robur*) leaflets. White oak roots collected from four soil monoliths (30 cm x 30 cm x 25 cm) ca. 1-1.5 m distance from the stem were pressure washed, scanned, and fine roots quantified using the software WinRHIZO. *P. cinnamomi* was isolated from about 40% of the sites (15 out of 39). English oak leaf baits resulted in 13 additional *P. cinnamomi*-positive trees missed by rhododendron baits. All *P. cinnamomi* sites were located below 40°N latitude. Preliminary results showed that trees from *Phytophthora*-infested wet sites had approximately two times less fine roots than trees at drier sites. Furthermore, at infested sites, a positive trend between deteriorating crown status and fewer fine roots was noted. At non-infested sites, healthy and declining trees had no difference in total roots analyzed.

A comparison of clubroot resistance in *Brassica* vegetable crops
M. MCDONALD (1), K. Sharma (1), A. V. Nieuwelaar (1), B. D. Gossen (2)
(1) Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada; (2) Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada
Phytopathology 102:S4.78

Clubroot of crucifers, caused by the protist *Plasmodiophora brassicae*, is responsible for major yield losses in vegetable *Brassica* crops. Cultivars of several *Brassica* crops with resistance to clubroot have been developed recently. This study compared clubroot incidence and severity on resistant (R) and susceptible (S) cultivars of broccoli, Brussels sprouts and Shanghai pak choy grown in field soil naturally infested with pathotype 6 of *P. brassicae*, and on the same cultivars grown under controlled conditions and inoculated with pathotype 6 or pathotype 3. In the field trials, broccoli cv. Emerald Jewel (R) was compared to cv. Diplomat (S) in 2010 and 2011, Brussels sprouts cv. Crispus (R) with Jade Cross E (S) in 2011, and Shanghai pak choy line B 2834 (R) with cv. Mei Qing Choy (S) in 2011. In the field trials, all of the susceptible vegetables had 100% clubroot incidence and very high severity. The resistant Shanghai pak choy and Brussels sprouts had no symptoms of clubroot, while the resistant broccoli had a low severity rating (10–14%). Each of the resistant cultivars had higher yield or shoot weight than the susceptible cultivar of the same crop. Under controlled conditions, the reaction to pathotype 6 was consistent to the field assessments, and resistance or susceptibility was similar for pathotype 3. Growing clubroot resistant cultivars appears to be an effective method for managing clubroot on *Brassica* vegetables.

***Phytophthora infestans* transmitted to seedlings growing from tomato fruit rotted by late blight but not their seed**
M. T. MCGRATH (1)
(1) Cornell University, Riverhead, NY, U.S.A.
Phytopathology 102:S4.78

Study impetus was outbreaks of late blight for which volunteer tomatoes or saved seed appeared to be possible pathogen sources. Fruit naturally infected with genotype US-23 were used. Fruit were held for 11 days after collecting, then put in potting mix in trays. Seedlings began emerging within 7 days in trays kept in a greenhouse. Late blight symptoms appeared on lower stems starting 13 days after planting in cultivar 'Sweet Treats', which emerged first. Asymptomatic seedlings were transplanted in groups into pots. Seedlings were

removed as symptoms appeared. Last were on 'Juliet' 67 days after collecting fruit. Very few sporangia were observed on stems examined microscopically. Abundant sporulation developed on stems incubated in humid plastic bags and on cotyledons when infection progressed up stems reaching this tissue. A similar set of trays was kept cool and dark for 75 days to delay germination. Symptoms did not develop on any seedlings that grew in these trays after they were put in the greenhouse. Seed was removed from symptomatic fruit of 'Mt Fresh Plus' and 'SunGold'. Some seed was planted immediately. Other seed was first either incubated for 8 or 15 days in the fruit's juice, held dry for 65 days, rinsed in water before drying, or incubated in either water or soapy water for at least 4 days before drying. No symptoms were seen on the seedlings that grew. While *Phytophthora infestans* survived on fruit, it was not present or able to survive in or on tomato seed.

Predicted phytochelatin synthase (EAM_2936) and gamma-glutamyl transpeptidase (EAM_2935) are required for full virulence in *Erwinia amylovora* Ea1189

R. R. MCNALLY (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.79

Erwinia amylovora is the causative agent of fire blight, a disease of rosaceous plants such as apple and pear. Multiple pathogenicity factors have been identified in *E. amylovora* including the type III secretion system (T3SS), a needle-like apparatus that reprograms host cells via the translocation of bacterial proteins directly into the host cytoplasm. HrpL, a transcription factor, coordinates the expression of all known components of the T3SS and is required in fire blight development. The role of HrpL in T3SS regulation does not preclude the possibility that HrpL may also regulate other virulence factors in *E. amylovora*. Our previous analyses of the HrpL regulon in *E. amylovora* identified multiple genes exhibiting HrpL-mediated expression without known roles in the T3SS. One such gene cluster includes putative phytochelatin synthase (EAM_2936) and gamma-glutamyl transpeptidase (EAM_2935) as annotated in the genome of *E. amylovora* ATCC 49946. EAM_2936 and EAM_2935 are predicted to detoxify xenobiotics like heavy-metals. While both exhibit signal peptides, the GC content of EAM_2936 is markedly lower than the genome of *E. amylovora*. Here we report novel virulence roles for EAM_2936 and EAM_2935 in *E. amylovora* Ea1189 as both Ea1189?EAM_2936 and Ea1189?EAM_2935 were highly attenuated in virulence four and six days post-inoculation in immature pear fruit. We also report the cellular localization of EAM_2936 and EAM_2935 and contribution to exogenous xenobiotics.

Vector-borne cotton boll disease transmitted by *Nezara viridula* nymphs

E. G. MEDRANO (1), J. F. Esquivel (2)
(1) USDA-ARS CPRU, College Station, TX, U.S.A.; (2) USDA-ARS, College Station, TX, U.S.A.
Phytopathology 102:S4.79

Previously we demonstrated transmission of an opportunistic *Pantoea agglomerans* (Rifampicin [Rif] resistant strain Sc 1-R) into cotton bolls by adult *Nezara viridula*, resulting in disease. Here, we report the potential for 5th instars of *N. viridula* nymphs to acquire, retain, and vector strain Sc1-R into bolls. Nymphs reared from eggs in the laboratory were fed fresh green beans (*Phaseolus vulgaris*). Transmission tests consisted of: 1) providing 3rd instars with sterile green beans (control) or Sc1-R contaminated beans for 2 d; 2) replacement of all food with sterile beans (2 and then 3 d); and 3) caging of insects with bolls at 2 wks post-anthesis (3 d). Bolls were harvested 2 wks following exposure to the insects. Seed and lint were plated on nonselective media and media with Rif. Control insects deposited only bacteria innately present within the stink bug (reaching 10^7 cfu/g tissue) and the bolls lacked disease symptoms. Nymphs fed beans contaminated with Sc1-R transmitted the pathogen (reaching 10^4 cfu/g tissue) into bolls and resulting in necrosis. Collectively, these results indicated that both adults and 5th instars are capable agents of vector-borne boll disease.

Assessing the genetic diversity of *Cherry leaf roll virus* with coat protein gene from different hosts

T. A. MEKURIA (1), K. C. Eastwell (1)
(1) Washington State University, IAREC, Prosser, WA, U.S.A.
Phytopathology 102:S4.79

Cherry leaf roll virus (CLRV; family *Secoviridae*; subfamily *Comovirinae*, genus *Nepovirus*) has a wide host range and is distributed worldwide. Recently it was observed that the virus occurs in orchards in the Pacific Northwest of USA where it contributes to the decline of sweet cherry trees. Information on possible sources of the virus that infect cherry trees is limited. The complete coat protein sequences of twelve isolates from sweet cherry, elderberry and walnut were determined and compared with each other and with published sequences from rhubarb and birch isolates. The overall sequence variability was as high as 29% and 23% at the nucleotide (nt) and amino acids (aa) levels, respectively. Five sweet cherry isolates from three separate locations were 94 to 100% identical to each other suggesting the virus might spread over long distances from the same source. When compared to CLRV from other hosts, the sweet cherry isolates showed greatest nucleotide identity (84 to 85%) to the birch isolate. Sequences of the walnut, elderberry and rhubarb isolates are more close related to one another (84% nt identity) than to the sweet cherry or rhubarb (71 to 79% nt identity) isolates. Isolates segregated into three distinct phylogenic clades. Elderberry isolates form a monotypic group; whereas rhubarb and walnut isolates together, and sweet cherry and birch isolates together form two separate clades.

Identification and characterization of a new bipartite begomovirus associated with yellow mosaic disease of *Jatropha* sp. in Dominican Republic

T. A. MELGAREJO (1), T. Kon (1), R. L. Gilbertson (1)
(1) University of California, Davis, CA, U.S.A.
Phytopathology 102:S4.79

A sample of jatropha leaves with yellow mosaic symptoms was collected in Clavellina, Dominican Republic in 2009. PCR with degenerate begomovirus primers revealed the presence of DNA-A and DNA-B components. Full-length DNA-A and DNA-B components were amplified using rolling circle amplification, and cloned and sequenced. The complete sequences of the DNA-A and DNA-B components had the highest nucleotide sequence identities with *Tobacco mottle leaf curl virus* (TbMoLCV, Genbank accession number: FM160943) and *Wissadula golden mosaic St Thomas virus* (WGMSTV, Genbank accession number: EU158095) at 88% and 77%, respectively. When delivered by agroinoculation, the DNA-A and DNA-B clones were infectious and induced symptoms of yellow mosaic, curling and crumpling in leaves of *N. benthamiana*, common bean (cv. Topcrop) and tobacco (cv. Samsum) plants. These clones were not infectious in jatropha, tomato, pepper and small sugar pumpkin plants. The jatropha begomovirus also was sap-transmissible from *N. benthamiana* to *N. benthamiana*, common bean and tobacco plants, but not to jatropha plants. Jatropha plants inoculated with the DNA-A and DNA-B clones by particle bombardment developed stunted growth and leaf crumpling and yellowing symptoms. Together, these results indicate that jatropha yellow mosaic disease in the Dominican Republic is caused by a new bipartite begomovirus species, and the provisional name of *Jatropha yellow mosaic virus* (JYMV) is proposed.

WITHDRAWN

Peroxidase activity and total phenolics in basil (*Ocimum* spp.) in relation to resistance to *Peronospora belbahrii*, the causal agent of downy mildew of basil

Z. MERSHA (1), S. Zhang (2)
(1) University of Florida, Tropical Research and Education Center, Homestead, FL, U.S.A.; (2) University of Florida, Homestead, FL, U.S.A.
Phytopathology 102:S4.79

Seven cultivars of *Ocimum* spp. were evaluated in greenhouse experiments and field trials for their responses to infection by *Peronospora belbahrii*, the pathogen of basil downy mildew. Basil leaves were collected and tested for peroxidase (POX) activity and total phenolics (Phe). A significantly ($P < 0.0001$) different level of POX activity and Phe content was detected among the seven basil cultivars. 'Lemon basil' and the cultivar 'Red rubin' were found to be resistant with nearly no disease (< 1%) developed until 18-20 days after inoculation (DAI) in the greenhouse inoculations. They also exhibited higher POX activity, higher Phe content and longer period of time from inoculation to symptom occurrence compared to the other five cultivars. Disease severity was lower than 60% on the cultivars 'Genovese' and 'Large leaf Italian' until 14 DAI, but the cultivars 'Cinnamon', 'Thai' and 'Common basil' had a disease severity up to 85% within 14 DAI. In field trials, lemon basil sustained nearly a disease-free vigorous growth 14 weeks after planting, but 'Red rubin' was more prone to the natural infection than in the greenhouse experiments. POX activity in field grown 'Lemon basil' was also significantly ($P < 0.0001$) higher than other cultivars. It is found that POX activity in basil was inversely correlated with downy mildew disease epidemics. POX activity and Phe content of basil cultivars could potentially be used to rapidly assess resistance in basil against downy mildew disease.

Evaluation of SAR inducers, chitosan, and silicon for control of Phytophthora blight of tomato

Z. MERSHA (1), S. Zhang (1), X. Mo (1)
(1) University of Florida, Homestead, FL, U.S.A.
Phytopathology 102:S4.80

Phytophthora capsici infects tomato plants causing root, crown and fruit rot, and leaf blight. In this study, foliar sprays of acibenzolar-S-methyl (ASM, 50 and 100 mg/liter), β-aminobutanoic acid (BABA, 500 and 1000 mg/liter), chitosan (0.5 and 1% w/v) and silicon (50 and 100 mg Si/liter), applied individually and in combination, were tested for their potential to control *P. capsici* on tomato under greenhouse conditions. Four-week old seedlings were treated with these compounds four times once every other day prior to inoculation. Plants were afterwards inoculated with a foliar spray of *P. capsici* at 1 × 104 zoospore/ml and inoculated plants were maintained in a humid chamber for overnight. Symptoms of leaf blight started 3 days after inoculation (DAI). Disease severity was significantly ($P <0.0001$) lower in all treated tomato plants compared to the non-treated control. Disease severity, when monitored 7 DAI, was 95% on the non-treated control plants whereas it ranged between 1 and 60% on the other treated plants. Foliar treatment of tomato plants with ASM and BABA alone or in combination with chitosan or silicon drastically reduced the disease severity. However, BABA at tested concentrations caused phytotoxic symptoms on tomato plants. The fungicide fluopicolide (Presidio) effectively controlled the disease in the greenhouse experiments. Future studies are being planned with reduced rates of BABA to avoid the phytotoxicity and to investigate the role of enzyme activity of tomato plant tissues in resistance to *P. capsici* after treatment with SAR compounds, chitosan and silicon.

Controlling charcoal rot, an emerging disease of strawberry

J. C. MERTELY (1), T. Seijo (2), N. A. Peres (2)
(1) University of Florida, GCREC, Wimauma, FL, U.S.A.; (2) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.80

Charcoal rot, caused by *Macrophomina phaseolina*, is increasingly common on strawberry in warmer regions of the world. Infected plants develop a vascular dry rot in the crown that causes plant collapse and death. Emergence of this disease coincided with the replacement of methyl bromide by alternative soil fumigants. Replicated small plot experiments were conducted over several seasons to evaluate strawberry cultivars and selected products for charcoal rot control. In each case, plots were artificially inoculated with *M. phaseolina* grown on corn cob pet litter deposited into planting holes before transplanting. In trials conducted in an open field and a high plastic tunnel during the 2011-12 season, cultivars Camarosa, Florida Radiance, and Winter Dawn were resistant to plant collapse (DI = 3 to 15%), whereas Strawberry Festival and Treasure were susceptible (44 to 83%). 'Camino Real' was intermediate (33 to 38%) in an open field test in 2010-11 and the tunnel test in 2011-12. In chemigation experiments, products were injected into the soil through drip tapes next to the plant rows. Two applications were made after transplant in the fall, and 2 or 3 applications were made in the spring. No product provided excellent control, but thiophanate methyl (Topsin) showed limited disease suppression over 3 seasons. Disease suppression was occasionally noted for azoxystrobin (Abound), *Bacillus subtilis* (Serenade Soil), *Gliocladium virens* (SoilGard), *Streptomyces lydicus* (Actinovate), *Trichoderma harzianum* (BW-240, RootShield), and triflumizole (Procure). Additional work is needed to determine whether these products or others can provide economic control via chemigation.

Assessment of damage caused by in soybean crop in the 2011/2012 season in Brazil

R. M. MESQUINI (1), C. V. Godoy (2), A. Bergamin Filho (1)
(1) Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, Brazil; (2) Embrapa Soja, Londrina, Brazil
Phytopathology 102:S4.80

The soybean target spot caused by is a common disease in crops in all regions of soybean production in Brazil. Information losses in crop yield are the basis for economic analysis of disease quantification, studies for this pathosystem are little known. In this way, the aim was to evaluate the target spot effect on the soybean photosynthetic efficiency as a model to damage estimate. A trial was conducted at Embrapa Soja, Londrina, Brazil, where the Bmx Potencia, Bmx Forca, Na 5909 and Brs 295 cultivars were sown in two different sowing dates (11/02/11 and 12/08/11). The experimental design was completely randomized, which each plot consisted of eight rows of five meters in length. The trial had four replicates. To obtain different gradients of disease, sprays with fungicide and inoculated with pathogen were made. The evaluating components were: the severity of the disease (from R4 phenological growth stage), the leaf area in stages R2, R4 and R5.3 with the aid of Leaf Area Meter (Li-3000Li-Cor®); defoliation, using the radiometer GreenSeeker which provides the normalized difference vegetation index- NDVI and grain yield (kg). After analyzing healthy area duration (HAD), healthy leaf area absorption (HAA) parameters correlated with grain yield, it is possible to conclude that the target spot does not cause damage in soybeans under the studied conditions.

Efficient inoculation of *Rice black-streaked dwarf virus* to maize using the planthopper *Laodelphax striatellus*

H. MIAO (1), D. Di (1), Y. Lu (1), M. Redinbaugh (2), L. Tian (1), A. Zhang (1)
(1) Plant Protection Institute, Hebei Academy of Agriculture and Forestry Sciences, Baoding, Peoples Republic of China; (2) USDA-ARS, Corn and Soybean Research, Department of Plant Pathology, Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.80

Maize rough dwarf caused by *Rice black-streaked dwarf virus* (RBSDV) is among the most important diseases of maize in China. Although deploying disease resistant hybrids would be the most effective and economic way to control the disease, identification and development of resistant hybrids has been limited by virus transmission rates that are too low for effective screening. An artificial inoculation technique for RBSDV was developed using *L. striatellus* as vector and wheat seedlings as the insect rearing and virus culture host. A planthopper colony was developed using wheat as an effective feeding and reproductive host. Subsequently, RBSDV-infected leafhoppers were obtained by allowing 3rd and 4th instar nymphs a 3 to 4 day acquisition access period onRBSDV-infected wheat. Planthoppers were then allowed to feed on healthy wheat for a 25 to 28 day latent period. The viruliferous leafhoppers were then allowed a 3 day inoculation access period on maize seedlings (10 to 12 leafhoppers/plant in 60 x 40 x 58 cm cages). Plants of the susceptible hybrid Zhengdan 958 developed dwarfing and enation symptoms 10 to 14 days after transplanting into a screenhouse. At tassling, 100% of plants were symptomatic, with a disease index of 97.3 out of 100. The high efficiency of RBSDV transmission indicates this technique provides a reliable procedure to screen for RBSDV resistance in maize.

Pathogenicity and aggressiveness of isolates belonging to a new *Phytophthora infestans* sensu lato population in Colombia

M. F. MIDEROS (1), J. Bastidas (2), Y. V. Castillo (2), L. E. Lagos (2), A. Bernal (1), S. Restrepo (1)
(1) Universidad de Los Andes, Bogotá, Colombia; (2) Universidad de Nariño, Pasto, Colombia
Phytopathology 102:S4.80

New populations of *Phytophthtora infestans* sensu lato have been characterized in South America, and most of them have been found causing diseases in native hosts of the tropical region. Recently in Colombia, we have found a new population of this pathogen associated with tree tomato (*Solanum betaceum*). Unlike isolates previously reported in the Andean Region, this new population is highly virulent on its host and shows different morphological and genetic characteristics. To understand the impact of pathogenicity features on the epidemiology and evolution of *Phytophthora* in the North Andean highlands, we obtained 40 isolates collected from different cultivars of tree tomato in Colombia and 10 isolates from potato. Isolates were morphologically and molecularly characterized. Additionally, nine aggressiveness components were evaluated in a detached leaf assay on four cultivars of tree tomato and two cultivars of potato (*Solanum tuberosum*). Results indicated that *Phytophthora infestans* sensu lato in tree tomato is highly diverse. Morphological features and some molecular markers revealed unique patterns

among these isolates. There were significantly high levels of aggressiveness among the isolates evaluated. Using hierarchical clustering principal component analysis (HCPC), three clusters were identified according to the aggressiveness components. A significant correlation was obtained between the aggressiveness component and genotypes. Interestingly, only three isolates were able to infect a susceptible cultivar of potato indicating that there exists a high level of host specificity. Result of this study can be used to understand the evolutionary history and strategies of adaptation to new hosts of *Phytophthora infestans* population in South America, especially when strong events of hybridization are occurring to increase the possibility of modification of the initial host range.

Parasitic capacity of *Trichoderma* against root rot disease of chicory
P. A. MILLAS (1), R. A. France (1)
(1) Instituto de Investigaciones Agropecuarias, Chillán, Chile
Phytopathology 102:S4.81

Chicory (*Cichorium intybus* var. *sativum*) is grown in Chile for inulin production. The crop is affected by *Phoma exigua* var. *exigua* and *Phytophthora cryptogea*, causing root rot disease, it reduces the inulin production and losses may reach up to 100%. Both pathogens are soil-borne; therefore fungicide control is difficult on farm. The objetive was to evaluate parasitic capacity of 60 strains of *Trichoderma* against both pathogens causing the root rot of chicory. The strains evaluated belong to INIA Collection, including strains from several species of *Trichodema* collected from a wide range of environmental conditions. The parasitic capacity of *Trichoderma* was evaluated by dual cultures, where the mycoparasite and the pathogen grew toward each other. A block agar from hyphae intermingled was observed with a light microscope for detection of coiling around and/or penetration of *Trichoderma* into the pathogen hyphae. The results showed that 52% of the *Trichoderma* strains had parasitic capacity against *P. cryptogea* and 60% against *P. exigua*. Moreover, 28% of the strains were coiled around of both pathogen hyphae, thus demonstrating the potential of *Trichoderma* as biocontrol against root rot disease of chicory.

Managing early-season stripe rust in soft red winter wheat
E. MILUS (1), J. Kelley (2), K. Lee (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) University of Arkansas Cooperative Extension Service, Little Rock, AR, U.S.A.
Phytopathology 102:S4.81

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, has been an important disease of soft red winter wheat in south-central United States since 2000 when a new aggressive strain of the pathogen emerged. Adult-plant resistance (APR) in soft red winter wheat has been useful for managing stripe rust. However, mild weather during the winter allows initial fall infections to develop into large "hot spots" of infected plants during tillering stage before APR is expressed. The objectives of this research were to determine the levels of resistance expressed by commonly-grown cultivars at various growth stages and the efficacies of commonly-used fungicides to eradicate existing infections and prevent the spread of viable spores. Replicated plots of 20 cultivars that were exposed to natural inoculum during tillering stage were evaluated for stripe rust severity and infection type from jointing to soft dough growth stages, and levels of APR differed among cultivars. Fungicides were evaluated on several days after treatment by collecting spores from treated plants and testing the spores for ability to cause disease on wheat seedlings under controlled conditions. Triazole fungicides such as propiconazole and tebuconazole appear to be the best choice for stopping the spread of viable spores from hot spots and providing time for APR to express.

Sublethal UV irradiation of *Arabidopsis thaliana* primes resistance to *Hyaloperonospora arabidopsidis*
S. J. MINTOFF (1), D. M. Cahill (2)
(1) Deakin University, Victoria, Australia; (2) Deakin University, Waurn Ponds, Victoria, Australia
Phytopathology 102:S4.81

Exposure of plants to high levels of UV-C irradiation causes DNA damage and cell death, yet irradiation with sub-lethal doses results in an increase in pathogen resistance, presumably through a cross-tolerance stress response. Our study aims to characterise this response and to determine what resistance factors are involved. *Arabidopsis* ecotype Col-0 was exposed to sub-lethal UV-C at doses up to $1kJm^{-2}$, and pathogen resistance-related parameters were examined (H_2O_2 production, callose and lignin deposition and defence gene activity). Furthermore plants (Col-0 or Ler) pre-treated with UV-C were inoculated 24h later with virulent isolates of *H. arabidopsidis*, and the same parameters examined. When *Arabidopsis* was irradiated with UV-C alone, hydrogen peroxide levels increased, the defence-related genes PR1, PDF1.2 and GST1 were up-regulated and callose was deposited along cell walls. There was no measurable change in lignin production. Inoculation of irradiated leaves with the virulent isolates resulted in the formation of hypersensitive-like lesions around sites of attempted entry demonstrating a switch from compatibility to what resembled incompatibility. UV-C irradiation appears to be priming the plants for resistance by activation of both the SA and JA pathways.

Induction of plant defense enzymes by plant growth-promoting rhizobacterium *Bacillus subtilis* IN937b in relation to suppression of Phytophthora blight on squash
X. MO (1), S. Zhang (1)
(1) University of Florida, Homestead, FL, U.S.A.
Phytopathology 102:S4.81

Phytophthora blight of squash caused by *Phytophthora capsici* is a common disease in South Florida and other squash production regions. The objective of this study was to evaluate the effect of plant growth promoting rhizobacteria (PGPR) on growth promotion and induced systemic resistance in squash against *P. capsici*. In the greenhouse experiments, 16 PGPR strains were evaluated for their effects on plant growth and suppression of Phytophthora blight. Drench applications of PGPR strain IN937b (*Bacillus subtilis*) significantly ($P<0.05$) reduced the disease incidence of Phytophthora blight by 40% when compared to the nontreated control. IN937b also significantly increased dry weight of shoot compared to the nontreated control. To explore the mechanisms underlying the disease suppression by IN937b, plant defense-related enzymes i.e. peroxidase (PO), phenylalanine ammonia lyase (PAL), and catalase (CAT) were investigated in squash plants. Prior to the pathogen inoculation, the bacterial treatment did not stimulate levels of PAL, PO, and PPO activities compared to the nontreated control. Two days after challenged with *P. capsici*, PAL, PO, and PPO activities were significantly increased in leaves treated by IN937b compared to the nontreated control. A similar induction pattern of PAL, PO, CAT activities were observed in the untreated leaves suggesting that IN937b root colonization could systemically induce defense-related enzymes activities in squash leaves. Reduced disease severities coupled with enhanced enzyme activities elicited by PGPR indicate that its mode of action for suppressing Phytophthora blight in squash is at least in part through induced systemic resistance.

Modulation of plant resistance to viral pathogen by abiotic stress factor
N. A. Moldakimova (1), G. S. Mukiyanova (1), D. G. Yarmolinsky (2), G. G. Brychkova (2), H. B. Scholthof (3), M. Sagi (2), R. T. OMAROV (1)
(1) Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, Astana, Kazakhstan; (2) Plant Stress Laboratory, The Albert Katz Department of Dryland Biotechnologies, French Associates Institute for Agriculture and Biotechnology of Drylands, Blaustein Institutes for Desert Research, Ben-Gurion University of Negev, Sede Boqer Campus, Israel; (3) Department of Plant Pathology and Microbiology, Texas A&M University, Houston, TX, U.S.A.
Phytopathology 102:S4.81

Plant resistance to simultaneous application of both biotic and abiotic stress factors applied simultaneously has not been well elucidated. The purpose of this study was to determine the effect of environmental stress factors, such as salinity, on viral systemic disease spread in *Nicothiana benthamiana*. Elevated concentrations of salinity in growth media in presence of *Eggplant mottled crinkle virus* (EMCV) were chosen as combined stress factors. Infection with *Eggplant mottled crinkle virus* systemic in *N. benthamiana* usually leads to systemic necrosis due to plant vascular collapse. We found that high concentrations of NaCl result in increased plant resistance to viral disease and its lower systemic accumulation, as was evident by immunoblotting and RT-PCR assays. Systemic symptoms have not been detected in plants initially exposed to 100mM and 150mM concentrations of NaCl 21 days prior inoculation. Furthermore, our studies indicate that plants exposed to dual stress factors exhibit increased levels of reactive oxygen species. The relationship between plant responses to biotic and abiotic stress factors may indicate the existence of universal defensive pathways of plant adaptation to unfavorable conditions.

WITHDRAWN

***Rose yellow mottle virus*, a novel virus that affects *Rosa* sp.**
D. MOLLOV (1), B. Lockhart (1), D. Zlesak (2)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) University of Wisconsin-River Falls, River Falls, WI, U.S.A.
Phytopathology 102:S4.82

A previously undescribed virus with filamentous 790-800 nm particles was associated with symptoms of yellow leaf mottling and premature defoliation in the rose cultivars 'June Bride' and 'Captain Harry Stebbings' in Minnesota and in 'Ballerina', 'Buff Beauty', 'Mozart', 'Cornelia', 'Nastarana', 'Dorothy-Perkins', and 'Sir Thomas Lipton' in New York. The virus was not transmitted by mechanical inoculation or by *Macrosiphum euphorbiae* but was transmitted by graft inoculation to healthy plants of 'June Bride', 'Love and Peace', 'Tropicana', and 'George Vancouver' in which typical symptoms developed and the presence of the virus was confirmed. The virus was provisionally named *Rose yellow mottle virus* (RoYMV). Graft inoculated 'Ballerina' developed severe necrotic cane lesions in addition to normal foliar symptoms. The necrotic symptoms were similar to those described for rose streak disease and appeared to be genotype specific. RoYMV was graft transmitted to 28 'Ballerina' related genotypes and caused leaf mottling in all and cane necrosis in 22 genotypes. Rabbit antibodies raised against purified RoYMV virions were used to detect the virus by ELISA and Immunosorbent Electron Microscopy (ISEM). RoYMV also was detected by RT-PCR using virus specific primers. Based on preliminary genome sequence analysis RoYMV appears to be a novel virus with sequence identity of 32-55% to known viruses in the family Potyviridae.

Identification, transmission, and genomic characterization of a novel member of the Caulimoviridae causing a yellow vein disease of cultivated rose
D. MOLLOV (1), B. Lockhart (1), D. Zlesak (2)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) University of Wisconsin-River Falls, River Falls, WI, U.S.A.
Phytopathology 102:S4.82

A previously undescribed virus with spherical 48-50 nm particles containing a circular ds DNA genome was associated with vein-yellowing symptoms in the rose cultivar 'Dr. Merkeley' in Minnesota and in the cultivars 'Madame Pierre Oget', 'Mozart', 'Prosperity', and 'Schneezwerg' in New York. The virus also occurred in *Rosa rugosa* showing leaf distortion. The virus was provisionally named *Rose yellow vein virus* (RYVV). The virus was transmitted by grafting to healthy plants of the variety 'George Vancouver' in which characteristic vein-yellowing symptoms developed and the presence of RYVV was confirmed. The virus was not transmitted by mechanical inoculation or by *Macrosiphum euphorbiae*. The RYVV dsDNA genome is about 8 kb in size and contains five putative ORFs, having similarity of 20-29% to 34-46% to corresponding regions of members of the Caulimoviridae. The genome organization of RYVV is distinct from those of known genera in the family Caulimoviridae, suggesting it may represent a new genus in this family.

Optimal timing of fungicide applications for control of citrus scab sporulation caused by *Elsinoë fawcettii*
S. N. MONDAL (1), M. M. Dewdney (2)
(1) CREC, University of Florida, Lake Alfred, FL, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.82

An effective citrus scab fungicide would reduce sporulation as well as prevent infection. Conidia production by *E. fawcettii* occurs in 2-3 hrs after a wetting event, but the pustule age where conidia continue to be produced is unknown. The optimal pustule age for sporulation of nine *E. fawcettii* isolates and how long conidia were produced was tested. Young flush was inoculated with 10^5 conidia/ml each of 7 Florida narrow host range (FNHR) pathotype isolates and 2 Florida broad host range (FBHR) on 2 reps/isolate/block in 2 blocks over time. Twenty pustules/rep were collected and sporulation induced at 5, 10, 20, 45, 60, 90, 120, 150 and 180 days post-inoculation (dpi) from 2 blocks. Pustules from all isolates produced conidia until 180 dpi and the optimal pustule age was between 10 to 60 dpi with average maxima of 6.7 X 10^6 conidia/ml at 20 days. Four fungicides [Kocide 2000 (1.4g metallic Cu/L), pyraclostrobin (0.25g ai/L), azoxystrobin (0.24g ai/L), ferbam (0.1g ai/L)] and an untreated control (UTC) were applied to 21 day-old scab pustules from 1 FBHR and FNHR isolate each on 2 reps/isolate/treatment/block in 2 blocks over time. Sporulation from 20 pustules/rep was induced 1, 3, 7 and 15 days post-application. The greatest sporulation suppression ($P < 0.05$) was with pyraclostrobin on all days for both pathotypes. All fungicide treatments significantly reduced the number of spores compared to the UTC ($P < 0.05$).

Comparison of methodologies used for the detection of grapevine viruses
J. MONIS (1), H. G. Stanghellini (1), Z. Morales (1), L. Abdelshahid (1)
(1) Eurofins STA Laboratories, Gilroy, CA, U.S.A.
Phytopathology 102:S4.82

Our lab developed the HealthCheck™ Panel A that includes the following assays for the detection of *Grapevine leafroll associated virus* (GLRaV -1, -2, -3, -4-9, -6, -7); *Grapevine virus A* (GVA), and *Grapevine fleck virus* (GFKV) by ELISA and End-Point RT-PCR that detects *Grapevine leafroll associated virus* -1, 2, GLRaV-2 Red Globe, -3, -4, -5, -6, -7, -9, *Grapevine virus A*, *Grapevine virus B*, *Grapevine virus D*, *Grapevine Syrah virus* (*GSyV-1*), *Grapevine fleck virus*, and *Rupestris stem pitting associated virus* (including the Syrah strain of RSPaV). Samples that were previously subjected to the HealthCheck™ Panel A were tested using Taqman RT-PCR specific for the detection of the following viruses: *Grapevine leafroll associated virus* -1, 2, GLRaV-2 Red Globe, -3, -4, -5, -7, -9, *Grapevine virus A*, *Grapevine virus B*, *Grapevine virus D*, *Grapevine fleck virus*, and *Rupestris stem pitting associated virus*. Our work showed that both the Taqman and End Point PCR had the same sensitivity as measured by the limit of detection using serial dilutions of known infected grapevine template RNA. However, Taqman RT-PCR failed to detect virus infection in many of the samples and positive controls tested. The lack of detection can be explained by the diverse genetic variant populations present in virus infected grapevines. We conclude that more than one complementary detection method should be used for accurate and sensitive pathogen detection.

Reaction of thirteen sugarcane varieties to orange rust, caused by *Puccinia kuehnii*
A. S. MOREIRA (1), C. R. Gonçalves (2), A. Ricci (2), A. Bergamin Filho (1)
(1) Escola Superior de Agricultura Luiz de Queiroz/Universidade de São Paulo, Piracicaba, Brazil; (2) Centro de Tecnologia Canavieira - CTC, Piracicaba, Brazil
Phytopathology 102:S4.82

Important varieties classified as intermediate or susceptible to *Puccinia kuehnii* are present in Brazilian's sugarcane production. Thirteen commercial varieties (SP89-1115, SP81-3250, RB86-7515, RB85-5156, RB92-5211, RB96-6928, and 7 CTC varieties coded by the CTC-Brazil) were sprayed with inoculum of *P. kuehnii* from grown sugarcane located in Ribeirão Preto, SP, Brazil. The experiment was conducted in growth room conditions at 25°C and 12-h photoperiod. The open/closed pustules proportion and the disease severity were evaluated by the scale described by Amorim et al. (1987) for 16 days. Three plants per variety were evaluated. Incubation period ranged from 7 to 8 days in the thirteen varieties. SP89-1115, RB92-5211, and CTC-G showed latent period of 9 days and RB85-5156, RB81-3250, CTC-A, C, D and E, showed latent period of 10 days. Open pustules were not observed for the other four varieties. CTC-A and SP89-1115 were more susceptible to *P. kuehnii* with the greatest potential for production of inoculum. More than 90% of open pustules were recorded at the end of assessments. RB85-5156, RB92-5211, SP81-3250, CTC-E, C, D, and G showed between 2% and 67% of open pustules. The highest disease severities occurred for RB85-5156, SP989-1115, CTC-A, and CTC-E (from 3.7% to 27.6%). In descending order of susceptibility we found SP89-1115~CTC-A~CTC-E > RB85-5156~RB92-5211~SP81-250~CTC-C~CTC-G > CTC-F~CTC-B~RB86-6928~RB86-7515.

Temporal dynamics of Asian soybean rust as influenced by leaf area index
E. N. MOREIRA (1), F. X. Vale (1), P. A. Paul (2), L. A. Maffia (1), F. W. Neves (1), P. Schulman (1), C. A. Silva (1)
(1) Universidade Federal de Viçosa, Viçosa, Brazil; (2) The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH, U.S.A.
Phytopathology 102:S4.82

Since first being reported in South America in 2001, Asian soybean rust (ASR), incited by *Phakopsora pachyrhizi*, has caused substantial losses in all major soybean production regions of Brazil. However, lots of questions still remain about the epidemiology of this disease, including the effect of leaf area index (LAI) on its temporal dynamics. Experiments were conducted in Lucas do Rio Verde, MT, Brazil during the 2010/2011 growing season to evaluate the effects of cultivar maturity and planting date, both of which affect LAI, on ASR development. The experimental design was a randomized complete block, with four replications and a split-plot arrangement of planting date and cultivar. Planting date (October 20, November 19, December 15, and January 5) was the as whole plot and cultivar maturity (early-, mid-, late-season), the sub-plot. All cultivars evaluated were susceptible to ASR, and all plots were naturally infected. ASR was first observed on the early- and mid-season cultivars of the first planting date. Preliminary results suggest that the rate of ASR development was slowest for the first planting date and fastest for the third and fourth planting dates. For all planting dates, the early-maturing cultivar had the highest rate of ASR progress, relative to the other cultivars.

Virus-induced gene silencing in *Nicotiana benthamiana* using a derivate vector of *Euphorbia mosaic virus Yucatan Peninsula* (EuMV-YP)

O. A. MORENO-VALENZUELA (1), H. J. Villanueva-Alonzo (1), L. A. Lopez-Ochoa (1), O. Guerra-Peraza (2), D. Robertson (2)
(1) Centro de Investigación Científica de Yucatán, Mérida, Yucatán, Mexico; (2) Department of Plant Biology, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.83

Virus-induced gene silencing (VIGS) is a process based on the sequence specific degradation of RNA and has become an effective method for reducing gene expression in plants. Geminiviruses are among virus families that have been used for the construction of VIGS vectors. They are relatively easy to modify into VIGS vectors, their inoculation is straight forward and also biosecure. In addition, they have a wide host range in economically important crops. A member of this family is the *Euphorbia mosaic virus Yucatan Peninsula* (EuMV-YP), which is infective in at least five species of different plant families (*Euphorbia heterophylla*, *Phaseolus vulgaris* cv. Top, *Capsicum annuum* cv. Anaheim, *Solanum lycopersicum* cv. Humaya and *Datura stramonium*). EuMV-YP: ΔCP:chlI virus vector was constructed replacing part of the gene of capsid protein by a multiple cloning site and then inserting a gene fragment of subunit I of magnesium chelatase (*chlI*). Virus induced gene silencing in *Nicotiana benthamiana* was efficient in the leaf vein and its surroundings. Silencing remained until the fruiting stage and it was extended to flowers and fruits. Silencing was affected by incubation temperature, yielding better results at 25 °C when compared with 20 °C and 30 °C, which coincided with a greater accumulation of viral DNA at this condition.

WITHDRAWN

***Neofusicoccum mediterraneum* causing cankers and *Diplodia mutila* and *Diplodia seriata* isolated from pomegranate (*Punica granatum*) in California**

D. P. MORGAN (1), T. J. Michailides (1)
(1) University of California-Davis, Kearney Agricultural Center, Parlier, CA, U.S.A.
Phytopathology 102:S4.83

Neofusicoccum mediterraneum, *Diplodia mutila*, and *Diplodia seriata* were isolated from 40% of cankers on the trunks and limbs of pomegranate (*Punica granatum*) in California from 2008-2011. Isolates matched published morphological characteristics for these species. The ribosomal internal transcribed spacer region using primers ITS 1 and 4, beta-tubulin gene using primers BT2A and BT2B, and elongation factor gene using primers EF1-728F and EF1-986R were amplified and sequenced. Sequences of California isolates of *N. mediterraneum* were identical with CBS 121558, *N. mutila* isolates with CBS 112553, and a *D. seriata* isolate nearly identical with CBS 112555. Inoculation of 10 one-year-old shoots of *P. granatum* cv. Wonderful with *N. mediterraneum* were made in July 2011 by removing 5-mm bark plug with a 5-mm cork borer and placing a 7-day-old 5-mm-diameter agar plug of *N. mediterraneum* mycelium side down. Inoculated wounds were covered with petroleum jelly first and then wrapped with Parafilm to retain moisture. Branches were harvested 6 months later, the cankers were measured, and isolations were made from the edge of discolored tissues to confirm Koch's postulates. Cankers averaged 5.5 cm in length. As far as we know, this is the first record of these three species of the Botryosphaeriaceae isolated from pomegranate in the world, and the first record of *N. mediterraneum* causing cankers on pomegranate shoots.

WITHDRAWN

Development and control of grape powdery mildew (*Erysiphe necator*) under different levels of ascosporic inoculum dose

M. M. MOYER (1), D. M. Gadoury (2), W. F. Wilcox (3), R. C. Seem (2)
(1) Washington State University, Prosser, WA, U.S.A.; (2) New York State Agricultural Experiment Station, Geneva, NY, U.S.A.; (3) Cornell University NYSAES, Geneva, NY, U.S.A.
Phytopathology 102:S4.83

There is a limited period of time early in an epidemic during which powdery mildew can infect fruit before berries develop ontogenic resistance. In this study, a *Vitis vinifera* 'Chardonnay' vineyard was infested with cleistothecia to yield a gradient of primary inoculum dose that spanned 3 orders of magnitude in 4 steps (1X, 10X, 100X and 1000X), where X = 22 or 17 cleistothecia per kg bark in year 1 or 2 of the study, respectively. An abbreviated fungicide program effectively suppressed disease on fruit up to the 100X level when in-season weather was moderately conducive for epidemic development (year 1), but suppression was degraded progressively above the 10X level when in-season weather was more conducive (year 2). In a subsequent study, disease foci established by inoculating foliage at key stages of vine development (cluster emergence, rachis elongation, and bloom)

caused severe fruit infection up to 1 m from the focus center when in-season weather was moderately conducive to disease, but up to 3.5 m from the focus center when in-season weather was more conducive. Thus, (i) favorable in-season weather can accelerate the development of powdery mildew on grape berries by increasing both the efficacy of primary inoculum quantity and the distance of significant spread from disease foci; and (ii) fungicidal control of berry infection can be influenced by an interaction between primary inoculum level and climate.

What is causing white pine needle damage in northeastern North America?

I. A. MUNCK (1), B. Burns (2), K. Lombard (3), J. Weimer (3), W. D. Ostrofsky (4)
(1) USDA Forest Service, Durham, NH, U.S.A.; (2) Vermont Department of Forests, Parks, & Recreation, Springfield, VT, U.S.A.; (3) New Hampshire Department of Natural Resources and Economic Development, Hillsborough, NH, U.S.A.; (4) Maine Forest Service, Augusta, ME, U.S.A.
Phytopathology 102:S4.84

White pine needle damage (WPND) is an emerging problem in New England and eastern Canada where *Pinus strobus* is of great historic, ecological, and economic importance. State agencies received hundreds of calls from concerned citizens during 2010 when WPND was particularly severe following a very wet spring. Foliar damage was attributed to a needle cast (*Canavirgella bandfieldii*) and a needle blight (*Mycosphaerella dearnessii*). Both these fungi cause similar symptoms, thus complicating diagnoses. In 2011 the USDA Forest Service (USFS) coordinated a survey with Forest Health State Cooperators (FHSC) from Maine, New Hampshire, and Vermont to investigate the cause of WPND. Sixty trees from 13 sites with foliar damage the prior year were sampled in May and July by FHSC and then diagnosed in the USFS Durham Field Office. The needles were infected with *M. dearnessii* and *C. banfieldii*, and another needle cast causing pathogen, *Bifusella linearis*. These three pathogens were present at the same site and more than one pathogen was found infecting the same tree. Long, black hysterothecia formed by *B. linearis* and *C. bandfieldii*, along with browning of the distal parts of the needles were present in samples collected in May. *Mycosphaerella dearnessii* was the most frequently observed and widely distributed pathogen, also the most consistently associated with chlorosis and defoliation in early July. WPND will likely remain a problem in years with wet springs.

Survey for '*Candidatus* Phytoplasma' species from nursery plants, urban shade trees, and agricultural crops in Nevada

A. Munoz (1), S. Wang (1), J. RASCOE (2)
(1) Nevada Department of Agriculture, Sparks, NV, U.S.A. (2) USDA-APHIS-PPQ-PHP-PSPI-NIS, Beltsville, MD, U.S.A.
Phytopathology 102:S4.84

In an effort to survey for exotic phytoplasma species such as '*Candidatus* Phytoplasma australiense', '*Ca.* Phytoplasma AP-MLO', '*Ca.* Phytoplasma prunorum' and other '*Ca.* Phytoplasma' species, more than 200 symptomatic plant samples were collected from ash trees, orchards, vegetable crops, and ornamental and nursery plants. To detect phytoplasma from suspected plant samples, petioles and midribs of each sample were disrupted, homogenized, and then preceded to genomic DNA extraction using a Qiagen DNeasy plant mini kit. Molecular detection was employed by amplifying fragments of DNA from the 16S, intergenic space region, and 23S rRNA phytoplasma genes using selected primers. To confirm the identity of PCR products, amplified DNA was subcloned into pGEM®-T vector and then sequenced. All the samples collected from yellowish ash trees, onion leaves, apples, pears, and other ornamental plants were negative for phytoplasma using multiple primer pairs. However, one composite sample collected from yellowish and stunted zucchini squash (*Cucurbita pepo* 'Seneca' XPG778) in southern Nevada generated a positive PCR product of 1647bp when P1/Tint primers were used. This phytoplasma belongs to the clover proliferation (16SrVI) group according to BLAST analysis and iPhyClassifier. The 16SrVI phytoplasma identified here could be the first record from zucchini in Nevada.

Three *Tobacco etch virus* strains that induce distinctly different disease phenotypes

J. F. MURPHY (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.84

Tobacco etch virus (TEV) strains HAT, Mex21 and N were compared for their disease induction in bell pepper (*Capsicum annuum* L). Pepper plants were inoculated with each TEV strain when at the 7 to 8 leaf stage and maintained in a greenhouse. Each virus induced systemic vein clearing as the initial symptom; however, subsequent symptoms differed as plants continued to develop. HAT induced mild systemic mosaic with no apparent plant stunting (avg. height 29 cm; weight 60 g). Mex21 induced systemic mosaic consisting of distinct light green and dark green areas with some leaf deformation. These plants were stunted relative to healthy controls and HAT-infected plants (avg. height 19 cm; weight 33 g). N induced severe chlorosis, leaf distortion and plant stunting (avg. height 11 cm; weight 14 g). Healthy control plants had an avg. height of 30 cm and weight of 53 g. Despite the milder symptoms induced by HAT, virus accumulation in systemically infected leaves was higher than for Mex21 and N infected plants.

Status of fluopyram resistance development

G. MUSSON (1), H. Young (1)
(1) Bayer CropScience, Research Triangle Park, NC, U.S.A.
Phytopathology 102:S4.84

Fluopyram is a fungicidal active ingredient recently approved by the U.S. Environmental Protection Agency for use in certain horticultural and row crops. Fluopyram, like all FRAC Group 7 fungicides, inhibits the action of the enzyme succinate dehydrogenase, which is a key component of the ATP generating function of the fungal mitochondria. There are currently seven different chemical groups classified within the Group 7 succinate-dehydrogenase inhibitors (SDHI's). Resistant populations have been selected for with certain pathogens where boscalid, a pyridine-carboxamide SDHI, has been in use for nearly ten years. Specific fungal isolates of several plant pathogens, including *Alternaria alternata* and *Botrytis cinerea* with varying levels of resistance to boscalid are often retaining sensitivity to fluopyram, a pyridinyl-ethyl-benzamide. Research into the potential causes of these different responses will be presented.

A predicted interactome for *Zea mays*

B. M. MUSUNGU (1), M. Geisler (2), R. Brown (3), D. Bhatnagar (3), A. M. Fakhoury (1)
(1) Department of Plant, Soil and Agriculture Systems, Southern Illinois University, Carbondale, IL, U.S.A.; (2) Department of Plant Biology, Southern Illinois University, Carbondale, IL, U.S.A.; (3) Southern Regional Research Center, USDA-ARS, New Orleans, LA, U.S.A.
Phytopathology 102:S4.84

An interactome is the genome-wide roadmap of protein interactions that occur within an organism. Interactomes for humans, fruit fly, and now the plant *Arabidopsis thaliana* have been generated using high throughput experimental methods. It is possible to use these experimentally derived interactomes to predict interactomes for other species based on orthology of genes between species. To build an interactome for maize, orthologs were identified to determine a one to one orthology between genomes of maize and reference species where both maize orthologs occurred for an interaction in the reference species, this implied that the proteins likely interacted in maize. This interlogs (interacting ortholog) method was used to find interactions between maize proteins. Experimental interactome studies have demonstrated that interacting proteins tend to be co-expressed, and that gene co-expression is a good predictor of physical interactions. We used microarray based gene expression data sets frommaize to produce a gene co-expression network. This was done both to identify co-expressed gene clusters, and to increase the confidence of the gene expression network. The maize interactome and gene expression networks will provide researchers with a tool for studying interactions of key proteins inmaize. This will facilitate identifying biomarkers linked to resistance to pathogens.

Mefenoxam sensitivity of recent strains of *Phytophthora infestans* in the United States

K. L. MYERS (1), R. Childers (1), D. Camuzeaux (1), G. Danies (1), I. M. Small (1), W. E. Fry (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.84

Since the late 1980s, populations of *Phytophthora infestans* in the United States have been dominated by mefenoxam-resistant strains. The predominant strains on potatoes since 1994/1995 have been in the US8 clonal lineage. US8 is an A2 mating type, very aggressive to potatoes and relatively resistant to mefenoxam. Populations of *P. infestans* on tomatoes have been more diverse. The pandemic of tomato late blight in 2009 caused us to test recently acquired strains for their sensitivity to mefenoxam. From 2009 through 2011, we tested the mefenoxam sensitivity of more than 275 isolates from more than 25 different states. The dominant strains were US22 (2009 and 2010), US23 and US 24 (2011). US8 and US11 have also been detected. In lab tests, members of the US22 and US23 clonal lineages have been generally sensitive to mefenoxam (growth suppressed to less than 40% of control at 5ppm mefenoxam). Members of the US8 and US11 clonal lineages have been generally resistant (growth on 5ppm mefenoxam at quite a bit more than 40% of growth in the

absence of mefenoxam). Most members of the US24 clonal lineage are sensitive to mefenoxam, but some isolates appear to somewhat resistant. In vivo assessments of selected isolates have confirmed the in vitro assessments.

Evaluation of resistance to *Phytophthora*-induced fruit rot and its correlation with fruit traits in *Capsicum annuum*

R. P. NAEGELE (1), A. Tomlinson (1), H. Gutting (1), S. Boyle (1), M. K. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.85

Phytophthora capsici is a major pathogen of pepper in field and greenhouse production causing fruit and root rot, and foliar blight. Breeding to incorporate disease resistance into pepper is essential for reducing losses long term. In this study, the inheritance of fruit rot was investigated in an F6 RIL population of CM334 and the cultivated variety 'Early Jalapeno', developed by Dr. Paul Bosland at the New Mexico Chili Pepper Institute. Phenotypic characteristics of the fruit were evaluated to identify potential correlations with disease symptoms. The two parents and 68 progeny lines were evaluated for fruit rot resistance using 3 different *P. capsici* isolates at 3 and 5 days post inoculation. Phenotypic traits of the population (fruit color, shape, firmness, gloss, and pericarp thickness) were evaluated separately for each line. Differences in lesion area, density of pathogen growth, and the phenotypic traits were detected between lines. Correlations between fruit phenotypic traits and disease susceptibility were tested. Positive correlations were detected between each of the measurements of disease (lesion area, growth per day and density of pathogen growth). No correlation was evident between fruit phenotypic traits and lesion area or density of pathogen growth. Pathogen growth per day was positively associated with fruit firmness only. These results suggest that breeding for fruit rot resistance in pepper will have minimal linkage drag with the fruit phenotypic traits measured.

High-grafted tomatoes to control bacterial wilt caused by *Ralstonia solanacearum*

K. NAKAHO (1), H. Kajihara (2), M. Maeda (3), A. Notsu (4), T. Kawara (5)
(1) National Agricultural Research Center, NARO, Tsukuba, Ibaraki, Japan; (2) Yamaguchi Prefectural Agriculture and Forestry General Engineering Center, Yamaguchi, Yamaguchi, Japan; (3) Niigata Agricultural Research Institute, Nagaoka, Niigata, Japan; (4) Hokkaido Ornamental Plants and Vegetables Research Center, Takikawa, Hakkaido, Japan; (5) Bergearth Co. Ltd., Uwajima, Ehime, Japan
Phytopathology 102:S4.85

Bacterial wilt caused by *Ralstonia solanacearum* is a major constraint in tomato production. In Japan, grafting of susceptible but high quality tomato cultivars onto highly resistant rootstocks has been adopted to manage bacterial wilt. Recently, however, bacterial wilt in grafted tomato plants has occurred in intensive greenhouse cultivation. Our previous report showed that the resistance in rootstocks is due to suppressed vertical and horizontal movement of the pathogen in plants. To enhance the resistance performance of rootstocks, we developed "high grafting" as a new strategy for controlling bacterial wilt. High grafting was defined as grafting at a higher position (at the stem above the second or third leaf) than usual (at the epicotyls). In greenhouse experiments, high grafting resulted in slow disease development and less severe wilting than usual grafting. In healthy plants, plant growth, yield and quality of tomato fruit in high-grafted plants were not significantly different from those produced by usual grafting. We further developed sustainable practices in combination with high grafting and soil reduction disinfection using molasses. These results indicate that high grafting could be an effective integrated management strategy against bacterial wilt.

***Alternanthera mosaic virus* TGB1 interaction with chloroplast beta ATPase is necessary for viral replication**

J. Nam (1), C. Jang (1), M. Li (1), H. Kim (1), S. Cho (1), H. Kim (1), D. K. Lakshman (2), J. Hammond (2), H. LIM (1)
(1) Chungnam National University, Daejeon, South Korea; (2) USDA-ARS FNPRU, Beltsville, MD, U.S.A.
Phytopathology 102:S4.85

The potexvirus *Alternanthera mosaic virus* (AltMV) has multifunctional triple gene block (TGB) proteins, among which our studies have focused on the functions and basis of TGB1 protein interactions. The TGB1 of AltMV has functions including RNA binding, RNA silencing suppression, and cell-to-cell movement, and is known to form homologous interactions. The helicase domains of AltMV TGB1 were separately mutated to identify which regions are involved in homologous TGB1 interactions. The yeast two hybrid system and Bimolecular Fluorescence Complementation (BiFC) *in planta* were utilized to examine homologous interactions of the mutants. As a result, residue 45(G) in helicase motif I was found to be critical to maintain homologous interaction. Mutations in the remaining helicase motifs did not inhibit TGB1 homologous interactions. In order to identify interacting host proteins, the extracted inclusion body proteins from AltMV-infected *Nicotiana benthamiana* were separated by 2-D gel electrophoresis. MALDI-TOF mass spectrometry of isolated protein spots identified TGB1, and chloroplast α and β ATPase subunits associated with inclusion bodies. Chloroplast β ATPase was confirmed to interact with TGB1 by BiFC. *Tobacco rattle virus* induced β ATPase gene silencing in *N. benthamiana* is being used to confirm the relationship between β ATPase interaction and AltMV replication. BiFC is revealing how TGB1 homologous interactions and other mutations affect the interaction with β ATPase.

WITHDRAWN

Selective RNA packaging of *Maize rayado fino virus* viruslike particles transiently expressed in *Nicotiana benthamiana* plants

A. Natilla (1), R. W. HAMMOND (1)
(1) USDA-ARS MPPL, Beltsville, MD, U.S.A.
Phytopathology 102:S4.85

Maize rayado fino virus (MRFV), genus *Marafivirus*, family *Tymoviridae*, is composed of isometric particles of 30 nm diameter. Each particle has 180 copies of the capsid protein (CP) forming a T=3 structure, stabilized by protein-protein interactions. MRFV CPs self-assemble in *Escherichia coli* and in *Nicotiana benthamiana* into virus-like particles (VLPs) identical in size and shape to MRFV native capsids. VLPs are known to incorporate cellular RNAs, with the level of each RNA species dependent on the level in the RNA pool. We investigated the ability of plant-produced MRFV-VLPs to encapsidate host cellular RNAs as well as a foreign reporter genes. High level production of MRFV-VLPs was obtained by transient expression in *N. benthamiana* using a pGD-based vector (agroinfiltration) and a *Potato virus X* (PVX)-based vector. Purified VLPs were subjected to IC-RT-PCR using polyclonal antisera to MRFV and primers specific to the MRFV-CP gene as well as the replicase, triple gene block and CP genes of PVX. VLPs produced by agroinfiltration resulted in amplified products corresponding to the MRFV CP gene, whereas VLPs produced using the PVX-based vector resulted in products corresponding to the PVX genome. Experiments to determine if reporter gene RNAs were encapsidated into MRFV-VLPs resulted in amplified products corresponding to the sequence of the foreign RNA. Our results suggest that highly represented foreign RNAs may drive MRFV-VLP assembly toward a programmed packaging.

Influence of zinc on growth and biofilm production of *Xylella fastidiosa*

F. NAVARRETE (1), L. De La Fuente (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.85

Xylella fastidiosa (Xf) is a xylem-restricted bacterium that is the causal agent of several economically important diseases in woody and herbaceous plant

hosts. Diseased plants show leaf scorching and/or chlorosis, in some cases resembling mineral deficiencies in Zn or Fe. Biofilm production inside xylem vessels is known to be important for both disease development and pathogen acquisition by the insect vector and has been shown to be affected by the chemical composition of the environment. To elucidate the potential roles of Zn in Xf biology, the effects of Zn amendments on Xf growth and biofilm production in vitro were evaluated. Total growth (including planktonic and biofilm cells) was assessed in test tubes, and biofilm production was also evaluated in microfluidic chambers. In test tubes, Zn amendments decreased planktonic growth and biofilm formation. However, microfluidic chamber assays showed dramatic increases in biofilm-like aggregates when the basal medium was supplemented with 0.4 mM Zn and cells were fed constantly into the system. Cell viability of aggregates formed inside the chambers was assessed by propidium iodide staining, which showed that most of the cells in the biofilm-like aggregates formed in the Zn-amended medium were non-viable. The effects of Zn on cell adhesion force and exopolysaccharide production is currently being tested. This information will ultimately be important in the development of nutritional approaches to disease management.

Effects of glyphosate application rates on soybean sudden death syndrome
S. S. Navi (1), L. Jing (1), X. YANG (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.86

Outbreaks of soybean sudden death syndrome (SDS) have been reported by farmers following applications of glyphosate at rates greater than label rate. Early study in our lab showed that higher rate applications of glyphosate increased SDS. Rate of glyphosate applications have been increasing since the introduction of Roundup Ready (RR) soybeans. USDA data show that commercial application rate of glyphosate was 0.57 A.I./year (lb/acre) in 1996 and the number was 1.23 in 2006. In recent years use of higher rates of glyphosate for weed management have been practiced by farmers because of the occurrence of glyphosate resistant weeds and availability of cheaper generic glyphosate. We conducted experiments in the greenhouse to investigate the effects of amount of glyphosate on occurrence of SDS in RR soybean. Treatments were established using different glyphosate application rates and application times. Results of this study were consistent with what reported 12 years ago by Sanogo, Yang and Scherm in 2000. Incidence and severity of SDS increased when soybean plants received higher rates of glyphosate, which suggests that over years, the nature of glyphosate effects on the occurrence of SDS in RR soybeans remains unchanged.

Relating aerial concentration and escape of *Pseudoperonospora cubensis* sporangia from a cucumber canopy to disease severity during cucurbit downy mildew epidemics
K. Neufeld (1), S. Isard (2), P. OJIAMBO (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.86

Pseudoperonospora cubensis causal agent of cucurbit downy mildew is disseminated aerially on a large spatial scale via asexual sporangia. A prerequisite to development of models to predict disease spread is the quantification of aerial concentration and escape of sporangia from infected fields. Sporangia concentrations, *C*, were monitored using Rotorod samplers deployed at 0.5 to 3.0 m above a infected cucumber canopy at two field sites with 1 to 40% disease severity. Mean sporangia concentration (*mC*) was higher at moderate than at low or high disease severity. Values of *mC* decreased rapidly with height above the canopy and values at 2.0 m were only 7% of those measured at 0.5 m when disease severity was moderate. No sporangia were collected at height of 3.0 m above the canopy. Escape of sporangia, *F*, varied with hour of the day and disease severity with a maximum value of 926.4 sporangia m^{-2} s^{-1}. Daily total flux, *DF*, was also dependent on disease severity and ranged from 5.9 to 2,242.3 sporangia m^{-2}. The fraction of available sporangia that escaped the canopy increased from 0.028 to 0.171 as average wind speed above the canopy at high *C* increased from 1.7 to 3.6 m s^{-1}. Variations of *mC* and *DF* with disease were well described ($P < 0.0001$) by a log-normal model with a severity threshold of 15%. Results will be incorporated in a regional-scale spore transport model currently used to predict the risk of spread of downy mildew among cucurbit fields in the eastern U.S.

Amplification of *Meloidogyne minor* with primers reported to be specific for *Meloidogyne fallax*
C. NISCHWITZ (1), M. Schmitt (2), M. McClure (2)
(1) Utah State University, Logan, UT, U.S.A.; (2) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.86

Based on an analysis of the 28S, D2-D3 expansion region, infective juveniles of root-knot nematodes in a routine soil sample from turf grass were identified as belonging to the group that includes *Meloidogyne chitwoodi* and *M. fallax*. To distinguish *M. chitwoodi* from *M. fallax*, specimens were amplified with primers designed by C. Zijlstra to be species-specific for these two taxa. The specimens did not amplify with the *M. chitwoodi*-specific primers but did amplify with the *M. fallax*-specific primers. The 28S D2-D3 region of additional nematodes from the same population was then sequenced and aligned with sequences of authenticated *Meloidogyne* species. The D2-D3 sequences of the nematodes that amplified with *M. fallax*-specific primers matched authenticated *M. minor* sequences. To confirm the findings, specimens from the authenticated *M. minor* populations were also tested with the *M. fallax*-specific primers and they also produced a band of the correct size (530bp). No other *Meloidogyne* species found on turf grasses that we tested were amplified with the *M. fallax*-specific primers. At the time these primers were developed, *M. minor* had not been described. Reliance on the *M. fallax*-specific primers for regulatory and research purposes could be misleading.

Bioinformatics and expression analysis of *Mycosphaerella fijiensis* reveals candidate polyketide synthase gene clusters for production of phytotoxins
R. D. NOAR (1), S. Herrero (1), M. E. Daub (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.86

Mycosphaerella fijiensis is a fungal pathogen which causes black Sigatoka, a leaf spot disease of banana that is responsible for up to 40% of the total banana production cost due to the fungicide sprays needed to control it. *M. fijiensis* is in the same teleomorph genus (sensu lato) as *Cercospora* species which are known to produce cercosporin, a light-activated phytotoxin. Although cercosporin production has not been reported for *M. fijiensis*, is it known that banana plants infected with black Sigatoka are less severely affected in the shade than in full sunlight. Furthermore, *M. fijiensis* has similar resistance as *Cercospora nicotianae* to many photosensitizing dyes, including high concentrations (at least 10 mM) of cercosporin. In *Cercospora* species, cercosporin is produced by a polyketide synthase and other enzymes encoded by a gene cluster. To identify gene clusters that may produce similar toxins in *M. fijiensis*, a manual BLAST search with the cercosporin biosynthetic cluster against the *M. fijiensis* genome was performed. The results were confirmed using the web tool SMURF (secondary metabolite unique regions finder), which identifies polyketide synthase and non-ribosomal peptide synthase gene clusters from sequence data. RT-PCR analysis was performed from infected leaf samples, showing that several of the polyketide synthases are expressed during infection.

Summary of methyl bromide alternatives research in Florida strawberries
J. W. NOLING (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.86

The primary objective of a 5-year USDA ARS Areawide Pest Management project was to evaluate cultural and chemical alternatives to methyl bromide soil fumigation within Florida strawberry production. Many different fumigants were evaluated individually or in combination with herbicides and compared to methyl bromide. Yields of chemical alternatives were averaged across years and locations and compared to the performance of methyl bromide. Strawberry yields were often near equivalent or higher than yields with methyl bromide when combinations of fumigants such as methyl iodide, chloropicrin, metam sodium or potassium, 1,3-dichloropropene, and dimethyl disulfide were evaluated. Pest control efficacy for the alternative fumigants was generally less than that of methyl bromide and more highly dependent upon application methods and conditions. Unlike methyl bromide, prevailing soil edaphic and climatic conditions before and after fumigant application were important determinants of efficacy and crop response with the alternative chemicals. It is hypothesized that some inconsistency in pest control is unavoidable with alternatives to methyl bromide. The study demonstrated that applications of various preemergent herbicides are needed to effectively broaden the spectrum of weed control and that improved control of plant parasitic nematodes and crop yields are dependent upon the adoption of early crop destruction as an IPM practice.

Phenotypic and genotypic characterization of *Rhizoctonia solani* isolates from zoysiagrass in Kansas
K. OBASA (1), P. St. Amand (2), G. Bai (2), M. Kennelly (1)
(1) Kansas State University, Manhattan, KS, U.S.A.; (2) USDA-ARS, Manhattan, KS, U.S.A.
Phytopathology 102:S4.86

Large patch, the most common and severe disease of zoysiagrass, (*Zoysia* spp.) is caused by *Rhizoctonia solani* anastomosis group (AG) 2-2 LP. Thirty six isolates from zoysiagrass in Kansas were characterized based on

anastomosis pairing, in-vitro mycelial growth rates, nuclear counts, virulence, PCR, and amplified fragment length polymorphism (AFLP). We developed a modified AFLP protocol involving two selective amplifications, the first with non-labeled *Eco*RI primers with two selective-nucleotide extensions and the second using labeled primers with one nucleotide extension. All the *R. solani* isolates belonged to AG 2-2 LP. Variations were observed among the isolates in their average number of nuclei per cell, mycelial growth rates, and virulence. We observed a positive correlation between virulence and in vitro mycelial growth rate. There was also variation in the isolates' amplified fragment length polymorphism (AFLP) DNA fingerprint, suggesting possible underlying genetic differences of biological significance among members of AG 2-2 LP. The modified AFLP method reduced noise peaks relative to allele peaks, resulting in more reliable and objective results.

WITHDRAWN

WITHDRAWN

Polyubiquitin is required for growth, development, and pathogenicity in the rice blast fungus *Magnaporthe oryzae*

Y. OH (1), W. Franck (1), E. Gokce (1), D. C. Muddiman (1), R. A. Dean (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.87

Protein ubiquitination, which is highly selective, regulates many important biological processes including cellular differentiation and pathogenesis in eukaryotic cells. Here, we integrated pharmacological, molecular and proteomic approaches to explore the role of ubiquitination in *Magnaporthe oryzae*, the leading fungal disease of rice world-wide. Inhibition of ubiquitin-mediated proteolysis using the 26S proteosome inhibitor, Bortezomib, significantly attenuated conidia germination, appressorium formation and pathogenicity in *M. oryzae*. Gene expression analysis revealed that many of genes associated with protein ubiquitination were developmentally regulated during conidia germination. Only a few, including a polyubiquitin encoding gene, MGG_01282, were more abundantly expressed during appressorium formation and under nitrogen starvation. Targeted gene deletion of MGG_01282 in addition to a significant reduction in protein ubiquitination as determined by immuno blot assays, resulted in pleiotropic effects on *M. oryzae* including reduced growth and sporulation, abnormal conidia morphology, reduced germination and appressorium formation, and the inability to cause disease. Mutants were also defective in sexual development and were female sterile. GFP subcellular localization studies revealed that polyubiquitin was highly expressed in intact conidia and accumulated in vacuoles during conidia germination and appressorium development. Examination of 510 candidate polyubiquitinated proteins under nitrogen starvation revealed overrepresentation of proteins involved in protein degradation and the components of the ribosome. Our study suggests that ubiquitination of target proteins plays an important role in nutrient assimilation, development and pathogenicity of *M. oryzae*.

A target-specific approach for screening fungal symbionts of native *Sirex* populations

R. OLATINWO (1), J. Allison (2), J. Meeker (3), W. Johnson (3), D. Streett (4), C. Carlton (5)
(1) Louisiana State University, Pineville, LA, U.S.A.; (2) Natural Resources Canada, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada; (3) USDA Forest Service, R8-FHP, Pineville, LA, U.S.A.; (4) USDA, Forest Service, Southern Research Station, Pineville, LA, U.S.A.; (5) Department of Entomology, Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.87

Two *Sirex* woodwasps (Hymenoptera: Siricidae) native to southern United States, *Sirex edwardsii* (Brullé) and *Sirex nigricornis* Fabricius are currently considered distinct species, though some consider them conspecific. As part of an attempt to verify whether or not they are distinct species, target-specific PCR primers were developed to identify and differentiate fungal symbionts isolated from mycangia of females of both species. The objective of this study was to develop specific primers for amplification of the conserved regions (ITS and IGS) of ribosomal DNA, useful for routine screening of fungal isolates from native *Sirex* species. A total of 100 females of both species were collected between late October and early November, 2011 in Grant Parish, Louisiana. The identity and frequency of *Amylostereum* species carried in the mycangia of the two *Sirex* species were determined. Results from DNA sequences from PCR products thus far have positively identified approximately 80% of fungal symbionts isolated as *Amylostereum chailletii* and 20% as *Amylostereum areolatum*. The results may confirm presence of *A. areolatum* (symbiont of *Sirex noctilo*) in native *Sirex* populations in the southern U.S., with potential implications for biocontrol efforts and management of *Sirex* in the United States.

Ambrosia beetle populations and associated fungal symbionts in central Louisiana

R. OLATINWO (1), J. Allison (2), D. Streett (3), C. Carlton (4)
(1) Louisiana State University, Pineville, LA, U.S.A.; (2) Natural Resources Canada, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada; (3) USDA, Forest Service, Southern Research Station, Pineville, LA, U.S.A.; (4) Department of Entomology, Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.87

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) are known to live in symbiosis with fungi that provide nutrition for young larvae from cultivated galleries as they excavate tunnels in living and dead trees. Some ambrosia beetle species are pests of valuable tree species. The objective of this study was to investigate ambrosia beetle diversity and abundance in central Louisiana and identify associated fungal symbionts. Ambrosia beetles were collected from traps located around Pineville, Louisiana between June 2011 and March 2012. Fungal symbionts were isolated and cultured. Amplification of DNA extracted from fungal symbionts was conducted using the ITS region fungal primers (ITS1F and ITS4) in PCR reactions. Specific primers for *Raffaelea lauricola* T. C. Harr., Fraedrich & Aghayeva (a symbiont of *Xyleborus glabratus* Eichhoff and causal organism

of Laurel wilt of Redbay (*Persea borbonia* (L.) Spreng.) were also included. Positive PCR products from symbionts' rDNA were sequenced and used for phylogenetic analyses. Over 80% of ambrosia beetles collected were identified as *Xyleborinus saxeseni* Ratzeburg. Thus far, no *X. glabratus* were found at the two locations, however other ambrosia beetles identified included *Xyleborus ferrugineus* Fabricius, *Xyleborus pubescens* Zimmermann, and *Cnestus mutilatus* Blandford (=*Xylosandrus mutilatus*). Although, *X. saxeseni* has been associated with *R. lauricola*, the PCR results indicated that no beetles had *R. lauricola* as symbionts.

Detection of resistance to QoI fungicides in *Rhizoctonia solani* isolates from rice

G. OLAYA (1), C. Buitrago (2), D. Pearsaul (1), H. Sierotzki (2), A. Tally (3)
(1) Syngenta, Vero Beach, FL, U.S.A.; (2) Syngenta, Stein, Switzerland; (3) Syngenta, Greensboro, NC, U.S.A.
Phytopathology 102:S4.88

A major loss of performance in the control of sheath blight was observed in 2011. Isolates of *Rhizoctonia solani* (causal agent of rice sheath blight) with resistance to QoI fungicides were identified in several fields in Acadia Parish, Louisiana. Twenty *R. solani* isolates were retrieved from rice sheath blight samples that were collected from 4 fields where performance of Quilt Xcel was compromised. Four azoxystrobin and propiconazole baseline sensitive isolates collected before the commercial introduction of Quadris, Tilt or Quilt Xcel were also included in the fungicide sensitivity studies. The *R. solani* isolates collected were all identified to be in the anastomosis group AG-1 IA. No changes in the propiconazole sensitivity of the *R. solani* isolates were detected. But, changes in the azoxystrobin sensitivity were identified. The sequencing of the cytochrome *b* gene revealed that QoI resistant *R. solani* isolates had phenylalanine to leucine substitutions at codon 129 in the cytochrome *b* gene (F129L mutation). A total of 457 isolates were collected from 23 fields located near the problem area and their sensitivity was determined using a Perennial ryegrass (*Lolium perenne*) bioassay. In 8 fields no resistant isolates were detected. In 15 fields the frequency of resistant isolates varied from 7 to 100%. Nine isolates from Arkansas were sensitive to azoxystrobin. This is the first report of QoI fungicides resistance in *R. solani* isolates from rice.

WITHDRAWN

WITHDRAWN

WITHDRAWN

The effects of diverse *Xylella fastidiosa* isolates on the model host *Nicotiana tabacum*

J. E. OLIVER (1), T. T. Arnold (1), P. A. Cobine (1), L. De La Fuente (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.88

The xylem-limited bacterium *Xylella fastidiosa* (Xf) can cause devastating disease in numerous crops including grape, citrus, and blueberry. The symptoms caused by Xf include leaf scorching and yellowing which are similar to those caused by prolonged drought stress or nutrient deficiencies. However, the exact causes of Xf symptoms in plant hosts remain elusive, and drought or nutrient deficiencies alone are insufficient to reproduce typical symptoms caused by Xf. Previously, *Nicotiana tabacum* cv. SR1 (Petite Havana) has been shown to be a useful model species in which to study Xf effects *in planta*, and we have shown that infection with Xf isolate 'Temecula' perturbs the allocation of mineral nutrients within the host plant. Calcium and magnesium concentrations are significantly elevated in leaves of plants infected with Xf 'Temecula' while potassium and phosphorous levels are decreased. A diverse collection of Xf isolates from naturally infected grape and blueberry plants was screened in infection studies in *N. tabacum* and exhibited differential symptoms in host plants. To further characterize the effects of these isolates on host plants, additional parameters including bacterial quantities, plant height, leaf chlorophyll, and mineral nutrient status were examined. Variability between isolates is indicated, and results from comparisons amongst isolates will be presented.

WITHDRAWN

Utility of multilocus "DNA barcodes" for identification of switchgrass rust populations

G. ORQUERA (1), K. Choi (1), C. D. Garzon (1), S. M. Marek (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.89

Switchgrass (*Panicum virgatum*) is a perennial C4 grass native to the central prairies of North America and a sustainable feedstock crop for cellulosic biofuels. Most cultivars are susceptible to foliar rusts, reportedly caused by *Puccinia emaculata*, *P. graminis* or *Uromyces graminicola*. This complex etiology complicates development of effective management strategies. To define the molecular identity of the pathogen(s), the following barcode loci were amplified and sequenced from numerous urediniospore collections: internal transcribed spacer region of ribosomal DNA (ITS-rDNA), β-tubulin (bTub), translation elongation factor 1-α (TEF1a), and mitochondrial cytochrome b (cytb). While cytb was readily sequenced directly from PCR products, ITS-rDNA, bTub, and TEF1a products could not be sequenced directly due to heterozygosity present in template DNA. Subcloning of PCR products was required to sequence individual alleles. Polymorphism was highest among ITS-rDNA, followed by TEF1a, and bTub. Cytb was monomorphic. ITS-rDNA sequences were 99% similar to a *P. emaculata* sequence at NCBI, and grouped with *P. asparagi*, *P. andropogonis* and *P. sorghi*. Far fewer sequences from rust species were available for the other loci. Also, whole genome amplification from single pustules or urediniospores generated template DNA suitable for PCR towards identifying homozygous genotypes. Thus far, only *P. emaculata* has been found among multistate collections.

***Aspergillus flavus* AF36 in Mexico: Distribution of an endemic biocontrol agent for mitigation of aflatoxin contamination of maize**

A. ORTEGA-BELTRAN (1), K. A. Callicott (2), P. J. Cotty (2)
(1) School of Plant Sciences, University of Arizona, Tucson, AZ, U.S.A.; (2) USDA-ARS, School of Plant Sciences, University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.89

Aflatoxins are naturally occurring carcinogenic mycotoxins produced by several members of *Aspergillus* section *Flavi* that frequently infect diverse crops in warm production areas. In 2011, aflatoxin contamination outbreaks occurred in Sinaloa, the state with the greatest maize production in Mexico. Maize provides over half of the total caloric intake in Mexico. Use of atoxigenic strains of *A. flavus* to reduce aflatoxin contamination is effective in commercial production of maize, peanut, cottonseed, and pistachio. Atoxigenic strains competitively displace aflatoxin-producing fungi resulting in lower aflatoxin accumulation in crops. One such atoxigenic strain that has been registered as a biopesticide with the USEPA is AF36. It is unknown if AF36 is endemic to Mexico. In order to assess incidence of AF36 in Mexico, 5292 isolates of *A. flavus* collected from Tamaulipas, Sonora, Sinaloa, and Nayarit were subjected to vegetative compatibility analyses. Sixty-two members of the VCG to which AF36 belongs were detected in maize and soil collected between 2005 and 2008. The current work demonstrates that AF36 is endemic to Mexico. Endemic atoxigenic strains well adapted to target regions are optimal resources for the development of effective biopesticides. Existence of an EPA registration for this endemic biocontrol agent may facilitate rapid registration within Mexico, providing an important tool that can rapidly be applied to aflatoxin management programs in Sinaloa and other affected states.

Incidence of five common pests in nurseries participating in three different certification schemes for pest risk mitigation

N. OSTERBAUER (1), M. Lujan (1), G. McAninch (1)
(1) Oregon Department of Agriculture, Salem, OR, U.S.A.
Phytopathology 102:S4.89

In Oregon, there are three different regulatory schemes available to certify nursery stock as pest-free, the audit-based US Nursery Certification (USNCP) and Grower Assisted Inspection (GAIP) programs and the standard shipping point inspection program (SPI). Potted plants grown within two USNCP, two GAIP, and two SPI nurseries were inspected for five common pests; Phytophthora root rot, Phytophthora foliar blight, root weevils, snails/slugs, and bittercress. Surveyors walked random transects within each nursery, establishing three survey plots at equidistant points along each transect. Within each plot, plants were inspected for the target pests and root and foliar samples collected for laboratory testing. *Phytophthora* presence was confirmed in samples with ELISA and PCR, while the other pests were visually identified in the field. Over two sampling periods, the incidence of Phytophthora root rot averaged 24.1%, 32.0%, and 34.2% for GAIP, USNCP, and SPI nurseries, respectively. For the other pests, incidence in GAIP, USNCP, and SPI nurseries averaged (in order) 2.6%, 7.3%, and 5.8% for Phytophthora foliar blight, 16.9%, 8.0%, and 6.5% for bittercress, 29.0%, 51.6%, and 34.0% for snails/slugs, and 6.9%, 6.7%, and 10.4% for root weevils. Based on these results, the most common pests found were snails/slugs and Phytophthora root rot. Additional surveys are planned to verify these findings.

RT-PCR assays for the detection and discrimination of *Maize dwarf mosaic virus*, *Sugarcane mosaic virus*, and *Sorghum mosaic virus*

Y. ÖZAKMAN (1), M. Arif (2), D. R. Caasi (2), F. M. Ochoa Corona (2)
(1) Biology Department of Hacettepe University, Ankara, Turkey; (2) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.89

Sugarcane mosaic virus (SCMV), *Maize dwarf mosaic virus* (MDMV) and *Sorghum mosaic virus* (SrMV) are important and responsible for causing disease to sugarcane, maize and sorghum plants. The Aim of this study is to develop a method for early, accurate, sensitive detection and discrimination of SCMV, MDMV, and SrMV. These methods are needed for inspection of products in quarantine locations, and by extension officers for management of the disease. SCMV, MDMV, SrMV have very similar capsid protein sequences. Specific primers were designed after aligning complete genomes seeking diagnostics signatures useful for discrimination. Reference sequences for this study were retrieved from the National Center for Biotechnology Information (NCBI). Primer design was done with Primer3, mFOLD and BLASTn, and reference and validated thermodynamic parameters were used. All primer pairs were validated *i*, against published sequences, and *i* against reference infected plant tissues used as positive controls. Preliminary results showed positive amplifications by RT-PCR with all set of primers.

Effect of drying methods on incidence of mycotoxins and mycotoxigenic strains of *Aspergillus* section *Nigri* in California raisins

J. D. PALUMBO (1), T. L. O'Keeffe (1), S. J. Vasquez (2)
(1) USDA-ARS WRRC, Albany, CA, U.S.A.; (2) University of California Cooperative Extension, Fresno, CA, U.S.A.
Phytopathology 102:S4.89

Grapes and raisins are susceptible to contamination by ochratoxin A (OTA) and fumonisin B_2 (FB_2), two mycotoxins produced by *Aspergillus* section *Nigri* species. To determine whether raisin drying methods influence mycotoxin content, raisins dried by traditional tray, continuous tray, and dried-on-vine methods were sampled from commercial vineyards in Fresno County, California and analyzed for mycotoxin content. OTA was detected in 28% of raisin samples, at levels from 0.15 ng/g to 3.2 ng/g. Fumonisins were detected in 90% of raisin samples, at levels from 0.04 µg/g to 0.68 µg/g. Mycotoxin incidence and levels were not significantly different among raisin drying methods ($P>0.05$). *Aspergillus* strains were isolated by plating raisin washes on DRBC agar, and mycotoxin production was assessed by HPLC analysis of agar plug extracts. Among 390 *Aspergillus* section *Nigri* isolates, only one OTA-producing *A. carbonarius* strain was recovered. In contrast, 267 strains of *A. niger* were isolated, 56% of which produced FB_2. The distribution of FB_2-producing *A. niger* strains, and the relative amounts of FB_2 produced by these strains, did not differ significantly among raisin drying methods ($P>0.05$). These results suggest that potential fumonisin contamination may be more widespread than OTA contamination in California raisins, and that different drying methods have little effect on the mycotoxigenic potential of *Aspergillus* section *Nigri* populations on raisins in California.

Photocatalysis: Effect of light-activated, antibacterial nanoscale formulations on *Xanthomonas perforans*, the causal agent of bacterial spot on tomato

M. PARET (1), G. Vallad (2), J. Jones (3), D. Averett (4), S. Olson (1)
(1) NFREC, University of Florida, Quincy, FL, U.S.A.; (2) GCREC, University of Florida, Wimauma, FL, U.S.A.; (3) Department of Plant Pathology, University of Florida, Gainesville, FL, U.S.A.; (4) EcoActive Surfaces Inc., Pompano Beach, FL, U.S.A.
Phytopathology 102:S4.89

Photocatalysis is a process where chemically reactive oxygen species are catalytically generated by certain minerals in the presence of light leading to anti-bacterial action. The anti-bacterial potential of photocatalytic nanoscale TiO_2; nanoscale TiO_2 doped with silver (TiO_2/Ag), and zinc (TiO_2/Zn; AgriTitan™) on *Xanthomonas perforans*, the causal agent of bacterial spot disease on tomato was evaluated in this study. In vitro experiments on photocatalytic activity, and studies on kinetics in antibacterial activity; and greenhouse and field trials were conducted after coating plants with the nanoscale formulations. The impact of the treatments on the total marketable yield was also assessed. TiO_2, TiO_2/Ag, and TiO_2/Zn had high photocatalytic activity on *X. perforans* strain Xp-F7 within 10 minutes of exposure to $3x\ 10^4$ lux leading to a significant drop in bacterial population compared to untreated control. TiO_2/Ag had the highest photocatalytic activity followed by TiO_2/Zn. In the preliminary greenhouse experiment TiO_2, TiO_2/Ag, and TiO_2/Zn significantly reduced the number of bacterial spot lesions compared to untreated control. TiO_2/Ag had the highest effect followed by TiO_2/Zn. Further greenhouse and field studies confirmed the anti-bacterial properties of TiO_2/Zn. The use of TiO_2/Zn did not cause any adverse effects on the yield in any of the field trials.

Survival of '*Candidatus* Liberibacter asiaticus' in different media over time
J. K. PARKER (1), S. R. Wisotsky (1), K. R. Sims (2), M. E. Hilf (2), L. De La Fuente (1)
(1) Auburn University, Auburn, AL, U.S.A.; (2) USDA-ARS, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.90

Huanglongbing disease (aka Citrus Greening Disease) of citrus, associated with infection by the bacterium '*Candidatus* Liberibacter asiaticus' (LAS), has spread rapidly in Florida since its identification in 2005. Infection with LAS, vectored by the Asian citrus psyllid (*Diaphorina citri* Kuwayama), is incurable; therefore, knowledge regarding LAS biology and pathogenesis is essential to develop a treatment. However, LAS cannot currently be successfully cultured, limiting its study. To gain insight into the conditions required for growth of LAS in vitro, LAS inoculum obtained from seeds of fruit from infected pomelo trees (*Citrus grandis* 'Mato Buntan') was added to different media, and cell viability was monitored for 18 days using quantitative polymerase chain reaction (qPCR) in conjunction with ethidium monoazide (EMA). Media tested were 100% King's B (K), King's B with 50% juice from the infected fruit (J50), King's B with 50% store-bought grapefruit juice (G50), and 100% store-bought grapefruit juice (G). Results show the J50, G50, and G media all prolong viability compared to K, with G50 and G being the most successful. Slides placed in inoculated media developed biofilm over time, with the most biofilm formed in G50 and G media. Fluorescence in situ hybridization (FISH) of this biofilm with LAS-derived 16S rRNA gene probes showed the biofilm contains aggregates of LAS cells. Results will contribute to future development of a culture medium for LAS.

Characterizing oomycetes in irrigation ponds associated with vegetable production in southern Georgia
V. PARKUNAN (1), M. Purvis (1), P. Ji (1)
(1) University of Georgia, Coastal Plain Experiment Station, Tifton, GA, U.S.A.
Phytopathology 102:S4.90

Irrigation ponds associated with vegetable production in southern Georgia were assessed to characterize populations of oomycetes in pond water including *Phytophthora* and *Pythium* species. Baiting with camellia leaves and other methods were used to isolate target oomycetes. Different groups of isolates were obtained and identified based on colony growth patterns and other morphological characteristics. Representative isolates in different groups were further identified by amplification and sequencing of the internal transcribed spacer (ITS) regions from rRNA gene of the isolates with the universal primers ITS1 and ITS4 using colony polymerase chain reaction (PCR) technique. The majority of isolates was identified as *Phytopythium* spp. and *Pythium* spp., with about 15% of the isolates being *Pythium litorale*, *P. sterilum*, and *P. helicoides*. A number of *Phytophthora* spp. was obtained such as *P. cryptogea*, *P. megasperma*, *P. hungarica*, *P. drechsleri*, *P. cactorum*, and *P. gonapodyides*. Some unidentified species are being further characterized. A year-round monitoring of various oomycetes in different irrigation ponds is being conducted and recovery efficiency of different methods is compared. Additionally, pathogenicity of selected isolates on vegetable crops is assessed to determine potential threat to vegetable production.

Tracking plant diseases by monitoring a sentinel plant system at the National Ornamentals Research Site at Dominican University of California
T. Pastalka (1), S. A. Johnson-Brousseau (1), K. L. KOSTA (2), S. Rooney-Latham (2), C. Blomquist (2), K. Suslow (3), R. Bulluck (4), S. Ghosh (1)
(1) Dominican University of California, San Rafael, CA, U.S.A.; (2) California Department of Food and Agriculture, Sacramento, CA, U.S.A.; (3) Hines Growers LLC, norCal, Winters, CA, U.S.A.; (4) USDA APHIS PPQ CPHST, Raleigh, NC, U.S.A.
Phytopathology 102:S4.90

The National Ornamentals Research Site at Dominican University of California is a field research nursery designed to study quarantine pests and pathogens of ornamentals. Current research focuses on the federally-quarantined pathogen, *Phytophthora ramorum* (*Pr*). The NORS-DUC facility has a triple-layer sentinel plant system to facilitate *Pr* detection capabilities. This system was developed by the NORS-DUC Nursery Committee for monitoring *Pr* inoculum movement, allowing inoculum source identification from either the native landscape or the nursery. Plants bought for use in the perimeter system were received from a nursery operating with a Compliance Agreement, held in quarantine and inspected routinely for disease development. When demonstrated *Pr*-free and disease-free, these plants were incorporated into the sentinel plant system. These plants have remained free of fungicide treatments. Monthly nursery inspections of perimeter sentinel plants have been carried out in collaboration with a CDFA liaison and the CDFA Plant Pest Diagnostics Center. As research activity increased or seasonal conditions warranted, onsite monitoring activities shifted to weekly inspections. Plant inspections and tissue samples began spring 2010 and were assayed by approved APHIS diagnostic protocols. Thus far *Pr* has not been detected. Whereas, other fungi were detected causing similar symptoms to *Pr*. These data reveal how sentinel monitoring at NORS-DUC allow for seasonal assessments of disease incidence and are providing longitudinal data to assess the threat of *Pr* movement in nurseries.

Genetic analysis of broad-spectrum resistance in Mesoamerican common bean accession PI 310762 to the hypervariable bean rust pathogen
M. PASTOR-CORRALES (1), S.-H. Shin (1)
(1) USDA-ARS, Beltsville, MD, U.S.A.
Phytopathology 102:S4.90

The devastating rust disease of common bean (*Phaseolus vulgaris*) is caused by *Uromyces appendiculatus*, a pathogen known for its extensive virulence diversity. Hundreds of races of this pathogen have been identified throughout the world. The Mesoamerican bean PI 310762 has remarkable broad-spectrum rust resistance to 89 of 90 races from different parts of the world and which are maintained at Beltsville, Maryland. Moreover, PI 310762 is resistant to races that render susceptible all ten named and mapped rust resistance genes known in common bean. These races also infect other common bean cultivars with broad-rust resistance but with unnamed and unmapped genes. To determine the inheritance of rust resistance in PI 310762, it was crossed with Pinto 114, a common bean cultivar that is susceptible to 88 of the 90 races maintained at Beltsville. Seedlings of the parents, F_1, F_2, and BC_1 populations, were inoculated with two Mesoamerican and two Andean races under controlled greenhouse conditions. The resistant reaction of PI 310776 to all races used in this study was expressed as minute uredinia, smaller than 0.3 mm in diameter. The susceptible reaction of Pinto 114 was expressed as large uredinia, larger than 0.5 mm in diameter. Genetic analysis of the rust resistance based on the infection type observed on 157 plants of the F_2 population fit a 3:1 resistant-susceptible ratio, suggesting that resistance in PI 310761 to *U. appendiculatus* is conferred by a single and dominant gene.

The type IV pilus plays a major role during interactions between the bacterial biological control agent *Lysobacter enzymogenes* and the fungal host *Cryphonectria parasitica*
N. PATEL (1), D. Lambert (1), N. Donofrio (2), B. Hillman (1), D. Kobayashi (1)
(1) Rutgers The State University of New Jersey, New Brunswick, NJ, U.S.A.; (2) University of Delaware, Newark, DE, U.S.A.
Phytopathology 102:S4.90

Lysobacter enzymogenes is a biological control agent known to produce lytic enzymes and antibiotics. Recent studies indicate the bacterium also uses pathogenicity mechanisms commonly used by pathogens of animals and plants during its interactions with fungal hosts. Amongst these mechanisms is the Type IV pilus (T4P), which is a dynamic filamentous appendage that functions as a major virulence structure. Genes encoding for the assembly and function of the T4P are located in at least six clusters throughout the *L. enzymogenes* genome. Seven strains containing deletion mutations in different *pil* genes were constructed and evaluated for changes in phenotypes associated with T4P. While most mutants were affected in polar attachment to fungal host cells, mutants varied in gliding motility and degree of virulence and fungal cell killing during interactions with the fungus *Cryphonectria parasitica*. These results strongly suggest that T4P contributes an important pathogenicity role during interactions between *L.enzymogenes* and fungal hosts. Effects of T4P mutants on fungal host gene responses are being assessed.

Development of loop-mediated isothermal amplification method for detection of *Rhizoctonia* spp.

J. S. Patel (1), M. Brennan (1), G. S. ALI (1)
(1) University of Florida, Apopka, FL, U.S.A.
Phytopathology 102:S4.91

Rhizoctonia spp. cause many destructive crop diseases resulting in significant yield and quality losses. Early detection of infection caused by *Rhizoctonia* spp. at initial stages of disease is critical for disease management and preventing the spread of infected plant material to new locations. In contrast to culture-plate and traditional PCR methods, loop-mediated isothermal amplification (LAMP) assays are rapid and can be performed using a simple water bath. A 60 minute LAMP method combined with a lateral flow device (LFD) was developed to detect *Rhizoctonia* spp. from infected plant samples. The amplified LAMP products were checked by naked eye, agarose gel electrophoresis and LFD. Positive infected samples resulted in characteristic turbidity in LAMP reactions compared to negative samples. These results were corroborated by electrophoresis in agarose gel. For implementation in the field, this LAMP method was tested in LFDs using biotin-labeled primers in the LAMP reactions. The biotin-labeled LAMP products were hybridized with a fluorescein amidite (FAM)-labeled hybridization probe and detected with a generic anti-biotin and anti-FITC antibody-based LFD. The developed LAMP-LFD assay provides a rapid and inexpensive diagnostic tool for detection of *Rhizoctonia* spp. from infected plant samples in the field.

Identification and characterization of soybean seedborne fungi in Kansas

R. PEDROZO (1), C. R. Little (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 102:S4.91

Screening for seed pathogens, such as *Fusarium* spp. and *Phomopsis longicolla*, is necessary in order to understand their mechanisms of infection and transmission. The objective of this work was to determine the identity of seedborne pathogens of soybeans in Kansas. Seed was collected from seven soybean varieties ('KS3406RR', 'KS5507NRR', 'K04-3083RR', 'G2 Genetics 7392', 'Midland 4770NRR', 'KS4607', 'KS4626') produced in nine counties (Cherokee, Neosho, Shawnee, Franklin, Republic, Saline, Reno, Pottawatomie, Crawford) and used for fungal isolations on selective media. Fungal identification was based upon morphological characters and PCR. The multi-location screening efforts showed that seed from 56% of the counties were infected with *Fusarium* spp. Seeds from Cherokee county (southeast Kansas) showed the highest incidence of *Fusarium* (54%), followed by Pottawatamie (16%), Neosho (12%), Republic (10%), and Franklin (8%) counties. The most frequently encountered *Fusarium* species were: *F. proliferatum*, *F. subglutinans*, *F. oxysporum*, *F. equiseti*, *F. graminearum*, and *F. semitectum*. Regarding *Phomopsis longicolla*, seed from 67% of the counties showed infection with the highest incidence found in Shawnee (32%) and Reno (27%) counties, followed by seed from Cherokee (25%), Neosho (8%), Pottawatamie (6%), and Republic (2%) counties. The soybean variety, 'KAES 4607', exhibited the highest infection by *Fusarium* and *P. longicolla* across sites. In addition, other fungi, such as *Alternaria*, *Aspergillus*, *Cercospora*, *Chaetomium*, *Cladosporium*, *Macrophomina*, *Penicillium*, and *Phoma* spp., were recovered in a location and variety-dependent manner. As a result, pathogenicity testing has been undertaken to examine the potential of these seedborne fungi to impact seedling vigor and seed quality.

Sustainable grape pest management for California using weather data and disease risk models

F. PEDUTO (1), L. B. Coop (2), J. F. Strand (1), L. J. Bettiga (3), J. C. Broome (1), W. F. Mahaffee (4), W. D. Gubler (1)
(1) University of California, Davis, CA, U.S.A.; (2) Oregon State University, Corvallis, OR, U.S.A.; (3) University of California Cooperative Extension, Monterey County, Salinas, CA, U.S.A.; (4) USDA-ARS HCRL, Corvallis, OR, U.S.A.
Phytopathology 102:S4.91

A Specialty Crop Block Grant was awarded to UC Davis from California Department of Food and Agriculture to develop sustainable grape pest management programs for raisin, table and wine grapes using science-based decision tools. A 3-year project was conducted in collaboration with the Western Weather Work Group to develop and demonstrate the use of weather data from public and virtual weather station networks to improve disease control and reduce fungicide use. Weather data ingest systems were programmed at Oregon State University to allow weather data to be incorporated into UC IPM weather and disease modeling infrastructures. Virtual weather stations were constructed and tested by a selected group of growers. As part of the same project, revisions to the high temperature threshold of the Gubler-Thomas model for grapevine powdery mildew were tested for 3 consecutive seasons in multiple locations in California, and combined with early season pathogen detection using spore traps coupled with quantitative PCR. Disease incidence and severity were assessed on leaves and clusters throughout the season to evaluate the efficacy of the models and to correlate estimates of aerial spore density according to spore trap with observations of visible mildew colonies. Among treatments, disease incidence and severity on clusters was statistically significantly lower in the 38°C x 2h, 36°C x 4h revision of the model. 38°C x 2h exhibited statistically significantly lower incidence and severity on leaves than did all other treatments. Also, there was a significant numerical relationship between spore density values according to the spore traps and disease incidence.

Characterization of virulence and genotypic diversity of *Colletotrichum acutatum* isolates recovered from apple in New Hampshire

C. Peralta (1), K. BRODERS (1)
(1) University of New Hampshire, Durham, NH, U.S.A.
Phytopathology 102:S4.91

Colletotrichum acutatum causes anthracnose on leaves and bitterroot on fruit of apple trees causing economic losses due to unmarketable fruit. In the autumn of 2011 there was greater than average rainfall, and warmer than average temperatures, which led to an outbreak of bitterroot in many orchards in New Hampshire. The objective of this study was to evaluate the phenotypic and genotypic diversity of twenty *C. acutatum* isolates recovered from five locations in New Hampshire. To evaluate genetic diversity the ITS, β-Tubulin, glyceraldehyde 3-phosphate dehydrogenase (GDPH), and actin and calmodulin genes were sequenced. All isolates were screened for virulence by inoculating detached apples of cultivar Cortland. A subset of eight isolates was used to evaluate the difference in resistance response of eight apple cultivars. Three replications of each isolate by cultivar inoculation were included in each experiment and the entire experiment was conducted three times. There was a significant effect of isolate ($P < 0.001$) and cultivar ($P < 0.001$) on lesion development, but no significant isolate by cultivar interaction was observed. Cultivar Pioneer Mac was the most susceptible and cultivars Honey Crisp, Mutsu, and Smoothie were the least susceptible. Sequence analysis revealed only a single polymorphic locus from all four genes located in the GPDH gene, indicating very little genetic diversity among isolates of *C. acutatum* infecting apple fruit in New Hampshire.

WITHDRAWN

WITHDRAWN

Multiplex detection of viruses infecting grapevine using a randomly primed, RT-PCR/macroarray platform

K. L. PERRY (1), M. Fuchs (1), K. Fisher (2), J. Thompson (1)
(1) Cornell University, Ithaca, NY, U.S.A.; (2) University of Utah, Salt Lake City, UT, U.S.A.
Phytopathology 102:S4.92

In grapevine, the control of virus infection and spread is achieved through the identification and elimination of infected vines, and the use of virus-free planting stocks. As such, much effort has been invested in developing reliable molecular diagnostic techniques. Here we report the development of a macroarray assay for the detection of most all recognized grapevine infecting viruses. A total of 1556 70-mer DNA oligonucleonucleotides were designed using full and partial genome sequences from 36 virus species. The oligonucleotides were spotted onto nylon membranes (21 x 8 cm) that can be re-used more than 20 times. The membranes were hybridized with randomly amplified cDNA generated by randomly primed RT-PCR from plant total RNA. More than 100 grapevine samples from New York State, elsewhere in the US, and Europe were tested. Infections by single and multiple viruses were observed, including members of the families that include betaflexivirids, closterovirids, secovirids and tymovirids. Results were largely consistent with those obtained by ELISA and PCR, and in some cases detected viruses not usually screened for. This work demonstrates a proof-of-principal for unbiased multiplex detection of viruses using a single robust macroarray platform. The macroarray technology complements the other available approaches for virus detection in grapevine.

Identification and characterization of two new *Pencillium* species causing blue mold of stored apple fruit in the United States

K. A. PETER (1), I. Vico (1), V. L. Gaskins (1), W. J. Janisiewicz (2), W. M. Jurick (3)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) USDA-ARS, AFRS, Kearneysville, WV, U.S.A.; (3) USDA-ARS, Food Quality Laboratory, Beltsville, MD, U.S.A.
Phytopathology 102:S4.92

Blue mold, caused by *Penicillium expansum* and *P. solitum*, is a postharvest disease that occurs during long term storage of pome fruit. In May 2011, decayed sporulating apples were collected from cold storage in a commercial packing house in Pennsylvania and 100 isolates of the blue mold fungus were recovered. Genomic DNA was obtained from each isolate, and the β-tubulin locus was amplified to determine species identity. The majority of the isolates were found to be *P. expansum*; however, 7 isolates were identified as *P. paneum* and 1 isolate as *P. carneum*, both of which were isolated from decayed 'Golden Delicious' apple fruit. To conduct Koch's postulates, 'Golden Delicious' apple fruit were wound-inoculated with the 8 *Penicillium* isolates. The fungi caused typical *Penicillium*-type lesions including: soft, watery lesions, with hard-defined edges. The fungi were recovered from the decayed apple fruit tissue and the ITS region was amplified using ITS 4 and 5 primers to confirm the species identification. Morphological characteristics were examined using CYA, YES, and MEA media. All isolates were susceptible to the 3 postharvest fungicides used to control *Penicillium* spp. Originally grouped as varieties of *P. roqueforti*, *P. paneum* and *P. carneum* were reclassified in 1996 as individual species. Neither species has been previously reported to cause postharvest decay on apple. This is the first report of blue mold on apple caused by *P. paneum* and *P. carneum* in the U.S.

Distribution and management of fungicide-resistant *Fusarium* spp. infecting potato seed tubers in Canada

R. D. PETERS (1), B. W. Beaton (2), T. Barasubiye (3), K. A. Drake (1), C. J. Banks (2), M. M. Clark (4)
(1) Agriculture & Agri-Food Canada, Charlottetown, PE, Canada; (2) Prince Edward Island Department of Agriculture, Charlottetown, PE, Canada; (3) Agriculture and Agri-Food Canada, Ottawa, ON, Canada; (4) Prince Edward Island Department of Agriculture, Kensington, PE, Canada
Phytopathology 102:S4.92

Fusarium spp. are important pathogens of potato that cause yield losses at planting and in storage following harvest. Surveys from 2007-2011 in Canada showed that *Fusarium sambucinum* was the most predominant seed-decay pathogen, followed by *F. coeruleum*, *F. avenaceum* and *F. oxysporum*. Isolates of the various *Fusarium* spp. collected during Canadian surveys were also tested for their sensitivity to thiophanate-methyl (Senator ® PSPT), thiabendazole (Mertect ® SC) and fludioxonil (Maxim® PSP). In 2011, most isolates of *F. sambucinum* recovered in the seed survey showed resistance to both thiabendazole/thiophanate-methyl and fludioxonil. By contrast, most other *Fusarium* spp. were sensitive to these products. Isolates of *F. oxysporum* recovered in these surveys were always sensitive to thiabendazole and thiophanate-methyl, but resistant to fludioxonil. Field and storage studies were conducted to ascertain the impact of fungicide-resistant strains on crop loss and to define potential management strategies. In all cases, treatment of potato seed pieces with mancozeb, difenoconazole or prothioconazole completely controlled seed-piece decay caused by a multi-class resistant isolate of *F. sambucinum*. Based on our research, knowing the predominant *Fusarium* spp. in a particular seedlot and their sensitivities to various chemical products would provide growers with important information to use to make disease management decisions.

Integrated management of foliar diseases of pyrethrum in spring

S. J. PETHYBRIDGE (1), T. Groom (1), S. Pilkington (2), F. S. Hay (2)
(1) Botanical Resources Australia, Ulverstone, Australia; (2) University of Tasmania, Burnie, Australia
Phytopathology 102:S4.92

Pyrethrum (*Tanacetum cinerariifolium*) is grown for the production of pyrethrins, used in many insecticidal products. In Australia, ray blight caused by *Phoma ligulicola* can cause severe dieback in spring. This disease has been controlled with a fungicide program designed to minimize the probability of resistance development. Recently, tan spot caused by *Microsphaeropsis tanaceti* has become increasingly prevalent in spring. One explanation for this may be differential sensitivity of *P. ligulicola* and *M. tanaceti* to fungicides. Isolates of *P. ligulicola* (n = 53) and *M. tanaceti* (n = 50) obtained from diseased plants in commercial fields in 2009/2010 were tested for sensitivity to boscalid *in vitro*. For *P. ligulicola*, 98% and 2% of isolates had EC_{50} of 0 to 0.05 and 0.05 to 5.0 µg/ml respectively. Conversely, 18, 26, 24, 18, and 14% of isolates of *M. tanaceti* had EC_{50} between 0 to 0.05, 0.05 to 0.5, 0.5 to 5.0, 5.0 to 50.0 and >50.0 µg/ml respectively. In spring 2011, severe disease occurred in eight fields in spring, despite application of boscalid, from which *M. tanaceti* was the predominant fungus isolated from symptoms. For isolates of *M. tanaceti* (n = 129) from these fields, 1, 3, 30 and 66% had EC_{50} between 0.0 to 0.5, 0.5 to 5.0, 5.0 to 50.0 and >50.0 µg/ml respectively. Sub-optimal disease control with boscalid appears to be associated with increased tan spot intensity in spring, and reduced sensitivity within the *M. tanaceti* population.

On-farm management factors associated with bacterial bulb rots of onion in Pennsylvania

E. E. PFEUFER (1), M. Mansfield (1), B. K. Gugino (1)
(1) Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.92

Sweet onions are increasingly being grown on diversified vegetable farms in Pennsylvania, however, in-field and storage losses due to bacterial rots can sometimes approach 50%. Commercial growers currently rely on copper-based products for their management. To expand the IPM toolbox through the identification of on-farm factors associated with increased bacterial disease incidence, 29 farms were visited three times each in 2011. Data was obtained for soil temperature and nitrogen, weed pressure, thrips feeding damage, tissue nitrogen, and harvest and postharvest storage losses as well as farm specific management practices. An isolate database containing nearly 1800 bacterial individuals was compiled from onion transplants, soil, weeds, and postharvest bulbs. A multiplex PCR method was used to identify isolates to species in addition to the causal agents of bulb rots; *Pantoea agglomerans* and *Pectobacterium caratovora* were frequently identified, often in combination with other pathogens. *P. agglomerans* was the most abundant pathogen species found on weed surfaces (17% positive), and 80% of redroot pigweed tested harbored this pathogen. Harvest soil nitrogen levels were related to harvest bacterial rots. Thus, targeting nitrogen and weed management may be additional ways growers can help reduce losses due to bacterial rots in their onion crops.

Induced systemic resistance in maize

C. PLANCHAMP (1), D. Balmer (1), C. Robert (1), C. Zwahlen (1), B. Mauch-Mani (1)
(1) University of Neuchatel, Neuchatel, Switzerland
Phytopathology 102:S4.92

Despite the economic importance of maize, studies about its induced systemic resistance are less common than those involving dicotyledonous plants. The

goal of the present study consists in a better understanding of the mechanisms that lead to defense reactions induced by beneficial bacteria in maize. For this purpose, various parameters affected by *Pseudomonas putida* KT2440 have been tested in maize plants. Root inoculation of maize seedlings with these bacteria induced defense reactions in leaves against *Colletotrichum graminicola*, the causal agent of corn anthracnose, showing the potential of *P. putida* KT2440 to induce resistance in maize plants. Moreover, experiments have been conducted to test the effect of the presence of *P. putida* KT2440 on the leaf herbivory. Plants were infested with larvae of *Spodoptera littoralis*, a generalist herbivore, and *Spodoptera frugiperda*, a specialist herbivore. There were no differences in larval weight gain in the case of the specialist *S. frugiperda* but larvae of the generalist *S. littoralis* grew better on untreated plants. These results indicate that in addition to inducing resistance against *C. graminicola* the bacteria induce anti-herbivore defense in the plants. Interestingly, this defense is effective against the generalist but seems to be suppressed or coped with by the specialist. Further studies to better understand these induced resistance mechanisms are currently in process.

Efficacy of trenching, rootstock, and compost to manage peach replant disease

R. POKHAREL (1)
(1) Colorado State University, Grand Junction, CO, U.S.A.
Phytopathology 102:S4.93

Replant diseases in peach caused by various pathogens are difficult to manage because of limited availability of effective broad spectrum chemicals. This study, part of an ongoing non-replant project at Western Colorado Research Center, investigated the effect of trenching, compost, and rootstock to manage replant disease on tree growth, compared with that of a non-replant site. Sierra Rich peach trees on Lovell, Nemaguard, St. Julian, and Viking and Zee Lady only on Viking rootstock were planted on a replant site with trenched (opened for a summer) ground with or without compost and a replant non-trenched ground (control) with 3-4 replications and a non-replant site with 40 replications in a completely randomized design. Tree growth, calculated from the tree circumferences measured at planting and after the first growing season, was analyzed using Proc GLM of SAS. Sierra Rich tree growth at non-replant site was significantly higher as compared to trees in replanted site irrespective of rootstocks whereas trees of both the varieties on Viking produced significantly higher growth. At replant site, trees on a trenched ground produced significantly higher growth as compared to control. Within trenched ground, trees on Viking and Nemaguard produced significantly higher growth with compost but not the trees on Lovell and St. Julian. The tree growth at non-replant site and on a replant trenched ground was 15 and 6.0 to 6.5 times higher, respectively than control.

Draft genome assembly of the ascomycete *Colletotrichum acutatum*

J. POLASHOCK (1), G. Cai (2), B. Hillman (2), P. V. Oudemans (3)
(1) USDA-ARS, Chatsworth, NJ, U.S.A.; (2) Rutgers The State University of New Jersey, New Brunswick, NJ, U.S.A.; (3) Rutgers University, Chatsworth, NJ, U.S.A.
Phytopathology 102:S4.93

Colletotrichum acutatum is an important pathogen of fruits and vegetables worldwide. The pathogen causes anthracnose on blueberry, strawberry, almond, avocado and other economically important crops. In New Jersey, anthracnose is the most important disease of blueberry and drives most of the fungicide use on that crop. To initiate a host-pathogen interaction project, the genome of an isolate of *C. acutatum* from blueberry was sequenced and assembled. Briefly, genomic DNA of *C. acutatum* was sheared, size selected (1-2 kb), and a 2x50bp mate-pair library was generated following standard protocols for sequencing on the Applied Biosystems SOLiD 3Plus. The sequencing run resulted in 326 million 50 bp reads with an approximate coverage of 326x. Using the Denovo2.2 pipeline and K-mer length 39 (other parameters default), the genome was assembled into 49,208,166 bases with a GC ratio of 52.7. The initial assembly consisted of 13,590 contigs of minimal length 100 bases, with half of the assembly in 1775 contigs of 8,113 bases or longer (contig N_{50}). The contigs were linked into 3214 scaffolds and singletons. Half of the assembled sequences were in 12 scaffolds of 1,326,933 bases (scaffold N_{50}) or longer. The final assembly, that was refined based on length, coverage depth, GC ratio, and blastx results, is comprised of 86 scaffolds totaling 48,513,324 bases (excluding Ns). Approximately 13 thousand protein-coding genes were identified in preliminary analysis of the genome.

Increasing the efficacy and effectiveness of application of disinfectants against persistent viruses of greenhouse vegetables

A. POLEATEWICH (1), G. Ferguson (2), M. Brownbridge (1)
(1) Vineland Research & Innovation Centre, Vineland Station, ON, Canada; (2) Ontario Ministry of Agriculture, Food & Rural Affairs Greenhouse & Processing Crops Research Centre, Harrow, ON, Canada
Phytopathology 102:S4.93

Plant viruses can have a devastating impact in a greenhouse. The environment and production system allows for a virus to invade a greenhouse and persist and spread to subsequent crops. Once a virus enters a plant, the only control option is to remove the plant and surrounding plants to prevent its spread. The lack of control options often results in significant yield losses associated with the presence of any virus. The best way to manage a virus is to prevent its infection in the first place, and therefore greenhouse sanitation is important to reduce losses due to plant viruses. Many of the disinfectant products on the market have not been tested against the more persistent plant viruses that are problematic in greenhouse vegetable crops. Many of the more efficacious disinfectants used in greenhouse sanitation are very corrosive and are not favored by greenhouse operators. Little information is available on the effectiveness of pre-disinfection washing of greenhouse surfaces, and some of the "off the shelf" commercial detergents currently being used by growers. This project examined several disinfectants and surfactants on aluminum, glass, and plastic substrates under laboratory and commercial conditions at different temperatures. By improving greenhouse sanitation, spread of mechanically transmitted viruses within greenhouses should be significantly minimized, thereby reducing losses caused by viruses and improving the competitiveness of the industry.

Characterization of two field isolates of *Bean common mosaic virus*

A. POPLAWSKY (1), O. V. Nikolaeva (1), X. Feng (1), J. R. Myers (2), A. V. Karasev (1)
(1) University of Idaho, Moscow, ID, U.S.A.; (2) Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 102:S4.93

Bean common mosaic virus (BCMV) and a closely related *Bean common mosaic necrosis virus* (BCMNV) are two aphid-transmitted potyviruses. Both BCMV and BCMNV are also transmitted by seed and represent a significant threat to bean production world-wide. BCMV and BCMNV isolates are classified into eight pathotypes according to their reactions in the presence of known resistance genes in common bean. Two serotypes, A and B, have also been distinguished, representing necrotic and non-necrotic isolates, respectively. In 2011, two isolates of BCMV were collected in the Willamette Valley of Oregon from bean plants exhibiting mild mosaic and stunting symptoms. The isolates appeared to be seedborne in the line L192, a brown-seeded dry bean with determinant growth habit. These two BCMV isolates were subjected to a biological characterization on twelve bean differential lines, and were classified as belonging to the non-necrotic pathotype VII, similar to a control isolate US10. When subjected to a serological testing against several antibodies produced in the laboratory, these two isolates were distinct from the necrotic control isolate TN1 (pathotype VI), and also distinct from isolate US10 (pathotype VII), both in TAS-ELISA and Western blots.

Host range studies of a *Beet curly top virus* (Logan) infectious clone

A. POPLAWSKY (1), S. Eid (1), A. V. Karasev (1)
(1) University of Idaho, Moscow, ID, U.S.A.
Phytopathology 102:S4.93

Curly top disease of sugar beet in Idaho is caused by a complex of at least three curtoviruses which are transmitted by the beet leafhopper. In the field, some of these viruses are found preferentially in sugar beet and others in beans. To address the mechanisms of these host preferences, an infectious, dimeric clone was constructed for *Beet curly top virus* (BCTV, Logan), and maintained in a binary vector suitable for agroinoculation. Infectivity of this BCTV clone was tested on a range of host plants following agroinoculation with subsequent ELISA, tissue printing, and PCR to confirm successful infection. *Nicotiana benthamiana*, two cultivars of sugar beet, and six cultivars of tomatoes supported replication of this BCTV infectious clone, and the typical symptoms of stunting, leaf curl and chlorosis at the growing point started appearing at 3 weeks post-inoculation in most cases. These symptoms correlated with the laboratory tests for the presence of BCTV. Both the infectivity rate and the relative level of virus replication were reduced for cultivars with partial resistance to curly top.

Reproduction of soybean cyst nematode on accessions of the core collection of *Phaseolus vulgaris*

S. H. POROMARTO (1), B. D. Nelson (1), R. S. Goswami (2), M. Welsh (3)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) DuPont Crop Protection, Stine Haskell Research Center, Newark, DE, U.S.A.; (3) USDA-ARS, Western Regional Plant Introduction Station, Pullman, WA, U.S.A.
Phytopathology 102:S4.93

Soybean cyst nematode (*Heterodera glycines*; SCN) is a potential threat to dry bean (*Phaseolus vulgaris*) in the major U.S. production area of North Dakota and northern Minnesota. Four hundred and sixteen accessions from the core collection of *P. vulgaris* in the Western Regional Plant Introduction Station, Pullman, WA, were evaluated in the greenhouse for host suitability for SCN HG type 0. Five-day-old healthy seedlings of uniform size were individually placed in a 2 x 1 cm deep hole in a conetainer containing autoclaved sand and 2,000 eggs were placed around the seedling. Conetainers were placed in plastic pots filled with sand and immersed in a water bath maintained at 27 degrees C inside the greenhouse. Plants were harvested at 30 days, and female nematodes were extracted and counted. The female index (FI) (FI = [the average number of females on the test plant divided by the average number of females on the susceptible soybean Lee 74] times 100) was calculated for each accession to assess host suitability. Approximately 23% and 31% of the accessions had FI's between 2-10 and 11-20, respectively. These results indicate sources of high levels of resistance to SCN in the core collection of *P. vulgaris*. Breeding efforts are currently underway to determine the genetics of resistance to SCN.

Salmonella enterica growth in the phyllosphere following synergistic interaction with virulent _Xanthomonas perforans_

N. POTNIS (1), J. B. Jones (1), J. D. Barak (2)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.94

Bacterial spot of tomato caused by four *Xanthomonas* species is a widespread and persistent problem in tomato fields and is responsible for significant yield losses. In addition, the ever-increasing risk of *Salmonella enterica* (*Se*) contamination of tomato increases the likelihood of economic loss and damage to the entire industry due to human illness. The fact that salmonellosis outbreaks have been linked to tomato fields infested with plant pathogens led us to hypothesize, that *Se* might benefit from co-colonization with bacterial plant pathogens in the phyllosphere. To test our hypothesis, we assessed the *Se* populations over two weeks with and without *Xanthomonas perforans* (*Xp*). Five-week old tomato plants were inoculated with a *Se* cocktail and virulent *Xp*, *Se* alone, or virulent *Xp* alone. *Se* and *Xp* populations were determined at multiple time points over two weeks. In the presence of virulent xanthomonads, *Se* phyllosphere populations were approximately one hundred-fold higher than *Se* populations on the leaves of tomato without xanthomonads by two weeks after inoculation. *Se* can benefit from the colonization of leaves by virulent *X. perforans* and can maintain populations high enough for human infection, which it would fail to reach by itself in the phyllosphere. Understanding the mechanisms involved in this synergy between plant pathogen and *Se* would be important for designing strategies to control risks associated with fresh produce.

Pathogenicity and disease development of tree tomato anthracnose in Ecuador

F. P. Poveda (1), J. B. OCHOA (2), E. Morillo (2), P. A. Backman (3)
(1) Central University of Ecuador, Quito, Ecuador; (2) National Institute of Agricultural Research (INIAP), Santa Catalina, Quito, Ecuador; (3) The Pennsylvania State University, Department of Plant Pathology, University Park, PA, U.S.A.
Phytopathology 102:S4.94

Tree tomato (*Solanum betaceum* Cav.) is an important commercial fruit for small farmers in the valleys of Ecuador. Anthracnose is the most important constraint to tree tomato cultivation in Ecuador. To better understand disease development and to identify the causal disease agent, pathogenicity studies and molecular identification were undertaken. A survey of tree tomato symptoms was performed in the main three tomato cultivation areas of Ecuador. The pathogen was isolated only from: sunken dark lesions on the stem, necrosis on the leaves, and sunken lesions on the fruit. In pathogenicity tests conducted on 3 month-old seedlings and ripe fruits of 'Amarillo Puntón,' the pathogen produced all the symptoms described, irrespective of isolate origin. Fifty isolates of *Colletotrichum* sp. obtained in this study were molecularly analyzed using CgInt-ITS4 and CaInt-ITS4 primers. The amplified DNA showed that isolates belonged to *Colletotrichum acutatum*, which corrects the previous identification of the pathogen as *Colletotrichum gloeosporioides*. These studies confirm that observed symptoms were caused by *C. acutatum*. A complementary pathogenicity study showed that the pathogen is highly virulent in younger leaves, causing large dark undefined lesions, while virulence decreased with the age of the leaf, with older leaves showing only small necrotic localized lesions. These studies will improve the understanding of disease epidemiology and assist in disease management.

WITHDRAWN

Effects of temperature on virus titer development and population growth of the wheat curl mite in wheat streak-resistant wheat cultivars

J. A. PRICE (1), A. Simmons (1), E. Evans (1), C. M. Rush (2)
(1) Texas AgriLife Research, Amarillo, TX, U.S.A.; (2) Texas AgriLife Research, Bushland, TX, U.S.A.
Phytopathology 102:S4.94

The majority of winter wheat production in the Southern Great Plains serves as a dual purpose crop for both grazing and grain production, which exposes plants to warmer fall temperatures and a variety of pathogens, including *Wheat streak mosaic virus* (WSMV). Wheat varieties have been developed with resistance to WSMV; however the resistance is ineffective above 25C. Little is known about plant/virus interactions as temperatures fluctuate during the growing season, therefore, a study was conducted to determine the affects of temperature fluctuations on virus titer development and mite population dynamics in resistant winter wheat cultivars. Wheat cultivars Ron L and Mace, containing resistance, TAM 112, containing field tolerance, and TAM 111 and Karl 92, control plants, were grown in a growth chamber at 27C, infested with viruliferous wheat curl mites and moved to 18C and then to 5C. After three weeks exposure to each temperature, three tillers were collected from each plant for mite population counts and virus quantification, using real-time PCR. Preliminary results revealed that once infection occurred at high temperatures, Mace and Ron L were not able to recover, even at temperatures below the resistance threshold. However, TAM 112, which possesses no known specific resistance genes, supported a reduced number of mites and lower virus titer when compared with the other cultivars, thereby exhibiting an uncharacterized tolerance to both the virus and its vector.

Increased CO_2 and temperature effects on Alternaria leaf spot and black spot of basil under controlled environment

M. Pugliese (1), E. Cogliati (2), A. Garibaldi (2), M. GULLINO (2)
(1) University of Torino, Grugliasco Torino, Italy; (2) Agroinnova-University of Torino, Grugliasco Torino, Italy
Phytopathology 102:S4.94

The pathosystems rocket (*Eruca vesicaria* subsp. *sativa*) – Alternaria leaf spot (*Alternaria japonica*) and basil (*Ocimum basilicum*) - black spot (*Colletotrichum gloeosporioides*) were chosen as models to assess the potential impact of increased CO_2 and temperature on disease under controlled environment. Potted plants were grown in phytotrons under 4 different simulated climatic conditions: (1) standard temperature (ranging from 18° to 22° C) and standard CO_2 concentration (400 ppm); (2) standard temperature and elevated CO_2 concentration (800 ppm); (3) elevated temperature (ranging from 22° to 26° C, 4° C higher than standard) and standard CO_2 concentration; (4) elevated temperature and CO_2 concentration. Each plant was inoculated with a spore suspension containing $1x10^5$ cfu/ml of the pathogen. Disease indexes and physiological parameters (chlorophyll content, fluorescence) were assessed. An increase of the chlorophyll content with higher CO_2 was observed. Disease incidence and severity were always positively influenced by the combination of rising CO_2 and increased

temperature, compared to standard conditions (400 ppm of CO_2 – 22° C). Plants grown at 800 ppm of CO_2 also showed a clear increment in the symptoms compared to 400 ppm. Considering the rising concentrations of CO_2 and global temperature, we can assume that this could increase the severity of *Alternaria japonica* on rocket and *Colletotrichum gloeosporioides* on basil.

Functional characterization of the gene *GzOch1* for mannosyltransferase in *Fusarium graminearum*

K. D. PURI (1), S. Zhong (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.95

The *Och1* gene encodes a key enzyme, α-1,6-mannosyltransferase, which is required for synthesis of N-glycans of cell wall proteins in *Saccharomyces cerevisiae*, *Aspergillus fumigatus* and *Neurospora crassa*, however, its function in plant pathogenic fungi is unclear. In this study, we identified and characterized the *Och1* ortholog (*GzOch1*) of *Fusarium graminearum*, the causal agent of Fusarium head blight in cereal crops. The gene knockout mutants of *GzOch1* were generated by replacing the coding region with a hygromycin B resistance gene cassette (*hph*). Three independent deletion mutants (ΔGzOch1-1, ΔGzOch1-2 and ΔGzOch1-3) were tested for vegetative growth, sporulation, sexual development, stress response, virulence, and mycotoxin production. Results indicated that the knockout mutants didn't grow on minimal medium (MM) with 1M KCl, on which the wild type were able to grow. Also, significant growth reduction was observed for the mutants grown on MM with 1.5 M sorbitol in comparison with the wild type. However, no significant difference in virulence was observed between the deletion mutants and the wild type when inoculated on the susceptible cultivar Briggs. In fact, the mutants produced a higher disease severity on Briggs although the difference was not significant as compared to the wild type. Comparison of the knockout mutants and the wild type in mycotoxin production on grains and rice culture, and sexual development on carrot agar will be presented.

Genetic analysis of worldwide *Sclerotinia homoeocarpa* populations with mating type and microsatellite markers

A. PUTMAN (1), I. Carbone (1), L. Tredway (2)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) Syngenta Crop Protection, Raleigh, NC, U.S.A.
Phytopathology 102:S4.95

Sclerotinia homoeocarpa causes dollar spot, the most economically important disease of turfgrass worldwide, but little is known about the population biology of this fungus. The objective of this research is to infer the population structure of *S. homoeocarpa* on a global scale. We performed genotyping at 14 microsatellite loci and determined mating type with a multiplex PCR assay. A total of 512 *S. homoeocarpa* isolates obtained from 43 locations on five continents and Oceania have been evaluated to date. Bayesian clustering analysis revealed limited admixture and clearly defined population structure between C3 and C4 host type isolates, with each type having two distinct groups. Within each type, some isolates assigned to the same group, and in select cases belonging to identical multilocus haplotypes, originated from multiple continents. A population sample from Puerto Rico formed a separate group that did not associate with isolates from either C3 or C4 grasses. Within each of 91 multilocus haplotypes, all isolates were of the same mating type. Clone correction showed that *MAT1-1* and *MAT1-2* were equally distributed among isolates from North America. Outside of North America the *MAT1-1*:*MAT1-2* distribution was approximately 1:2, but this deviation was not significantly different from 1:1. Initial results from this research suggest that *Sclerotinia homoeocarpa* has undergone long distance dissemination, sexual reproduction, and clonal amplification.

Early infection events in tomato roots predisposed to *Phytophthora capsici* by salt stress

M. F. Pye (1), J. D. MacDonald (1), R. M. BOSTOCK (1)
(1) University of California-Davis, Plant Pathology, Davis, CA, U.S.A.
Phytopathology 102:S4.95

Plants respond to a wide range of abiotic and biotic stresses with complex signaling networks, often under the control of phytohormones. These networks are impacted by many factors, as well as by positive and negative crosstalk that can occur among downstream effectors of the response. The phytohormone, abscisic acid (ABA), plays a central role in adaptation to water stress, such as imposed by water deficit or high salinity. Stress events, even those from which the plant fully recovers, elevate ABA levels to predispose plants to levels of pathogen inoculum they would normally resist. ABA's role in induced susceptibility to *Phytophthora* species is now generally recognized, but the underlying mechanisms and impact on host defense responses are unresolved. This study examines early infection events in tomato roots predisposed by salt stress to *P. capsici*. Confocal microscopy was used to examine if the hemibiotroph, *P. capsici* displays an altered infection strategy in predisposed roots. A change in the rate of root colonization and associated cell death was apparent in infected salt stressed roots. Abiotic and biotic stress related gene expression was profiled in predisposed roots following infection. Unlike some bacterial pathogens, *P. capsici* does not appear to be manipulating the ABA signaling pathway during infection. However, defense related gene expression is strongly suppressed in predisposed tomato roots relative to *P. capsici* infected controls.

WITHDRAWN

Transcriptome analyses of *Sclerotinia sclerotiorum* infecting chickpea and lentil using RNA sequencing

D. Qiu (1), G. Vandemark (2), W. CHEN (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.95

Sclerotinia sclerotiorum causes white mold of many important crops. To elucidate its pathogenic mechanisms, transcriptome analyses were used to study its interactions with chickpea and lentil. Five mRNA libraries were constructed from *S. sclertiorum* (strain WM-A1), healthy chickpea (cv. Spansih White) and lentil (cv. Pardina), and advancing disease lesions of chickpea and lentil infected with *S. sclerotiorum* 48 h post inoculation, and sequenced with the 454 Titanium RNA sequencing. The transcripts in the interaction transcriptomes were separated into either the plant RNA or pathogen RNA based on BLAST searches. The pathogen transcripts in the interaction transcriptomes that were not found in the transcripts of the pathogen alone were considered as induced transcripts (expressed in response to infection). About 50% of the >65000 unigenes in both interaction transcriptomes were from the pathogen. There were 704 and 589 induced unigenes of pathogen in the interaction transcriptomes with chickpea and lentil, respectively, in which 177 and 162 unigenes were highly expressed. Among the induced and highly expressed genes, 42 were in common in both transcriptomes, and 18 of them (43%) code for plant cell wall degrading enzymes. In addition, the induced unigenes with other functions like recognition and signaling, and transporter were also identified. Many of the induced transcripts were confirmed with qRT-PCR. The induced genes are potential pathogenicity factors and are subjects of further investigation.

Transcriptome analyses of the interaction between *Sclerotinia trifoliorum* and chickpea using RNA sequencing

D. Qiu (1), G. Vandemark (2), W. CHEN (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.95

Sclerotinia trifoliorum causes stem rot and white mold of many important crops, mostly cool season legumes. Its pathogenic mechanisms are not well

understood. We used transcriptome analysis to study its interaction with chickpea to gain mechanistic understanding of this pathosystem. Three mRNA libraries were constructed from *S. trifoliorum* (strain 06CWM-G47), healthy chickpea plant (cv. Spanish White) and advancing disease lesions of chickpea infected with *S. trifoliorum*, and sequenced with the 454 Titanium RNA sequencing. The transcripts in the interaction transcriptome were separated into either plant RNA or fungal RNA based on BLAST searches. The transcripts in the interaction transcriptome that were not found in the transcritpomes of the pathogen or the plant were considered as induced transcripts (expressed in response to the interaction). About 27% of the >26000 unigenes in the interaction transcriptome were from the pathogen. There were 392 induced unigenes of the pathogen, 50 of them were highly expressed, and 10 of the 50 induced, highly expressed unigenes code for plant cell wall degrading enzymes. Induced unigenes with other functions like recognition and signaling were also identified. Some of the induced transcripts were confirmed using qRT-PCR. Additionally, there were 946 induced unigenes of chickpea and 243 of them were highly expressed. These induced genes in the host and the pathogen provide clues for further investigating the interaction between chickpea and *S. trifoliorum*.

***Blueberry necrotic ring blotch virus* represents a unique genus of plant RNA viruses**

D. F. QUITO-AVILA (1), R. R. Martin (2)
(1) Centro de Investigaciones Biotecnologicas del Ecuador (CIBE)-ESPOL, Guayaquil, Ecuador; (2) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 102:S4.96

Blueberry necrotic ring blotch virus (BNRBV) possesses a 14kb nt genome divided in 4 ssRNA segments. Evolutionary relationships between BNRBV and other plant RNA viruses varied depending on the amino acid (aa) sequence used for the analysis. According to the methyl transferase domain, BNRBV clustered with members of the *Bromoviridae*. Whereas; the analysis of the polymerase grouped BNRBV with members of the *Virgaviridae* and *Citrus leprosis virus* (CiLV), genus *Cilevirus*. The movement protein revealed that BNRBV is closest to CiLV and two tentative members of the *Cilevirus*: *Passion fruit green spot virus* and *Ligustrum ringspot virus*. An unusual genetic feature found in BNRBV was the presence of two helicase (HEL) domains; one at the 3'end of RNA 1 (HEL-1) and the second one located at the 5' end of RNA 2 (HEL-2). More importantly, the two HEL domains did not cluster in the same clade. Instead, HEL-1 was found to group with members of the *Virgaviridae*, whereas, HEL-2 showed a closer relationship with members of the genera *Potexvirus*, *Carlavirus*, and *Closterovirus*. Furthermore, searches for conserved motifs revealed that HEL-1 contains motifs (I, II, III and V) commonly found in members of the *Alphavirus*-like superfamily; whereas HEL-2 showed motifs (I and II) highly conserved among members of the *Flavivirus*-like superfamily. These findings suggest the creation of a new genus for the classification of plant RNA viruses with characteristics similar to BNRBV.

WITHDRAWN

WITHDRAWN

Mechanisms of silicon-induced resistance in gray leaf spot-perennial ryegrass pathosystem

A. RAHMAN (1), W. Uddin (1)
(1) The Pennsylvania State University, State College, PA, U.S.A.
Phytopathology 102:S4.96

Integration of plant defense response elicitors and biofungicides along with compatible traditional fungicides into a disease management strategy is one of the most sustainable solutions for effective disease control. Among various plant defense elicitors, silicon has been found to be able to induce resistance in plants against different pathogens. When applied at a rate of 5 ton/ha, silicon (Si) was found to suppress infection of perennial ryegrass by *Magnaporthe oryzae*. High performance liquid chromatography (HPLC) analysis of treated and untreated perennial ryegrass leaves exhibited considerable up- and down-regulation of metabolites in Si-treated and *M. oryzae*-challenged plants when compared to untreated plants. Using mass spectrometer, further evaluation of the differential expression of the HPLC peaks revealed elevated presence of a particular phenolic compound with a structural similarity to that of cinnamic acid. The phenolic compound is known to serve as precursor for many simple phenylpropanoids such as *p*-coumaric, ferulic, sinapic and caffeic acids that are known to be induced during plant defense response. This study indicates that silicon may play a role in activating phenylpropanoid pathway in presence of *M. oryzae* infection. Further analysis of the separated compounds will be conducted in order to identify the exact molecular structure of the compounds that may be involved in defense response in gray leaf spot pathosystem.

Pathogenic and molecular characterization of a *Sclerotinia* isolate used as a mycoherbicide for selective control of ground ivy

M. RAHMAN (1), K. Lympus (1), B. MacDonald (1)
(1) West Virginia University, Morgantown, WV, U.S.A.
Phytopathology 102:S4.96

Ground ivy (*Glechoma hederacea*), a member of the mint family is a common invader of lawns in most parts of West Virginia and is regarded as the most difficult-to-control weed problem in home lawns. Lawn care Companies and home owners heavily rely on broad leaf herbicides to control the weed. Overuse of chemicals poses risk of resistance development in weed populations as well as injury to non-target plant species especially ornamentals. In summer 2011, a fungal isolate was recovered from diseased ground ivy. Colony morphology of the fungus and formation of sclerotia on PDA indicated it to be a member of the genus *Sclerotinia*. The initial isolate was then used to grow colonized oat grain inocula that were used to conduct a field trial at WVU agronomy farm plot infested with ground ivy with two different levels of inocula and a non-treated control in a randomized complete block design (RCBD) with 4 replicates. Within 60 days of inoculation ground ivy was significantly ($P<0.001$) suppressed at higher and lower levels of inocula by 70% and 55% compared to the non-treated control. Amplification effort of ITS region of the fungus with previously characterized isolate specific primers indicated it to be a unique isolate. Further molecular characterization and host specificity analyses results will be presented.

Management of C*ercospora apii* on celery using conventional and reduced-risk fungicides

R. N. RAID (1)
(1) University of Florida, Belle Glade, FL, U.S.A.
Phytopathology 102:S4.97

Early blight, caused by *Cercospora apii*, is the most serious disease of celery in Florida. Two field experiments were conducted during the 2011/2012 growing season to investigate the efficacy of conventional fungicides and chemistries that are considered "low-risk", primarily plant activators (Systemic Acquired Resistance or SARs) in combination with copper or biofungicides. Both experiments were randomized complete block designs with four replications of 12 treatments. In comparing conventional fungicides, two strobilurin fungicides, pyraclostrobin (Cabrio and Headline) and azoxystrobin (Quadris), provided high levels of disease control, particularly when applied in premixture with carboximide (Merivon and Priaxor) or sterol inhibiting (Quilt) fungicides. In the low-risk trial, three plant activators, acibenzilar (Actigard), anthraquinone (Regalia), and laminarin (Vacciplant), provided significant suppression by themselves when applied on a 9-10 day schedule, but blight management was significantly enhanced when topped with foliar applications of copper fungicides (Kocide 3000 and Phyton 27) or the biopesticide *Bacillus subtilis* QST713 (Serenade Max). Treatment of celery with two foliar applications of the foliar materials per week provided significantly better control than those receiving only weekly applications. Although providing significant control, none of the low-risk treatment combinations provided control equal to that provided by the strobilurin/ carboximide premixture Pristine. It is important to note that activators were applied prior to disease onset and no phytotoxicity or yield drag was observed.

Development of an immunoassay for detection of *Citrus psorosis virus*

C. RAMADUGU (1), M. Kayim (2), M. Keremane (3), R. F. Lee (4)
(1) University of California, Riverside, CA, U.S.A.; (2) University of Cukurova, Adana, Turkey; (3) USDA-ARS, Citrus Germplasm Repository, Riverside, CA, U.S.A.; (4) National Clonal Germplasm Repository for Citrus & Dates, USDA-ARS, Riverside, CA, U.S.A.
Phytopathology 102:S4.97

Psorosis is a widespread and economically significant disease of citrus caused by closely related viruses designated as Psorosis A and Psorosis B. Infected plants show reduced vigor, low yields and premature death. The disease is graft-transmissible, seed-borne, and the symptoms as well as severity of infection vary depending on the host and the strain of the virus. Psorosis A can protect against expression of bark scaling caused by the more severe Psorosis B; ringspot isolates of Psorosis cause local lesions on *Chenopodium quinoa*. Diagnosis of Psorosis based on field symptoms is unreliable. *Citrus psorosis virus* (CPV) is classified as an ophiovirus and has a multi-component single stranded RNA genome. We have cloned the coat protein gene of a California isolate of CPV into an expression vector, pET 101 D Topo® and expressed the recombinant coat protein in *Escherichia coli* BL21. The expressed coat protein consists of a carboxy-terminal histidine-tag enabling purification of the fusion protein using nickel columns. Purified recombinant protein was tested using anti-his antibody on western blots and used for raising antisera in rabbits and in chicken. The polyclonal antisera are useful for quick and reliable diagnosis of several isolates of CPV. We are currently testing different biologically characterized isolates of Psorosis in California and Turkey to evaluate the efficacy of the antisera.

Predominance of potato cultivar Agata in Brazil and its effect in the dissemination and variability of *Potato virus Y*

T. O. Ramalho (1), S. B. GALVINO-COSTA (1), A. R. Figueira (1)
(1) Federal University of Lavras, Lavras, Brazil
Phytopathology 102:S4.97

Since the introduction of the potato necrotic strains of *Potato virus Y* (PVY) in Brazilian fields, the planted area with susceptible cultivars, such as Baraka and Achat, was significantly reduced, from more than 50% in 1996 to less than 5% in 2005. They were replaced mainly with Monalisa and Atlantic, but with the introduction of new strains such as PVY^{NTN} it was again replaced by the tolerant cultivars. Nowadays Agata occupies more than 60% of the planted area, because when infected with low virus concentrations it is symptomless and does not present potato tuber necrosis (PNTDR), behaving as a silent source of inoculum. Several surveys in Brazilian potato fields have shown that more than 50% of samples are infected with PVY^{NTN}. As a consequence, new PVY recombinant isolates had been reported in Brazil, such as the recently described PVY^{E}-AGA/MON. Another atypical isolate, named MF-AG-52 was detected, inducing necrosis in tobacco plants and presenting PVY^{O} serotype, but with PVY^{NTN} profile in multiplex RT-PCR. Besides that, a specific genome fragment with 995pb was amplified with primers for the PVY^{E}-AGA/MON, showing that it could be a new recombinant. The propagation of seeds coming from symptomless PVY infected plants in Brazil, where the vector population is high and the potato is cultivated during three seasons per year, may be the reason for the increment of necrotic strains incidence, and also for the high variability of PVY strains seen in this country.

WITHDRAWN

Genetic structure of sympatric populations of *Rhizoctonia solani* AG-1 IA from *Brachiaria* and rice in Colombia

L. M. Ramos Molina (1), M. Zala (2), B. A. McDonald (2), P. C. CERESINI (3)
(1) University of São Paulo State (UNESP), Jaboticabal, Brazil; (2) ETH Zurich, Institute of Integrative Biology (IBZ), Zurich, Switzerland; (3) FAPESP-Biota/University of São Paulo State (UNESP), Ilha Solteira, SP, Brazil
Phytopathology 102:S4.97

In the early '90s, the fungus *Rhizoctonia solani* has emerged as an important pathogen associated with the death of *Brachiaria* pastures in the Colombian Llanos. Our goals in this study were: i) To determine the genetic diversity and the predominant reproductive system of *R. solani* AG-1 IA populations infecting *Brachiaria*; ii) To estimate the historical patterns of migration and the magnitude of current gene flow between populations of *R. solani* AG-1 IA from *Brachiaria* and rice. To study the population genetic structure of the pathogen, a total of 198 isolates of *R. solani* AG-1 IA were collected from fields of *B. brizantha* cv. Toledo, *Brachiaria* hybrid Mulato and from rice. These isolates were genotyped using ten microsatellite loci. A mixed mating system (which includes sexual reproduction and dispersal of adapted clones) characterized the populations of *R. solani* AG-1 IA infecting *Brachiaria*. The high clonal fraction and deviations from Hardy-Weinberg equilibrium found in three of the four populations were consistent with Wahlund effect associated with the mixing of populations. In fact, we observed high levels of gene flow between populations from *Brachiaria* in contrast with significant subdivision with the rice-infecting population. Historical patterns of migration indicated that the likely source of the current populations infecting *Brachiaria* in the Colombian Llanos was from populations that originally infected rice.

Evaluation of *Ralstonia* CANARY technology

K. RAPPAPORT (1), H. Bowman (1), J. Elphinstone (2), L. Levy (3), Z. Liu (1)
(1) USDA APHIS PPQ CPHST, Beltsville, MD, U.S.A.; (2) The Food and Environmental Research Agency, York, United Kingdom; (3) USDA APHIS, Riverdale, MD, U.S.A.
Phytopathology 102:S4.97

CANARY is a B cell-based technology that rapidly identifies low levels of pathogens. The B cell line is engineered with a bioluminescent protein and an antibody gene expressed and anchored on the outer membrane of the cells. The assay is carried out through detection of photons emitted by bioluminescent proteins upon crosslinking of antigens to engineered antibodies. This study expands our evaluation of the technology by testing 60 *Ralstonia solanacearum* isolates collected from a wide range of geographical origins and plant hosts; 20 potential cross-reacting bacterial taxa; greenhouse

grown eggplant, tomato and nightshade plants infected with 3 *R. solanacearum* isolates; 30 river water samples; and 24 potato tuber samples. All *R. solanacearum* isolates were positively identified while closely related bacterial taxa generated negative reactions, indicating high analytical specificity of this B cell line. All 30 river water samples produced negative reactions, which was consistent with results generated by standard *R. solanacearum* detection methods. CANARY detected the four positive potato tuber samples among the 24 samples tested and successfully detected *R. solanacearum* in all greenhouse grown infected plants. The results generated with the CANARY method are currently being compared with standard detection methods (CFU estimates and immuno-based assays) and amplification-based assays to determine the sensitivity, specificity, calling accuracy, cost and ease of use.

The prevalence and impact of *Fusarium* and *Microdochium* species in U.K. malting barley

R. V. RAY (1), L. Nielsen (1), S. G. Edwards (2), D. J. Cook (1)
(1) University of Nottingham, Loughborough, United Kingdom; (2) Harper Adams University College, Newport, United Kingdom
Phytopathology 102:S4.98

Fusarium head blight disease caused by *Fusarium* and *Microdochium* spp. can result in significant reductions of yield and quality of cereals. Mycotoxin levels in UK malting barley have been reported to be below the EU legislative safety limits, but the effect of sub-acute *Fusarium* infection on the malting quality of barley has remained relatively unclear. The SAFEMalt project (Strategies Against *Fusarium* Effective in MALTing barley) is a 3-year multi-partner research initiative spanning the malting barley supply chain from barley breeder through barley grower and merchant to brewer. The project incorporates two annual surveys of UK Spring malting barleys (2010, 2011 harvests) and also has retrospective access to UK spring barley samples collected for mycotoxin screening between 2007-2009. In 2010, Real Time PCR analyses identified that the main species present across 88 samples of UK malting barley were *F. avenaceum*, *F. langsethiae*, *F. poae*, and *F. tricinctum* with each species detected in 80-90% of all samples. The predominant mycotoxins detected in 2010 were HT2/T2 and nivalenol correlating positively with quantified DNA of *F. langsethiae* and *F. poae*, respectively. *Fusarium sporotrichioides*, known HT2/T2 producer, was not detected in any barley samples between 2007 and 2010. The immediate effects of *Fusarium*/*Microdochium* contamination on malting quality parameters of barley will be presented and discussed.

An update on grapevine viruses and viroids in Washington State vineyards

N. RAYAPATI (1)
(1) Washington State University, Prosser, WA, U.S.A.
Phytopathology 102:S4.98

Since virus diseases affect grapevine health and fruit quality, we have conducted state-wide surveys of Washington vineyards from 2005 through 2011 seasons for the presence of grapevine viruses. In red-berried cultivars, leaf samples with different symptoms were collected during the season. Because white-fruited cultivars do not exhibit apparent symptoms, leaf samples were collected randomly from individual grapevines. Samples were tested by single-tube one-step RT-PCR for the presence of viruses. Amplicons were cloned and sequenced, and nucleotide sequences compared with corresponding sequences available in GenBank to confirm RT-PCR results and assess genetic diversity. The data showed presence of six *Grapevine leafroll-associated viruses* (GLRaV-1, -2, -3, -4, -5, and -9), *Grapevine rupestris stem pitting-associated virus*, *Grapevine virus A*, *Grapevine virus B*, *Grapevine fanleaf virus*, *Grapevine fleck virus* and *Grapevine Syrah virus-1*. These viruses were present in individual grapevines as singled and/or mixed infections. GLRaV-3 was found to be the most prevalent and widely distributed. In addition, a limited number of samples tested positive for *Australian grapevine viroid*, *Hop stunt viroid* (HSVd), *Grapevine yellow speckle viroid-1* and *Grapevine yellow speckle viroid-2*. These viroids were detected as mixed infections with grapevine viruses mentioned above. HSVd was found to be widespread in several wine grape cultivars.

Beneficial effect of ectomycorrhiza on conifer seedlings colonized by dark septate endophytes

V. REININGER (1), T. N. Sieber (1)
(1) ETH Zürich, Zürich, Switzerland
Phytopathology 102:S4.98

Mycorrhizal roots are frequently colonized by dark septate endophytes (DSE), in particular by fungi of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex (PAC). These ascomycetes are very common and widespread colonizers of coniferous and ericaceous tree roots on the northern Hemisphere. Some PAC strains reduce growth increment of their hosts but are beneficial in protecting roots against strong pathogens. Nothing is known about the effects of mycorrhizal fungi on PAC and the PAC-mycorrhiza association on plant growth. Effects of four PAC genotypes and their endophytic biomass, mycorrhization by *Laccaria bicolor* (strain S238N) and two temperatures (19°C and 25°C) on biomass of *Pseudotsuga menziesii* (Douglas-fir) and *Picea abies* (Norway spruce) seedlings were studied. Temperature and the PAC genotype only had an effect on biomass of spruce but not on biomass of Douglas-fir. Higher temperature reduced mycorrhization of both hosts. Mycorrhization compensated the adverse effects of PAC on growth of Norway spruce at both temperatures but those on growth of Douglas-fir only at 25°C. The compensatory effects probably rely on the reduction of the PAC-colonization density by mycorrhizae. We conclude that ectomycorrhizae form physical and/or physiological barriers against PAC leading to reduced PAC-colonization of the roots. The results clearly showed that mycorrhization by *L. bicolor* is beneficial for the host plant.

Differential acquisition and transmission of Florida *Tomato spotted wilt virus* isolates by Western flower thrips

S. R. Reitz (1), S. ADKINS (2)
(1) USDA-ARS, Tallahassee, FL, U.S.A.; (2) USDA-ARS USHRL, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.98

Thrips-vectored *Tomato spotted wilt virus* (TSWV) is one of the most important insect-vectored plant pathogens globally. The virus host range encompasses many key vegetable, ornamental and agronomic crops. TSWV populations are highly heterogeneous, which has important implications for vector relations and epidemiology. Although several thrips species can vector TSWV, Western flower thrips (WFT; *Frankliniella occidentalis*) is the most important vector in vegetable crops in Florida. We assessed the ability of WFT to acquire and transmit five independent local lesion isolates of TSWV derived from tomato, pepper or peanut. Sequence analysis revealed minimal variation in the N gene but substantially more in the glycoprotein precursor. First instar larvae were allowed to acquire the virus from infected tomato plants and then placed individually as adults on petunia leaf discs for transmission. Individual thrips and leaf discs were assayed by commercially available enzyme linked immunosorbent assay to determine whether TSWV acquisition and transmission had occurred. WFT larvae differentially acquired the five TSWV isolates from infected tomato plants. WFT adults differentially transmitted these isolates to petunia leaf discs. The efficiency with which WFT acquired isolates was not necessarily related to the efficiency with which they were transmitted. Our results demonstrate the importance of TSWV heterogeneity in its interactions with thrips vectors.

WITHDRAWN

Projecting Monterey pine populations over time in the presence of pitch canker disease

G. J. REYNOLDS (1), N. McRoberts (1), T. R. Gordon (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.99

Monterey pine (*Pinus radiata*) is native to California and widely planted in Mediterranean climates around the world for production of timber and pulp. Pitch canker, a disease caused by *Fusarium circinatum*, is a serious threat to *P. radiata* both in native forests and in plantations. Because of its economic importance worldwide, conservation of *P. radiata* in native populations is a high priority. To better understand the impact of pitch canker, we developed a demographic matrix projection model to simulate the dynamics of naturally occurring *P. radiata* populations in California in the presence of the disease. Tree demography and disease intensity data for the model were collected from seven sites in a native forest on the Monterey Peninsula. The height, stem diameter, and number of pitch canker strikes were recorded for each tree included in the survey. Trees were divided into five size classes (seedlings, small saplings, medium saplings, large saplings, and mature trees). Transition probabilities were estimated from the data; numbers of cones and seeds and transition probabilities for these stages were estimated from a range of data sources. In the model, pitch canker influences state-specific fecundity and survival probabilities. Populations were projected over 30 decades from specified initial population sizes. Our results indicate that facilitating survival of mature trees will be critical to maintaining Monterey pine forests in their present native state.

Cercospora leaf blight disease of soybean: Variety differences, source of inoculum, and environmental effects

J. REZENDE (1), B. Buckley (2), Z. Chen (2)
(1) Louisiana State University, Baton Rouge, LA, U.S.A.; (2) Agcenter, Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.99

Cercospora leaf blight (CLB) is an economically important disease in soybean (*Glycine max* L.), and can cause substantial yield loss. Identification of soybean lines resistant to CLB is critical in the effective control of this disease. In this study, twenty different soybean varieties were screened for their level of CLB resistance under field conditions in Louisiana at two locations with four replicates using complete randomized block design. Soybean leaf samples were collected biweekly for the first four weeks after planting, then weekly until late R6, which resulted in fourteen collections for each location. Twenty leaves were collected randomly from each plot, visually inspected for disease symptoms, and examined for the presence of *Cercospora kikuchii* using quantitative real-time PCR (qPCR). Eight different soybean varieties were found to have significant levels of *C. kikuchii* growth two weeks after planting, suggesting the source of inoculum is most likely from the seeds. Visible CLB symptoms were first observed at the late R5 and early R6 soybean growth stages. Although both qPCR and visual ratings identified several soybean lines as the most and least susceptible to CLB, there is a significant difference in CLB development between the two locations, and a high variation of fungal DNA levels between collection dates. This suggests that fungal growth and CLB development in soybean is influenced by variety, environment and possibly other unknown factors.

WITHDRAWN

Investigation of *Salmonella enterica* survival in water

S. RICHARDSON (1), J. M. Cevallos-Cevallos (1), J. Hu (1), V. Zelenev (2), A. Wright (1), A. H. Van Bruggen (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) Moscow State University, Moscow, Russia
Phytopathology 102:S4.99

Survival of *S. enterica* in the environment has been studied, but survival in surface and irrigation water is not well understood. Therefore, the occurrence and survival of a gfp-marked, kanamycin-resistant strain of *S. enterica* in water was studied in two experiments. The first experiment had five eutrophication levels (30, 50, 100, 500 and 1000 mg Dissolved Organic Carbon (DOC) / L water) over a four-month period and the second one had three eutrophication levels (30, 100 and 200 mg DOC / L water) over a three-week period. Each flask was inoculated with a gfp-strain of *S. enterica* at 1010 CFU/ml. The flasks were placed in replicated incubators at 7, 17 and 27 °C in the first trial and at 27 °C in the second. In the first experiment, *S. enterica* colonies were counted at 3-day intervals after dilution plating on kanamycin-amended LB agar. In the 3-week study, *Salmonella* densities were determined daily by dilution plating and flow cytometer counts. *S. enterica* concentrations declined quickly within one week; then slowly during one month. The decline was significantly faster at the lower three carbon levels than at the higher two levels in the first experiment. Effects of eutrophication were not significant in the second experiment. When daily observations were plotted over time, a wave-like pattern was discerned in DOC contents over time and in some cases in *S. enterica* densities; these oscillations were verified by harmonics analysis.

WITHDRAWN

Evaluation of model plants for use in elucidation of *Sclerotinia homoeocarpa* pathogenesis

R. RIOUX (1), J. Kerns (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.100

Dollar spot of turfgrass, caused by *Sclerotinia homoeocarpa*, occurs on many turfgrass species worldwide and is among the most important diseases with respect to pesticide expenditures. Studies of *S. homoeocarpa* pathogenesis may decrease the costs of control but are limited by the amount of genetic information available for natural hosts. Various plants with well-characterized genomes as well as the natural host *Agrostis stolonifera* were utilized to study *S. homoeocarpa* infection. Leaves of all plants were inoculated with agar plugs of 7 day-old potato dextrose agar cultures of four *S. homoeocarpa* isolates to monitor the progression of infection. Results on all plants were similar and showed an initial 72-hour biotrophic phase prior to the onset of host tissue necrosis. These findings were corroborated by microscopic analysis of inoculated tissues. Isolation attempts from necrotic host tissue on monocot models were unsuccessful unless samples were within 10cm of the inoculation site. Consequently, semi-purified culture filtrates were determined to induce symptoms similar to those observed in the infection assays. Symptom development was not correlated with oxalic acid content of culture filtrates, which is typical of other *Sclerotinia* species. This research enhances understanding of the infection process and virulence mechanisms of *S. homoeocarpa* and demonstrates the usefulness of various model plant species for further study of *S. homoeocarpa* pathogenesis.

Development of a semiselective media for enhanced detection of *Sclerotinia homoeocarpa* from plant tissues

R. RIOUX (1), B. van Ryzin (1), J. Kerns (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 102:S4.100

Dollar spot, caused by the fungus *Sclerotinia homoeocarpa* is one of the most prevalent diseases on turfgrass worldwide. Due to difficulties identifying sources of inoculum, a more sensitive detection method for *S. homoeocarpa* is necessary. An improved semi-selective medium for isolation of *S. homoeocarpa* was developed by comparing growth inhibition on media with various amendments to the current standard, PDA+++ (potato dextrose agar with 50mg/L Tetracycline, Chloramphenicol, and Streptomycin). Amendments included various fungicides, lactic acid, and bromophenol blue for indication of fungal oxalic acid production. Seven-day old agar plugs of four *S. homoeocarpa* isolates and eight field contaminants were placed in the center of a 60mm diameter petri dish containing PDA with combinations of different amendments. Colony diameter was measured at 48 hours after transfer and percent inhibition was calculated relative to PDA+++. Validation of media that inhibit contaminant growth with minimal impact on *S. homoeocarpa* was performed by comparing isolation frequency from field and seed samples to PDA+++. Results demonstrate that azoxystrobin is a good amendment for semi-selective media while PCNB is poor because it significantly inhibits *S. homoeocarpa* growth. Adding bromophenol blue is unnecessary because the color change indicative of acid production is minimal and does not develop fast enough to aid in detection of *S. homoeocarpa*.

Use of sulfur dioxide (SO_2) as a postharvest treatment to control gray mold of blueberry (*Vaccinium corymbosum*)

S. A. RIVERA (1), J. P. Zoffoli (1), B. A. Latorre (1)
(1) Pontificia Universidad Católica de Chile, Santiago, Chile
Phytopathology 102:S4.100

Blueberry fruits shipments from Chile require >15 days to reach the international markets. Under these conditions, gray mold (GM) (*Botrytis cinerea*) can cause severe losses. The effect of SO_2 fumigation to control GM was studied. Mature fruits 'Brigitta', inoculated with *B. cinerea* (500 conidia/fruits), were fumigated with 25, 50 and 100 µL/L applied as a continuous SO_2 stream (150 mL/min) in 11 liter chambers at 20°C to attain an SO_2 concentration x time product of 50, 100, 300 and 400 µL/L x h. Treated and non-treated fruits were maintained for 15 days at 0°C + 3 days at 20°C prior to the determination of the GM incidence. SO_2 significantly ($p<0.0001$) controlled GM, and a significant interaction ($p<0.0001$) of the SO_2 concentration x time product was obtained. The GM was reduced by 41.2% using 25 µL/L for 6.0 h (equivalent to 100 µL/L x h) and 58.3% using 50 µL/L for 4.5 h (equivalent to 100 µL/L x h), but a 18.7% reduction was obtained using 100 µL/L for 2.0 h (equivalent to 100 µL/L x h). Therefore, the initial SO_2 concentration and exposure time to achieve a given SO_2 concentration x time product appears to be critical. The optimal GM control was obtained with >300 µL/L x h; obtained using either 25 µL/L for 14.0 h or 50 µL/L for 7.5 h. Similarly, the GM was reduced to 96.4% when using 100 µL/L for 5.0 h (400 µL/L x h). No sign of phytotoxicity was observed visually. These results demonstrate the effectiveness of SO_2 fumigation to control GM of blueberry.

Natural products for suppression of damping-off pathogens in organic cucumber production

D. ROBERTS (1), L. F. McKenna (2), J. E. Maul (2), D. K. Lakshman (2), J. Buyer (2), S. E. Emche (2)
(1) USDA, Sustainable Agricultural Systems Lab, Beltsville, MD, U.S.A.; (2) Sustainable Agricultural Systems Lab, Beltsville, MD, U.S.A.
Phytopathology 102:S4.100

Disease control measures must be developed for organically produced cucumbers as growers have few disease management options. Ethanol extracts of antibiotic-producing beneficial bacteria were tested for suppression of the damping-off pathogens *Pythium ultimum* and *Rhizoctonia solani*. Extracts from *Serratia marcescens* isolates N4-5 and N2-4 inhibited *P. ultimum* sporangial germination in Nutrient broth while extracts from *Burkholderia cepacia* BC-F and BC-2 inhibited growth of *R. solani* on Potato Dextrose Agar. Extracts from *B. cepacia* BC-1 and *Pseudomonas fluorescens* PF-5 were only slightly inhibitory to these pathogens. Ethanol extracts from isolate N4-5 also suppressed damping-off of organic cucumber seedlings caused by *P. ultimum* in growth chamber studies when applied as seed treatments. Greenhouse experiments are being conducted to determine if ethanol extracts from these beneficial bacteria suppress damping-off of cucumber caused by *R. solani*. PCR followed by DNA sequence analysis and TLC were used to confirm that pyrrolnitrin was produced by isolates N4-5, BC-F, BC-2, and PF-5. Prodigiosin was previously shown to be produced by N4-5. Pyrrolnitrin and prodigiosin are ethanol soluble and inhibitory to *P. ultimum* and/or *R. solani*. These studies demonstrate that the use of OMRI-approved solvents for the isolation and seed application of natural products is promising for use in organic cucumber production systems for the control of soilborne plant pathogens.

Eicosapolyenoic acid action in *Arabidopsis* and tomato: Novel PAMPs with reciprocal effects on plant defense signaling networks

S. M. Roberts (1), M. L. Bjornson (1), T. Savchenko (1), T. V. Roubtsova (1), M. F. Pye (1), T. Kasuga (2), C. Lazarus (3), K. Dehesh (1), R. M. BOSTOCK (1)
(1) University of California, Davis, CA, U.S.A.; (2) USDA-ARS, University of California, Davis, CA, U.S.A.; (3) University of Bristol, Bristol, United Kingdom
Phytopathology 102:S4.100

Eicosapolyenoic acids (EP) – arachidonic (AA) and eicosapentaenoic (EPA) acids – are common fatty acids in lipids and other cellular components of plant pathogenic oomycetes that function as conserved signaling molecules across eukaryotic kingdoms. EP released during infection of plants by *Phytophthora* and other oomycete species may serve as novel pathogen-associated molecular patterns (PAMPs) to engage defense signaling networks in a jasmonic acid-dependent manner. These changes are manifested as a generalized rapid stress response resulting in enhanced tolerance to pathogens and insects. Transcriptome analyses of *Arabidopsis thaliana* roots challenged with *Phytophthora capsici* or engineered to constitutively produce very low levels of EPs (EP plants) reveal a coexpression network of four strongly induced genes. These genes, including two that are known to function as deadenylases that degrade mRNAs, are normally expressed transiently. In addition, two of the four genes are constitutively expressed in EP plants without infection, suggesting a novel EP-mediated post-transcriptional regulation. The AA-induced expression pattern and known functions of these genes implicates them in a stress response network in *Arabidopsis* that serves some unique function in AA response. The action of AA and EPA in *Arabidopsis* and in a tomato root model will be presented.

Incidence and molecular detection of *Gooseberry vein banding associated virus* in the *Ribes* spp. collection of the National Clonal Germplasm Repository

N. L. ROBERTSON (1), C. J. Macknicki (1), T. A. Steinlage (1), J. D. Postman (2)
(1) USDA-ARS, Palmer, AK, U.S.A.; (2) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 102:S4.100

Gooseberry vein banding disease (GVBD) is closely associated with *Gooseberry vein banding associated virus* (GVBAV, genus *Badnavirus*, family *Caulimoviridae*), and is one of the most prevalent diseases in European gooseberry and currant crops. In 1993, leaf vein-banding was reported on 17.5% of the 630 clonal *Ribes* accessions from the USDA-ARS National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon. Nearly seven years later, PCR assays on 48 GVBD cultivars of gooseberries, black and red

currants, and wild *Ribes* spp. from NCGR confirmed the presence of GVBAV on all plants showing symptoms. In 2009-2011, clonal cuttings of *Ribes* accessions from NCGR were maintained in greenhouses for distribution at the Arctic and Subarctic Plant Gene Bank in Palmer, Alaska. GVBAV-specific PCR primers (GVBAV-3F/R) were used to assay over 200 accessions regardless of symptoms. Approximately 38% of the accessions tested were positive, which included about 57% of 56 *Ribes rubrum* L., 25% of 88 *R. nigrum* L., 44% of 36 *R. uva-crispa* L., and 34% of 26 *Ribes* crosses and wild species. PCR products with the expected ca. 527 bp segment and greatest concentration were well correlated with strong vein clearing symptoms. These fragments were directly sequenced, and their nucleotide identities ranged from 94% to 99% when compared in GenBank with five other GVBAV accessions by BLAST. The significance of these results implies that GVBAV is not genetically diverse on the 3' end of the genome, and that the population may be genetically similar from *Ribes* plants collected around the world.

Effect of Goss's leaf blight severity on grain quality and on *Clavibacter michiganensis* subsp. *nebraskensis* seed infection

A. E. Robertson (1), C. C. Block (2), C. R. Hurburgh (1), L. M. SHEPHERD (2)
(1) Iowa State University, Ames, IA, U.S.A.; (2) USDA-ARS, Ames, IA, U.S.A.
Phytopathology 102:S4.101

Goss's wilt and leaf blight of corn, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), has reemerged as an economically important disease across much of the Midwestern U.S. The widespread incidence of Goss's wilt has raised questions as to effects on grain quality and the role of seed infection in disease spread. In 2011, we selected nine fields with Goss's leaf blight and created sampling plots in areas of low disease severity (0-5% leaf area affected) and high disease severity (>5% leaf area affected). Disease severity was estimated at the R5 growth stage and stalk rot ratings made at harvest. Harvested ears were shelled and grain quality characteristics assessed, including test weight, 1000-seed weight, moisture, protein, starch, and oil content. Seeds were individually assayed for Cmn seed infection, and samples planted for seed to seedling transmission studies. No differences in test weight or protein content were found in grain samples from the low disease plots compared to the high disease plots. Stalk rot increased significantly in the high disease plots, and there was a significant reduction in 1000-seed weight in the same plots. No Cmn infection was detected in seeds from the low disease plot samples (0-5% leaf area). In the more heavily infected field plots (over 5% leaf infection), only low amounts of seed infection (<1%) were noted. No seed transmission was detected in greenhouse growouts.

Flagellar phylogenetics: A study of crown oomycete evolution

G. P. ROBIDEAU (1), T. L. Rintoul (2), C. Levesque (2)
(1) Carleton University, Ottawa, ON, Canada; (2) Agriculture and Agri-Food Canada, Ottawa, ON, Canada
Phytopathology 102:S4.101

The kingdom Straminipila (Stramenopiles) is defined by the possession of an ornamented "straminipilous" or "tinsel" flagellum. With flagella being central to the classification of these organisms, it stands to reason that the genes controlling flagellar structure and motility should be able to provide some interesting insight into the evolution of different species and genera of oomycetes. Ultrastructural studies have already revealed the connection between flagellar apparatus morphology and phylogeny of zoosporic fungi. This concept has now been explored at the molecular level, with DNA sequencing of an axoneme central apparatus gene (ACA) and a tinsel flagellum mastigoneme gene (OCM1) from a variety of Peronosporales (eg. *Pythium*, *Phytophthora*) and Saprolegniales (eg. *Saprolegnia*, *Aphanomyces*) species using degenerate primers. The phylogenetic reconstructions obtained from ACA and OCM1 are well supported and in accordance with current views of oomycete systematics. Interestingly, the OCM1 amino acid sequence is highly variable between species, but highly conserved within species. Such a species-specific nature suggests that OCM1 may play an important ecological role, possibly in oomycete interaction with hosts, and could be used to better define species boundaries in oomycetes.

Blueberry necrotic ring blotch: A new disorder of southern highbush blueberries

T. S. ROBINSON (1), P. M. Brannen (1), C. M. Deom (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 102:S4.101

Blueberry necrotic ring blotch disorder (BNRBD), a new presumptive viral disease of southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrids), was first reported in 2006 in Bacon County, GA. Since then, the disease has spread rapidly throughout the Southeast. Susceptible blueberry leaves display symptoms of irregularly shaped dark brown-black rings or blotches that coalesce to form necrotic tissue and result in premature defoliation. Symptomatic plants tested positive for the virus by RT-PCR. Studies are being conducted to fill critical knowledge gaps regarding the epidemiological processes as related to disease development and spread of BNRBD *in planta* and in-field. Preliminary results suggest that the disorder spreads from within the center on the plant and moves outward. Within a field, results suggest that the disorder moves in a gradient fashion, often displaying an edge effect. In addition, movement within rows appears to be more rapid than movement across rows, suggesting that a slow moving insect vector may be involved in transmission. More information about BNRBD needs to be obtained to better understand the disorder and to begin developing management strategies for controlling the disorder to assure the continued expansion of blueberry production in Georgia.

Incidence of virus infecting pepper (*Capsicum annuum* L.) in two main pepper-producing regions of Chihuahua, Mexico

L. ROBLES-HERNANDEZ (1), A. Karasev (2), A. Gonzalez-Franco (1)
(1) Universidad Autonoma de Chihuahua, Chihuahua, Mexico; (2) University of Idaho, Moscow, ID, U.S.A.
Phytopathology 102:S4.101

Pepper is an important crop of Chihuahua, Mexico with 66525.3 acres harvested in 2009. Thirteen virus species are reported to cause disease pepper in several States of Mexico but in Chihuahua. A survey was conducted to determine the incidence of virus infecting pepper in Chihuahua State. A total of 213 leaf samples were collected in 10 locations of the South-Central and North regions of Chihuahua, and tested by DAS-ELISA using specific polyclonal antibodies.Twelve different virus species were identified. *Cucumber mosaic virus*, *Tobacco mosaic virus*, *Alfalfa mosaic virus*, *Tobacco etch virus*, *Tomato bushy stunt virus*, *Pepper mild mottle virus*, *Potato virus Y*, and *Pepper mottle virus* were detected in the South-Central region, while *Tobacco ring spot virus*, *Tomato ring spot virus*, *Impatient necrotic spot virus*, and *Tomato spotted wilt virus* were identified in the North. *Cucumber mosaic virus* (73%) and *Tobacco mosaic virus* (36%) were predominant in the South-Central region, whereas *Tobacco ring spot virus* (55%) and *Tomato ring spot virus* (29%) were higher in the North. Disease incidence (21%) and severity index (17%) were higher in the North that in the South-Central region. Although predominately infected samples with one virus were observed, multiple viruses (6-8) were detected in several samples from the both regions. From our knowledge, this is the first report of viral incidence on pepper in the two important pepper-producing regions of Chihuahua.

Multilocus sequence analysis of xanthomonads associated with poinsettia production reveals pathogen variability

W. D. ROCKEY (1), D. J. Norman (2), J. B. Jones (1)
(1) Department of Plant Pathology, University of Florida, Gainesville, FL, U.S.A.; (2) Department of Plant Pathology, University of Florida, Apopka, FL, U.S.A.
Phytopathology 102:S4.101

As high value ornamental crops, species of Poinsettia (*Poinsettia*), Croton (*Codiaeum*), Crown of Thorns (*Euphorbia*) and Geranium (*Pelargonium*) are often grown in mixed production and in close proximity in commercial glasshouse facilities throughout the world. Because of its virulence and because of its ability to infect many genera, bacterial blight, caused by *Xanthomonas axonopodis* pv. *poinsettiicola* (Xap), is considered to be the most serious bacterial disease causing extensive crop losses. Multiple Xap isolates have exhibited the ability not only to cause a leaf spot on poinsettia, but these also may systemically infect geranium. In this study, seventy-two Xap isolates were characterized via pathogenicity tests, hypersensitive response, and sequence analysis. Multilocus Sequence Analysis/Typing (MLSA or MLST) using six conserved housekeeping genes were used to sequence the *Xanthomonas* isolates' DNA. The genes used were gltA, gap1, fusA, gyrB, lacF, and lepA. Phylogenetic analysis revealed in multiple clusters indicating significant genetic variation. Data can assist epidemiologists in identifying disease sources and tracking pandemics.

Modelling the effect of soybean rust on soybean yield using the CSM CROPGRO: Soybean

R. A. RODRIGUES (1), J. Pedrini (2), C. W. Fraisse (3), J. C. Fernandes (4), F. B. Justino (1), A. Heinemann (5), F. X. Vale (6), L. Costa (1)
(1) Agricultural Engineering Department, Federal University of Viçosa, Viçosa, MG, Brazil; (2) University of Passo Fundo, Passo Fundo, RS, Brazil; (3) University of Florida, Agricultural & Biological Engineering Department, Gainesville, FL, U.S.A.; (4) Embrapa Trigo, Passo Fundo, RS, Brazil; (5) Embrapa Arroz e Feijão, Santo Antônio de Goiás, Goiás, Brazil; (6) Plant Pathology Department, Federal University of Viçosa, Viçosa, MG, Brazil
Phytopathology 102:S4.101

In recent years, there has been a large increase in the use of simulation models in Agriculture. Currently, *Decision Support System for Agro-technology*

Transfer (DSSAT) is an important tool for modeling and simulation of crop growth. However, one of its limitations is the fact that simulation models do not take into account the effect of diseases and pests. Thus, this study aimed to calibrate and validate the CSM-CROPGRO-Soybean by estimating the growth, development, and yield of soybean cultivar BRS Valiosa at Viçosa city, Minas Gerais State. Indeed, it was evaluated the coupling module of asian soybean rust to CSM-CROPGRO-Soybean considering on and off soybean yield simulations. Experimental data for evaluation, testing, and fitting genetic coefficients of the model were obtained during the 2006/2007, 2007/2008, and 2009/2010 growing seasons. The Generalized Likelihood Uncertainty Estimation (GLUE) was used to estimate the genetic coefficients. The pedo-transference functions were used to estimate soil physical parameters. The soybean yield simulations for 5 sowing dates were based on the meteorological data from 1968 to 2009 obtained from stations of the National Institute of Meteorology. The model obtained presented high sensitivity to genetic coefficient variation as well as for phenological development and grain yield under the soil and climate conditions for Viçosa city. The soybean cultivar used demonstrated greater probability to obtain higher productivity in years with favorable weather conditions for epidemics. In the presence of soybean rust, productivity can be heavily affected in November when the relative humidity is higher than 90%. For all sowing dates analyzed, October was the month where great yield was obtained.

Characterization of novel secreted proteins from *Xylella fastidiosa*
E. ROGERS (1)
(1) USDA-ARS SJVASC, Parlier, CA, U.S.A.
Phytopathology 102:S4.102

Xylella fastidiosa is a bacterium that causes disease of agriculturally important crops, including Pierce's disease of grapevine. Little is known about virulence factors that are necessary for *X. fastidiosa* to grow and cause disease in the xylem vessels of a plant host. Any protein secreted by the bacterium has the potential to interact with the plant host and affect pathogen virulence and/or recognition. A number of novel *X. fastidiosa* proteins with predicted signal sequences for secretion were investigated. An *E. coli* model system and/or *X. fastidiosa* itself was used to confirm secretion for the majority of tested proteins. A *Tobacco rattle virus* (TRV) vector was used to express these proteins and examine effect on pathogenicity of TRV in *Nicotiana benthamiana*. Of 58 proteins assayed, 8 were shown to reproducibly and significantly increase the virulence of TRV, implying they function as pathogenicity factors in *X. fastidiosa*. Currently, mutations are being constructed in each of the 8 candidate pathogenicity factors in *X. fastidiosa*; once knock-out strains are available, disease assays in grapevine will be performed.

Survey of oomycete species associated with soybean seedling diseases in the United States
A. ROJAS (1), J. Jacobs (1), C. A. Bradley (2), P. D. Esker (3), L. Giesler (4), D. Jardine (5), B. D. Nelson (6), D. K. Malvick (7), S. Markell (6), A. E. Robertson (8), J. C. Rupe (9), L. Sweets (10), K. A. Wise (11), M. I. Chilvers (1)
(1) Michigan State University, East Lansing, MI, U.S.A.; (2) University of Illinois, Urbana, IL, U.S.A.; (3) University Wisconsin-Madison, Madison, WI, U.S.A.; (4) University of Nebraska, Lincoln, NE, U.S.A.; (5) Kansas State University, Manhattan, KS, U.S.A.; (6) North Dakota State University, Fargo, ND, U.S.A.; (7) University of Minnesota, St. Paul, MN, U.S.A.; (8) Iowa State University, Ames, IA, U.S.A.; (9) University of Arkansas, Fayetteville, AR, U.S.A.; (10) University of Missouri, Columbia, MO, U.S.A.; (11) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 102:S4.102

Soybean is the second most important crop inthe U.S., but yield is often reduced by diseases affecting stand establishmentand root health. Oomycete pathogens have been commonly associated with seedling damping off and root rots under conducive conditions. However, there is limited information regarding their frequency and abundance across the major soybean producing states in the Midwest. In order to determine the distribution and prevalence of oomycete species associated with soybean seedling diseases, a survey was conducted across 12 states. Nearly, 300 hundred oomycete isolates were recovered from five different fields in each state, using semi-selective medium (CMA-PARP+B). The resulting isolates were identified by sequencing of the ITS region of rDNA. Preliminary sequence results of 1812 isolates distinguished a total of 54 *Pythium* and 2 *Phytophthora* spp., with *Py. sylvaticum* (14%) and *Py. oopapillum* (16%) being the most frequent; even so, species composition frequency fluctuates between sampling locations revealing an east-west gradient. Pathogenicity and aggressiveness are being evaluated for representatives of each species. The distribution, and frequency of the species found in this study provide valuable information on abundance and prevalence of potential soybean oomycete pathogens across different geographical locations and conditions. This study will be the foundation for the development of diagnostic tools and the improvement of soybean seedling and root health.

Mapping of genes associated with Diplodia ear rot resistance in maize
M. P. ROMERO (1), C. P. Woloshuk (1), G. S. Johal (1), K. A. Wise (1)
(1) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 102:S4.102

Stenocarpella maydis is considered the most common ear rot pathogen in corn fields, and seed quality is compromised in infected ears. Resistance to *S. maydis* is quantitatively inherited and highly influenced by environment. A study was conducted near Lafayette, Indiana to locate potential quantitative trait loci (QTLs) and molecular markers involved in resistance to Diplodia ear rot in the corn intermediate B73 x Mo17 (IBM) population of recombinant inbred lines (RIL). The 302 lines were planted with one replication in 2010, and two replications in 2011. Each line was artificially inoculated at a vegetative growth stage (V7) with sterilized sorghum seed colonized with *S. maydis*. A set of 1339 Simple Sequence Repeats (SSR) or microsatellite, and Single Nucleotide Polymorphisms (SNPs) markers on the 302 lines were analyzed by composite interval mapping (CIM) with Windows QTL Cartographer version 2.5. Data collected from 2010 and 2011, were analyzed using a threshold LOD of 2.5 cM. When the data were combined, seven QTLs, on chromosome 9 and 6 were found from the susceptible parent B73; two more QTLs, on chromosome 3, were found from the resistant parent Mo17.

Multigene analysis of *Pythium* species causing carrot cavity spot in California and Michigan
N. ROSENZWEIG (1), X. H. Lu (1), H. Jiang (1), J. Hao (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.102

Carrot cavity spot (CCS) has a worldwide distribution. The disease is caused by a complex of *Pythium* spp. whose distribution varies based on geographic and environmental factors. A phylogeographic approach was used to resolve which *Pythium* species were associated with CCS in California (CA) and Michigan (MI) and to understand their phylogenetic relationships. Isolates of *Pythium* spp. were collected from cavity-spot symptomatic carrots in major carrot production areas of CA and MI, and subjected to DNA sequence, single and multi-locus phylogenetic and phenotypic analysis. PCR amplicons of the internal transcribed spacer, the beta-tubulin gene, part of the cytochrome c oxidase gene and the nitrogen dehydrogenase gene were sequenced. Two separate types of phylogenetic reconstruction, maximum-parsimony and Bayesian methods, were utilized. The Bayesian method used the combined DNA sequences from the four gene regions present in all isolates. The results indicated *P. violae*, *P. dissotocum* and *P. ultimum* distributions were restricted to CA whereas *P. sylvaticum*, *P. intermedium*, and an unidentified *Pythium* spp. were restricted to MI and separated by variability in fungicide sensitivity. The results of this study will provide information for chemical disease management in carrot production on the regional phylogenetic relationship of *Pythium*.

Transcriptome characterization of an Armillaria root disease pathogen reveals candidate pathogenicity-related genes
A. ROSS-DAVIS (1), J. Stewart (2), J. Hanna (3), M. Kim (4), R. Cronn (5), H. Rai (6), B. Richardson (7), G. McDonald (3), N. Klopfenstein (3)
(1) USDA Forest Service, Moscow, ID, U.S.A.; (2) USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR, U.S.A.; (3) USDA-FS, Rocky Mountain Research Station, Moscow, ID, U.S.A.; (4) Department of Forestry, Environment, and Systems, Kookmin University, Seoul, South Korea; (5) USDA-FS, Pacific Northwest Research Station, Corvallis, OR, U.S.A.; (6) Wildland Resources Department, Utah State University, Logan, UT, U.S.A.; (7) USDA-FS, Rocky Mountain Research Station, Provo, UT, U.S.A.
Phytopathology 102:S4.102

Armillaria species display diverse ecological behaviors ranging from beneficial saprobe to virulent pathogen. *Armillaria solidipes* (pending vote to conserve *A. ostoyae*), a causal agent of Armillaria root disease, is a virulent primary pathogen with a broad host range. The white rot pathogen attacks sapwood as mycelial fans under the bark and grows between trees as rhizomorphs. Armillaria root disease reduces forest productivity via direct tree mortality and non-lethal cryptic infections that impact tree growth. To better understand pathogenicity of the fungus, we characterized a transcriptome of an active mycelial fan from *A. solidipes* in northern Idaho. cDNA from polyA+ purified total RNA was sequenced using a paired-end read approach on the Illumina GAII platform which generated 24,166,534 reads. A BLASTx search against the NCBI NR database using 39,943 contigs assembled *de novo*

resulted in 19,599 sequences with significant alignments (≤ 1e-15), predominantly to fungi (79%). Signal P identified 19,792 putative signal peptides from the assembled contigs and 15,470 contigs were further characterized into Gene Ontology terms. Several putative genes showed strong homology to genes annotated to be involved in pathogenicity. This work is a primary step toward understanding pathogenicity of tree root-rot fungi and our results provide useful insights into identifying specific genes with potential roles associated with pathogenicity of *A. solidipes*.

WITHDRAWN

Episodic abiotic stress and *Phytophthora ramorum* blight in rhododendron: Impacts on root infection, symptom expression, and chemical management

T. V. ROUBTSOVA (1), S. A. Johnson-Brousseau (2), R. M. Bostock (3)
(1) Department of Plant Pathology, University of California-Davis, Woodland, CA, U.S.A.; (2) Department of Natural Sciences & Mathematics, Dominican University of California, San Rafael, CA, U.S.A.; (3) Department of Plant Pathology, University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.103

Phytophthora ramorum causes foliar blight and dieback of many nursery species. Of concern for disease management is that roots, when colonized by *P. ramorum*, may serve as a potential reservoir of inoculum. We hypothesized that mild abiotic stresses can compromise host resistance to trigger systemic disease development from cryptic root infections as well as influence the efficacy of chemical management. Three *P. ramorum* susceptible rhododendron cultivars were examined for their responses to chilling, water logging, water deficit, or salinity following root inoculation. For growth chamber experiments, plants were maintained at 22°C/15°C (day/night) with a 16 h photoperiod. There was no evidence for disease predisposition by waterlogging or by chilling (20°C/4°C, day/night, for 5 days). Brief episodes of salt or drought stress predisposed plants to enhance subsequent Ramorum blight development. A nursery trial was established to test the interaction of salt stress and fungicide treatments (Subdue Maxx or Aliette). Plants were inoculated by adding an infested V-8 broth/vermiculite-medium to the soil. Although plants did not show any above-ground symptoms of Ramorum blight after six months, roots were heavily colonized in all treatments. These results indicate that rhododendron can sustain extensive root infections without above-ground symptoms, and raise concerns about the adequacy of current practices for monitoring and managing *P. ramorum* in the nursery.

Identification of a new citrus cytoplasmic virus associated with citrus leprosis disease in Colombia using deep sequencing

A. ROY (1), N. Choudhary (1), G. A. Leon (2), D. Achor (1), J. Shao (3), G. Wei (4), D. D. Picton (4), L. Levy (4), M. K. Nakhla (4), J. S. Hartung (3), R. H. Brlansky (1)
(1) University of Florida, CREC, Lake Alfred, FL, U.S.A.; (2) Centro de Investigación La Libertad, Corpoica, Villavicencio, Colombia; (3) USDA-ARS, MPPL, Beltsville, MD, U.S.A.; (4) USDA-APHIS-PPQ-CPHST, Beltsville, MD, U.S.A.
Phytopathology 102:S4.103

Cytoplasmic *Citrus leprosis virus* (CiLV-C) is one of the causal agents of citrus leprosis disease (CiLD) in South and Central America. CiLV-C was reported from Colombia in 2006. In 2011, Valencia and Navel orange trees affected with CiLD from La Libertad and Villavicencio, Colombia were sent to the USDA-APHIS-PPQ-CPHST, Beltsville, MD, USA for testing. Antisera against known CiLV-C failed to react with CiLD samples from these areas in ELISA tests. In addition, CiLV-C RT-PCR specific primers also failed to produce amplicons. However, virus like particles similar to CiLV-C was observed in the cytoplasm of the infected leaves utilizing transmission electron microscopy. Total RNA was isolated from symptomatic and healthy leaves and was sent to Fasteris, S.A. (Plan des Ouates, Switzerland) where siRNAs were isolated and analyzed by deep sequencing with the Illumina GA IIX platform. Potential virus sequences were identified by subtracting the citrus genome sequences and known plant virus sequences from the pool of reads. Sequence reads between 15-35 nt in size were assembled by tiling using the Velvet and Oases sequence programs. The contigs were compared with the NCBI non-redundant protein database with blastx. The complete nucleotide sequence and genomic organization of this new CiLV-C associated with CiLD in Colombia was determined. The viral genome is composed of RNA1 (~8.8 Kb) and RNA2 (~5.0 Kb) and is structurally identical to CiLV-C isolates from Brazil and Panama. Both of the RNAs of the new Colombian CiLV-C showed <70% nucleotide identities with known CiLV-C sequences and appear to be a novel virus infecting citrus and causing symptoms similar to citrus leprosis.

Novel *Pseudomonas syringae* strains associated with leaf spot diseases on watermelon (*Citrullus lanatus*) and squash (*Cucurbita pepo*) in California

I. RUBIO (1), H. Bouzar (2), T. M. Jardini (3), S. T. Koike (4), C. T. Bull (3)
(1) California State University-Monterey Bay, Salinas, CA, U.S.A.; (2) Sakata Seed America, Inc., Salinas, CA, U.S.A.; (3) USDA–ARS, Salinas, CA, U.S.A.; (4) University of California Cooperative Extension, Salinas, CA, U.S.A.
Phytopathology 102:S4.103

In 2006 and 2011, bacteria, fluorescent on KMB, were isolated from leaf spots on greenhouse-grown watermelon (*Citrullus lanatus*) and field-grown squash (*Cucurbita pepo*), in coastal California. Biochemical characterization of the isolates indicated that they belonged to *Pseudomonas syringae*. Multilocus sequence typing (MLST) of four housekeeping genes revealed that these isolates were members of genomospecies 1 and were distinct from fluorescent pseudomonads previously identified as cucurbit pathogens. The BLAST tool in the Plant-Associated Microbes Database was used to compare gene sequences of the isolates to those in the public database. Gene sequences from the watermelon and squash isolates were similar but different from the gene sequences of *P. syringae* pv. *aceris* or *P. syringae* pv. *solidagae* and *P. syringae* pv. *papulans*, respectively. Additionally, DNA fragment banding patterns for the isolates generated by rep-PCR using the BOXA1R primer were distinct from members of genomospecies 1 and the pathotypes of fluorescent pseudomonads pathogenic on cucurbits. These data indicated that novel *P. syringae* isolates in genomospecies 1 were associated with the foliar diseases on watermelon and squash. Further taxonomic data are needed, including host range evaluations, to determine if these isolates are variants of previously named pathovars or represent new pathovars.

Extent of variability of the internal transcribed spacer region within *Phakopsora pachyrhizi*

T. A. RUSH (1), B. Kennedy (1), A. McTaggart (1), G. Heller (1), M. Toome (1), G. L. Hartman (2), R. W. Schneider (1), M. C. Aime (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.; (2) USDA-ARS, University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.103

Phakopsora pachyrhizi, causal agent of soybean rust, was first discovered in the U.S. in 2004. This pathogen has devastated soybean crops worldwide, leading to concern that the U.S. soybean crop would be at risk. A large spore dispersal study was conducted by others, which utilized a qPCR detection assay based upon the internal transcribed spacer (ITS) rDNA locus of the pathogen. However, this assay apparently did not always detect pathogen spores in rain water collected from areas with high disease incidences. The goal of the present project was to assess infraspecific variation within the ITS of *P. pachyrhizi*, especially within the region of the locus where the existing detection assay primers and probes were placed. Numerous samples of infected soybean and kudzu leaves were collected from the southern U.S. from which the ITS locus was cloned and sequenced. Our ITS sequences were then compared to those generated by other studies and deposited in GenBank. We found an unexpectedly large amount of infraspecific variation, including the presence of divergent ITS copies within single leaf samples in our collection. This diversity was attributable to single nucleotide polymorphisms and indels of different lengths. Our results offer a possible explanation for the apparent discrepancies in the spore dispersal study. We also conclude that asexual reproduction from a single source introduction may not be the best explanation for the level of variation observed throughout the southern U.S.

Effects of rootstock grafting on root-knot nematode (*Meloidodyne arenaria*, race 2)-infested soil

Y. RYU (1)
(1) Gyeongbuk Agricultural Research and Extension Service, Uisung-gun, Gyeongbuk, South Korea
Phytopathology 102:S4.104

Root-knot nematodes cause a significant yield loss and quality deterioration in field and green house crops. Grafting with resistant rootstock is one of control method for nematode management. To asses the effect of eggplant rootstock for root-knot nematode, rootstock cultivars,'Torvum vigor' (*Solanum torvum*), 'Taibyou VF' (*Solanum* spp.) and 'Daitaro' (*Solanum melongena*), were grafted on eggplants (*Solanum melongena* cv. *Chookyang*) and planted in root-knot nematode infested microplot in a green house and compared with non-grafted control for fruit yield, quality and plant growth. Eggplants grafted on rootstock produced 51 to 73% more fruits than non-grafted eggplant and eggplant grafted on 'Torvum vigor' had the highest fruit yield and plant growth. On the other hand, non-grafted eggplant had lower yield but had higher root weight because of heavy root gall resulted from root-knot nematode infection. Whereas, eggplant grafted on rootstock had fewer egg mass and galled roots than non-grafted eggplants. In conclusion, rootstock grafting in eggplant farming is a good alternative technique in root-knot nematode infested green houses without compromising fruit yield and also can be applicable for organic farming practice.

First report of *Cryphonectria parasitica* on chestnut in Lebanon

A. T. SAAD (1), M. Temsah (2), L. T. Hanna (1)
(1) American University of Beirut, Beirut, Lebanon; (2) Biology Department, Lebanese University, Beirut, Lebanon
Phytopathology 102:S4.104

Chestnuts *Castanea sativa* have long been cultivated on a limited scale in Lebanon. During the last ten years, however, chestnut plantings have been gaining importance on the sandy mountainous areas over 1200 meters above sea level. Last summer, growers reported the death of new plantings as well as deterioration in the health of the older chestnut trees. Affected chestnut trees showed dieback symptoms and sunken stem cankers of various sizes on the main trunks and limbs, with yellow to orange blisters. Tissues beneath the upper surface of the cankers were dead, sometimes with mycelial mats. Laboratory examinations of affected tissues revealed the presence of scattered or aggregated yellow to buff colored unilocular or complex multilocular pycnidia embedded in the bark tissue that has become particularly spongy. The conidiophores were hyaline, septate and branched. The conidia (3x1.5µm) were hyaline, unicellular, and ellipsoidal in shape. The perithecia were seen immersed in the stromatic tissues, in aggregates and were mostly oblique. Perithecia were dark brown to black, globose (300-400µm in diameter), slightly depressed with long ostiolar necks up to 800µm long and 200µm wide at the base. Ascospores (9-12x3-5.5 µm) were hyaline, two celled, rounded at their ends, smooth and slightly constricted at the septum. This is the first report of the occurrence of the quarantine chestnut blight disease in Lebanon.

Molecular characterization of an isolate of *Japanese holly fern mottle virus* from leatherleaf fern

S. SABANADZOVIC (1), R. A. Valverde (2)
(1) Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.; (2) Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.104

Several plants of leatherleaf fern (*Rumohra adiantiformis*) displaying virus-like symptoms were observed at a local nursery in Baton Rouge, LA. Double-stranded RNAs were extracted from infected tissue and analyzed by gel electrophoresis. dsRNA analyses revealed banding patterns similar to those associated with infections of Japanese holly fern with *Japanese holly fern mottle virus* (JHFMoV). Due to the worldwide economic importance of leather leaf fern (LLF) and the limited information available on fern viruses we decided to completely characterize the genome of the putative virus affecting LLF. The complete sequence of the genome confirmed that the virus from LLF is a strain of JHFMoV. While RNA2 (3006 nt) of the LLF strain is almost identical (99% identity) to RNA2 of JHFMoV, RNA1 (6231 nt) shared only 88% of the nucleotide sequence. Specific differences between isolates of JHFMoV isolated from holly fern and those infecting leatherleaf fern were observed in the putative protein product encoded by ORF1b, which could be involved in host specificity. The economic importance of this virus for the fern industry is yet to be determined.

Examining normalized changes in the expression of selected genes in pea in response to infection by *Aphanomyces euteiches*

G. SAHA (1)
(1) Department of Crop and Soil Sciences, Washington State University, Pullman, WA, U.S.A.
Phytopathology 102:S4.104

The most globally destructive disease of pea is root rot caused by the soil borne oomycete *Aphanomyces euteiches*. Commercial cultivars have limited tolerance to Aphanomyces root rot and can suffer extensive losses when planted in infested fields. Tolerance to the pathogen is conditioned by several genes and their environmental and epistatic interactions. Associating distinct disease reactions with differential expression of specific genes would provide robust evidence of gene function. Our objective was to examine gene expression in the *Aphanomyces*-tolerant PI accession 90-2079 and the susceptible cultivar Dark Skin Perfection (DSP). The expression of 39 selected genes was examined using real time PCR (Sybr Green) at 1, 2, 4 and 6 days after inoculation of plants with *A. euteiches.* Several genes, including disease resistant response protein 206 and B-1,3 glucanase-2 were expressed at significantly higher levels in inoculated plants than in healthy controls. Conversely, a gene for ethylene responsive element binding protein was down regulated in inoculated 90-2079 (tolerant) at all time points. Translation initiation factor (TIF) and 18S rRNA genes were determined to be suitable reference genes for normalizing real time PCR gene expression data obtained from pea subjected to infection by *A. euteiches*. Work will continue to identify genes differentially expressed in tolerant reactions and validate possible patterns of differential gene expression across a range of pea genotypes.

Comparative genome analysis of members of the Magnaporthaceae using DSYND, a novel syntenic detection tool

J. SAILSBERY (1), B. Clay (2), C. Jackson (2), L. Ma (1), R. Dean (2)
(1) University of Massachusetts-Amherst, Amherst, MA, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.104

Magnaporthe oryzae (rice blast disease), *Gaeumannomyces graminis* var. *tritici* (take-all disease in grasses including wheat), and *Magnaporthe poae* (summer patch on turf grasses) are three closely related pathogenic fungi with vast economic importance. Comparative genomics provides a powerful method to rigorously evaluatethe genetic and evolutionary basis of structure-functional relationships associated with these pathogens. We conducted a genome-scale comparative study across these three genomes, including refined genome annotation, RNA transcriptional analyses, and protein homology analyses. Further, we developed novel software (DSYND) to identify syntenic regions without reliance on gene models. We found that *M. poae* and *G. graminis* were more closely related than to *M. oryzae*, sharing a higher number of homologs, more conserved gene function, larger syntenic regions and exhibited more dynamic transcriptional profiles.

DSynD: Dimensional synteny detection, identification of syntenic regions between multiple genomes

J. Sailsbery (1), B. CLAY (2), C. Jackson (2), D. Brown (2), L. Ma (1), R. Dean (2)
(1) University of Massachusetts-Amherst, Amherst, MA, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.104

DSynD is a software package that can detect regions of syntenic DNA between multiple large genomes simultaneously. The software allows many user options, such as defining the preferred syntenic region size and complexity. DSynD assigns each syntenic region a probability and determines those that are the most significant. The measures used to determine probability compensate for the effects of repetitive DNA. DSynD determines syntenic regions based entirely on homologous DNA and thus requires no gene models and makes no assumptions with regards to gene order or orientation. Here, we present two test cases (Sordariomycetes and Hominidae) using DSynD, each comparison involving three related species. DSynD is released as an open-source software package and is available at http://www.fungalgenomics.ncsu.edu.

Comparative analysis of techniques for detection of quiescent *Botrytis cinerea* in grapes by quantitative PCR

S. SAITO (1), L. Cadle-Davidson (2), W. F. Wilcox (1)
(1) Cornell University NYSAES, Geneva, NY, U.S.A.; (2) USDA-ARS, Grape Genetics Research Unit, Geneva, NY, U.S.A.
Phytopathology 102:S4.104

Quantitative PCR (qPCR) can be used to detect and monitor pathogen colonization, but early attempts to apply the technology to quiescent *Botrytis*

cinerea infections of grape berries identified some specific limitations. In this study, four DNA extraction methods, two tissue-grinding methods, two grape (*Vitis vinifera*) organs, two probe sets and two enzymes were compared in order to improve the sensitivity of *B. cinerea* detection in grapevine. Furthermore, duplex qPCR for concurrent detection of *B. cinerea* and *V. vinifera* DNA was developed as a check against false negative assays, and to establish a Pathogen Coefficient (PC) that addresses variability among samples caused by DNA quality, PCR efficiency and pipetting error. The resulting optimized qPCR technique was highly sensitive, providing a Ct value < 33 for as little as 0.001 ng of *B. cinerea* DNA, and could be applied as a tool to monitor quiescent *B. cinerea* infections in vineyards.

WITHDRAWN

Integrating grain harvesting and preharvest management strategies to minimize losses due to Fusarium head blight and deoxynivalenol in wheat
J. D. SALGADO (1), P. A. Paul (1), K. T. Willyerd (1), L. V. Madden (1)
(1) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.105

The integration of fungicide application, host resistance, and cultural practices is the most effective approach for managing Fusarium Head Blight (FHB) and deoxynivalenol (DON) in wheat. However, under favorable conditions, even when the best pre-harvest management practices are used, Fusarium damaged kernels (FDK) and DON contamination of grain cannot be avoided. Grain harvesting strategies have also been recommended as a way of reducing losses due to FHB and DON. In 2011, the influence of varying combine harvester configurations on FDK and DON was evaluated as part of an integrated management approach. Plots of two moderately resistant and two susceptible cultivars were treated with the fungicide Prosaro (475 ml/ha) at anthesis and then inoculated with a spore suspension of *Fusarium graminearum* 36 hours after. Non-inoculated and non-treated plots were used as checks. FHB intensity was rated at soft dough, and FDK, DON, grain yield and test weight were quantified at harvest. Two different combine harvester configurations (C1, the default, and C2, modified to increase air flow through the combine) were used to harvest different subsets of the plots of each resistance x treatment combination. The main and interaction effects of fungicide and cultivar were significant for all measured responses. C2 resulted in numerically lower FDK and DON, and significantly higher test weight than C1. Together, resistance, fungicide, and C2 resulted in the highest test weight.

Role of a putative amino acid transporter in fungal disease resistance in alfalfa
D. SAMAC (1), M. Dornbusch (1), D. Foster-Hartnett (2), Z. Tu (2), S. Gantt (2)
(1) USDA-ARS, St. Paul, MN, U.S.A.; (2) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 102:S4.105

Accessions of the model legume *Medicago truncatula*, a close relative of alfalfa, were identified that are resistant to several foliar pathogens and microarray technology was used to identify genes specifically expressed in the resistance response. A large proportion of the up-regulated genes had only weak similarity to known genes or had no significant matches in the database. Transgenic alfalfa (*M. sativa*) plants were produced that expressed interfering RNA (RNAi) constructs of selected genes to evaluate their role in disease resistance. Expression of RNAi resulted in a 60-98% reduction in specific transcripts. Down-regulation of a putative transmembrane lysine-histidine transporter (LHT) and hydroxyisoflavanone dehydratase (HID) resulted in susceptibility to the anthracnose pathogen *Colletotrichum trifolii*. HID catalyzes the production of formononetin, a precursor in synthesis of the phytoalexin medicarpin. Expression profiling of an LHT RNAi line using RNA-seq with and without inoculation with *C. trifolii* found mis-regulation of genes involved in secondary metabolism, carbohydrate metabolism, protein modification, transport, and amino acid metabolism. Down-regulation of early nodulin 12A and a putative BZIP transcription factor had no effect on resistance to *C. trifolii*. These results provide further evidence for a critical role of isoflavonoid compounds in disease resistance and identify a novel gene involved in resistance to fungal pathogens in alfalfa.

A potential multidrug ABC transporter gene from field isolates of *Sclerotinia homoeocarpa* involved in propiconazole resistance
H. SANG (1), J. Hulvey (1), J. T. Popko (1), G. Jung (1)
(1) University of Massachusetts, Amherst, MA, U.S.A.
Phytopathology 102:S4.105

An ABC-G transporter gene potentially involved in multi-drug efflux, *ShABC1*, was identified from RNA-Seq data of *Sclerotinia homoeocarpa*. Analysis of the *ShABC1* amino acid sequence indicates that *ShABC1* is a homolog of *PMR1*, a multi-drug resistance ABC transporter from *Penicillium digitatum*. In a previous study, overexpression of an ABC-G transporter gene (*ShatrD*) in *S. homoeocarpa* was associated with propiconazole insensitivity. For assessing the involvement *ShABC1* in propiconazole sensitivity, the relative expression (R.E.) of *ShABC1* was assayed with quantitative Real-Time PCR before and after treatment of propiconazole (0.1 µg ml^{-1}) for 1h in a panel of eight isolates from five sites. Constitutive R.E. of *ShABC1* in two sets of 8 isolates sampled from two golf courses with previously confirmed practical field resistance were also assayed. Propiconazole insensitive isolates significantly overexpressed *ShABC1* before and after treatment. The linear relationship between $\log_{10}$ R.E. of *ShABC1* and propiconazole EC_{50} was highly significant ($P < 0.0001$). *ShABC1* and *ShatrD* expression levels were compared in two insensitive isolates after propiconazole treatment at varying concentrations (0, 0.1, 1, and 5 µg ml^{-1}). R.E. of *ShABC1* was significantly higher than R.E of *ShatrD* at the 5 µg ml^{-1}. R.E. of eight isolates in the panel were assayed 1h after exposure to boscalid at 10 µg ml^{-1} to investigate their potential roles in multi-drug resistance. R.E. of *ShABC1* was significantly induced within insensitive isolates in response to boscalid, however, *ShatrD* was not. We hypothesize that both genes might play similar roles in propiconazole sensitivity, but may each have unique substrate specificities.

First approach to molecular phylogeny within the genus *Phyllachora*
M. D. Santos (1), M. E. Fonseca (2), L. S. Boiteux (3), J. C. DIANESE (1)
(1) Universidade de Brasília, Brasília, DF, Brazil; (2) Embrapa Hortaliças, Brasília, DF, Brazil; (3) Embrapa Hortaliças/Universidade de Brasília, Brasília, DF, Brazil
Phytopathology 102:S4.105

A collection of phyllachoraceous fungi associated with host members of the family Myrtaceae was established and deposited at the Herbarium UB (Mycological Collection). Total DNA was extracted, and the ITS1-5.8S-ITS2 genomic region was amplified via polymerase chain reaction with universal primers. Direct sequencing of the amplicon was performed and analysed using the SeqMan and Megalign programs (Lasergene Package). Phylogenetic analyses were performed using PAUP (version 4.0b10; Sinauer Associates). Heuristic searches for the most-parsimonious trees were conducted with random stepwise addition (1,000 replications) and branch swapping algorithm using tree bisection-reconnection. Clade stability was assessed using 1,000 bootstrap replications. So far, there is no other *Phyllachora* ITS sequence data available in GenBank. Therefore, *Xylaria* (GenBank GU324757) was used as outgroup. Two *Phyllachora* clades were identified with high bootstrap support. Clade #1 showed *Phyllachora* species associated with species in host genera *Myrcia*, *Psidium*, and with *Eugenia punicifolia*. Clade #2 included *Phyllachora* species associated only with *Myrcia* species. Additional analyses with a broader collection of *Phyllachora* samples combined with SSU mitochondrial DNA and 18S DNA are underway to confirm the phylogenetic tree of the myrtaceous-parasitic members of the genus *Phyllachora*.

Characterization of glycoside hydrolase-producing bacteria isolated from Thailand soils
S. SARAIHOM (1), D. Kobayashi (2), P. Lotrakul (1), S. Prasongsuk (1), D. Eveleigh (2), H. Punnapayak (1)
(1) Chulalongkorn University, Bangkok, Thailand; (2) Rutgers University, New Brunswick, NJ, U.S.A.
Phytopathology 102:S4.105

Biological control strains of *Lysobacter* and *Stenotrophomonas* spp. are known for their ability to produce extracellular lytic enzymes and antibiotics. While several strains belonging to these genera have been isolated from a broad range of geographically distinct habitats, most have been obtained within temperate climates. To determine their prevalence in tropical climates, soil collected from different areas of Thailand were sampled for the presence of *Lysobacter* and *Stenotrophomonas* spp. Initial isolation procedures utilized a fungal mycelial baiting method and an enrichment culture broth procedure that used a minimal salts medium with fungal mycelium as a sole carbon source. Isolated bacteria were further selected based on morphological and physiological phenotypes consistent with the genera, including production of extracellular lytic enzyme activities. After initial screens, nine bacterial isolates were found to be taxonomically related to *Lysobacter* or *Stenotrophomonas* spp. as determined by 16S rRNA gene sequence analysis. Two strains, R06 and R08, displayed high levels of cellulase activities using carboxylmethylcellulose and filter paper as substrates compared with other isolated strains. Characterization of the molecular basis of these activities is currently in progress.

USABlight and fungicide sensitivity of recent genotypes of *Phytophthora infestans* to oomycete-targeted compounds

A. Saville (1), C. Pearce (1), J. B. RISTAINO (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.106

Phytophthora infestans causes potato late blight, an important and costly disease of potato and tomato crops. USABlight (www.usablight.org) has been launched to provide a communication medium between research and extension faculty, growers, the industry and the public about late blight. Visitors can learn about disease symptoms, examine current and past disease reports, learn about disease management options, and submit samples for genotyping and fungicide testing. We have begun screening recent genotypes of *P. infestans* for sensitivity to fungicides. Nineteen isolates of *P. infestans* from locations in the US representing the current genotypes were collected between 2009 to 2011. Isolates were tested for sensitivity to a range of concentrations of mefenoxam, fluopicolide, cyazofamid, or etridiazole on rye V-8 media and allowed to incubate at 18°C in the dark for two weeks. The percentage growth compared to a nonamended control was determined and plotted against the log of the fungicide concentration to determine the mean EC_{50}. The baseline mean EC_{50} values for fluopicolide were lower for US-22 (0.14 mg/L) than for US-8 (0.43 mg/L). The relative sensitivity of the common US genotypes of *P. infestans* to each fungicide will be presented.

Observations on blueberry leaf rust, caused by *Thekopsora minima*, in Michigan

A. C. SCHILDER (1), T. D. Miles (1), J. M. Gillett (1), R. W. Sysak (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.106

Over the past few years, leaf rust has become widespread in highbush blueberries in Michigan, sometimes resulting in premature defoliation. Symptoms are most common in late summer and fall. Lesions are small and yellow at first and then turn dark brown with yellowish orange uredinia on the abaxial surface. Uredinia are dome-shaped, erumpent, and 100 to 400 µm in diameter. Urediniospores are yellow, obovate, and 19 to 25 × 16 to 20 µm (ave. 22 × 18 µm). Spore walls are hyaline, echinulate, and 1 to 1.5 µm thick with obscure germ pores. Based on morphology and DNA homology with known isolates, the pathogen was identified as *Thekopsora minima*. This fungus has also been reported on highbush blueberry in Japan, South Africa, Delaware, and New York. In Michigan, the pathogen was previously assumed to be *Pucciniastrum vaccinii*. Hemlock (*Tsuga canadensis*), the alternate host, is a common and valuable conifer in the Michigan landscape. No aecia were seen on hemlocks near infected fields but it may have been too late in the season to observe them. Susceptibility of 12 blueberry cultivars was assessed in a young planting, Cultivar Bluecrop was the most susceptible, followed by Liberty, Rubel and Jersey. Aurora, Berkeley, and Huron were the most resistant. A fungicide efficacy trial showed that Pristine (pyraclostrobin + boscalid) had excellent activity; Indar (fenbuconazole), Orbit (propiconazole), and Quash (metconazole) had good activity. Serenade (*Bacillus subtilis*), Sonata (*B. pumilis*) and Bravo (chlorothalonil) had moderate activity.

Rainfastness of fungicides on grape leaves

A. C. SCHILDER (1), J. M. Gillett (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.106

Grape growers regularly use fungicides that are subject to wash-off by rain. However, the removal of fungicides from grape tissues by rain has not been well-quantified. Rainfastness of the following fungicides: Dithane (mancozeb), Ziram (ziram), Captan (captan), Abound (azoxystrobin), Pristine (pyraclostrobin + boscalid), Prophyt (potassisum phosphite), and Procure (triflumizole) was studied by exposing treated grape leaves to simulated rainfall of 0, 0.1, 0.5, 1.0, and 2.0 inches. In general, fungicides were not substantially removed until about 1 to 2 inches of rainfall, as measured by residue analysis and disease control efficacy using *Phomopsis viticola* as a test pathogen. Surprisingly, systemic fungicides were also washed off, despite the assumption of greater rainfastness. However, at low rainfall amounts, systemic fungicides were more rainfast than protectant fungicides. Effects of fungicide age (1 or 7 days) or addition of an spreader-sticker (Nu-Film P) were not consistent. In general, disease control efficacy of remaining residues was better than the actual residue amount would suggest. Further research is needed to determine if rainfall intensity and frequency play a role.

WITHDRAWN

Survey of wood decay fungi of *Casuarina equisetifolia* (ironwood) on the islands of Guam and Saipan

R. L. SCHLUB (1), R. C. Mendi (1), C. C. Aiseam (1), R. C. Mendi (1), J. K. Davis (1), M. C. Aime (2)
(1) University of Guam, Mangilao, Guam; (2) Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.106

As a result of statistical modeling of data from individual trees and tree sites, the occurrence of basidiocarps consistently emerged as the dominant explanatory variable for Guam's declining ironwood trees (*Casuarina equisetifolia*). A survey was conducted in February 2012 in the Mariana Islands to elucidate which of the known basidiocarp-forming genera are most consistently correlated with the decline. Species from five basidiomycete genera of the class Agaricomycetes, belonging to the orders Polyporales (*Ganoderma*, *Favolus*, *Pycnoporus*), Hymenochaetales (*Phellinus*) and Thelephorales (*Sarcodon*) were previously identified from Guam based on macro- and micromorphology and DNA sequencing. As a result of the February survey, *Ganoderma* sp. (*G. australe* complex) was the basidiocarp found to be most frequently associated with unhealthy trees. Conks of the fungus were commonly found on Guam where they appeared on roots and butts of declining and stumps of dead trees. On Saipan where decline does not exist and where the trees are considerably healthier, *Ganoderma* sp. was rarely found. In contrast, *Phellinus* sp. was the most widespread fruiting basidiocarp on Guam and Saipan. Though the actual species of *Phellinus* remains to be determined, it does not appear to represent *P. noxious*, and is not consistently associated with trees in decline. These and other species associated with ironwood trees in the Mariana Islands will be discussed.

Resistance to *Phytophthora* in new rootstocks for almond and stone fruits

L. S. SCHMIDT (1), R. G. Bhat (2), D. A. Kluepfel (1), G. T. Browne (1)
(1) USDA-ARS CPGRU, Davis, CA, U.S.A.; (2) University of California, Davis, CA, U.S.A.
Phytopathology 102:S4.106

Almonds and stone fruits suffer serious losses to several soilborne diseases, including Phytophthora crown and root rots. Restrictive soil-fumigation mandates are motivating the development of fruit and nut tree rootstocks with improved disease resistance. Accordingly, we tested 17 new almond/stone fruit rootstock genotypes and five widely-planted rootstocks for resistance to *Phytophthora* taxon '*niederhauserii*' (*Ptn*), an aggressive pathogen of almond in the San Joaquin Valley of California. Rootstock clones were transplanted into potting mix that was non-inoculated (control) or inoculated with *Ptn*. All plants received bi-weekly 48-hour soil flooding periods. Resistance was assessed 8 weeks after transplanting. Susceptibility to *Ptn* was relatively high among rootstocks with: peach parentage (Empyrean #1; Harrow Blood x Okinawa clones 1, 10, 28, 32, and 50; Lovell; and Nemaguard); peach x almond parentage (Bright Hybrid clones 5 and 106; Garnem; and Hansen 536); and (peach x almond) x peach parentage (Rootpac 20 and Tempropac); the mean percentages of crown rot (CR) were 37-100%. In contrast, rootstocks that included some plum parentage (Controller 5; Krymsk clones 1, 2, 9, and 86; Marianna 2624; Myrobalan; and Replantpac) were less susceptible (CR 1-30%). Our results suggest that the inclusion of some plum parentage in a rootstock's genetic background may increase resistance to *Ptn*.

Effects of application timing and rates of application of a demethylation inhibitor fungicide on Cercospora leaf blight of soybean

R. W. SCHNEIDER (1), C. L. Robertson (1), N. A. Ward (2)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.; (2) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 102:S4.107

Cercospora leaf blight (CLB), caused by *Cercospora kikuchii*, is a major disease of soybean in Louisiana and the Gulf South. Previous studies demonstrated varietal tolerance and resistance, but resistance was overcome within two years. Varieties that did well in one location showed considerable differences in other parts of Louisiana. Timing of disease onset is a key factor in crop impact, which can result in substantial reductions in yield and seed/grain quality. Previous work, in which latent infection was monitored using real-time PCR protocols, showed that infection occurred during vegetative growth stages even though symptoms were usually not observed until late reproductive stages. The purpose of this study was to evaluate rates and application timings of a demethylation inhibitor fungicide (flutriafol). Several application protocols at up to 14 oz/A were assessed including first applications at first flower (R1) continuing through late pod fill (R6). In addition, multiple applications with below-label rates were assessed. Plots were rated for disease severity at mid-R6. Findings indicated that an early application with at least 7 oz/A, applied no later than R1, will be required for effective control of CLB. Rates of less than 7 oz/A were ineffective, regardless of when they were applied or the number of applications.

Onion ipmPIPE network—Interactive resource for onion stakeholders

H. F. SCHWARTZ (1), B. K. Schroeder (2), J. VanKirk (3), K. Douce (4), G. Jibilian (5), J. Lafferty (6), G. W. Norton (7)
(1) Colorado State University, Fort Collins, CO, U.S.A.; (2) Washington State University, Pullman, WA, U.S.A.; (3) Southern Region IPM Center, Raleigh, NC, U.S.A.; (4) Center for Invasive Species & Ecosystem Health, Tifton, GA, U.S.A.; (5) Multigrain International, LLC, Fort Collins, CO, U.S.A.; (6) Planalytics, Inc., Berwyn, PA, U.S.A.; (7) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 102:S4.107

The Onion ipmPIPE Project was initiated recently with support provided by the USDA-NIFA Specialty Crop Research Initiative and matching funds from U. S. onion stakeholders. Its goal is to incorporate existing pest management programs and pest risk assessment models into an internet platform for national implementation and validation. It is also expanding innovative diagnostic tools available for priority diseases caused by various pathogens and their disease complexes, including: *Iris yellow spot virus* (IYSV); *Thrips* (emphasis on onion thrips) – as a vector and pest; and foliar and storage fungal and bacterial diseases. The Onion ipmPIPE currently consists of a network of annual sentinel plots and production surveys in 7 states. The Project Web site includes a series of menus, maps, reports, illustrations, and management links for topics that include: *Allium* Crops, Diseases, Insect Pests, Forecasts and Market Pricing Tools. It emphasizes IPM strategies including selection of disease resistant varieties, planting clean seed, suitable crop rotation, scouting and confirmation of economic threats from disease organisms and insect pests, and timely application of pesticides as needed.

Root-knot nematode infection rates are reduced in roots of tobacco engineered to express RNAi targeted to a nematode parasitism gene

K. SCHWERI (1), G. Huang (2), M. G. Mitchum (3), T. J. Baum (4), R. S. Hussey (2), R. Lewis (1), E. L. Davis (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) Univeristy of Georgia, Athens, GA, U.S.A.; (3) University of Missouri, Columbia, MO, U.S.A.; (4) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.107

Root-knot nematodes (RKN), members of the genus *Meloidogyne*, cause severe economic losses in multiple crop species, including *Nicotiana tabacum*. RKN invade plant roots and transform selected plant cells into multinucleate feeding cells using effector proteins that are produced in specialized esophageal gland cells and secreted from the nematode stylet into the host plant root cells. Previous studies have shown that the 16D10 parasitism gene transcript can be silenced using host-derived RNA interference (RNAi), and *Arabidopsis thaliana* plants that expressed 16D10 double-stranded RNA (dsRNA) were highly resistant to all four major RKN species. Using the same 16D10-RNAi constructs developed for *Arabidopsis*, two haploid lines of *Nicotiana tabacum* were transformed and midvein tissue culture of mature leaves was used to produce double-haploids. T1 tobacco progeny were produced through self-fertilization. When *M. arenaria* and *M. incognita* where inoculated onto roots of T1 16D10-RNAi tobacco plants, their egg production was significantly reduced as compared wild-type plants. Correlation analyses of 16D10 small RNA expression levels in transformed tobacco with nematode infection severity are now in progress. New constructs using different promoters and different introns for the RNAi hairpin have been created and are currently being transformed into both tobacco and *Arabidopsis* in an attempt to improve siRNA production and further reduce RKN infection of tobacco roots.

Solanaceous weeds as potential hosts for new clonal lineages of *Phytophthora infestans*

A. C. SEIDL (1), A. J. Gevens (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.107

Weed hosts of *Phytophthora infestans* in the US have been noted, but the potential of new clonal lineages to infect common solanaceous weeds, which may influence the epidemiology of potato and tomato late blight, has not been elucidated. Representative isolates of the clonal lineages US-22, US-23, and US-24 collected in WI in 2009 and 2010 were used to inoculate wounded or intact fruits and detached leaves of black nightshade (NS), hairy NS, bittersweet NS and 'Juliet' tomato. Sporulation incidence on fruit was low for all hosts and lineages, except for wounded black NS fruits inoculated with US-22 (39%). This overall low fruit incidence may still be important due to the large number of fruits produced and their ability to persist beyond foliage senescence. Mycelial density, averaged across lineages, was low on infected black NS and bittersweet NS leaves (<17%) but high on hairy NS (89%), which was statistically similar to tomato. To further compare hosts, sporangial production by US-22, US-23, and US-24 on leaf disks of 'Brandywine Red' tomato, 'Katahdin' potato and hairy NS was quantified. On hairy NS sporangial production by US-23 was significantly greater than by US-24, but across lineages was not significantly different than potato. This work demonstrates the potential of new clonal lineages of *P. infestans* to infect and sporulate on fruits and leaves of common solanaceous weeds, which may have important epidemiological consequences for late blight management.

Phytophthora crown rot of strawberry: Cultivar resistance and chemical and cultural control

T. SEIJO (1), J. Mertely (1), M. Oliveira (1), V. Whitaker (1), N. A. Peres (2)
(1) University of Florida, GCREC, Wimauma, FL, U.S.A.; (2) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 102:S4.107

Fungicides were evaluated on strawberry plants inoculated with *Phytophthora cactorum* for control of Phytophthora crown rot (PhCR) for 3 seasons in Florida. Ridomil Gold, phosphite products and Presidio were all effective, but Ridomil Gold and Kphite (phosphite) controlled the disease most effectively. Isolates of *P. cactorum* collected in the 2011-12 season were evaluated for resistance to Ridomil Gold, the current industry standard for treatment of PhCR. No resistance was detected. Various strawberry cultivars and advanced selections of the UF breeding program were evaluated over 2 seasons for susceptibility to *P. cactorum*. 'Strawberry Festival' and 'Treasure' were the most resistant, with final percent mortalities of 0 to 1%. 'Florida Radiance' and 'Winterstar' were the most susceptible with final mortalities reaching 69%. In Florida, strawberries are grown annually from bare-root, green-top transplants. Transplants dug too early in the nurseries (smaller, weaker plants) may be more susceptible to PhCR. The most susceptible cultivars, 'Florida Radiance' and 'Winterstar', each had more disease in transplants dug in September rather than October during one of two seasons in which dig dates were evaluated. *P. cactorum* comes on infected transplants. Some growers were concerned that recycling irrigation water from infected fields might infect healthy transplants in adjacent fields, however, no *P. cactorum* was detected in 96 water samples from 4 infected commercial farms over a 6-week period.

Fungi isolated from diseased inflorescences of mango in Puerto Rico

L. M. SERRATO-DIAZ (1), L. I. Rivera-Vargas (2), E. I. Latoni-Brailowsky (2), R. D. French-Monar (1)
(1) Department of Plant Pathology and Microbiology, Texas AgriLife Extension Service, Texas A&M System, Amarillo, TX, U.S.A.; (2) Department of Crops and Agro-Environmental Sciences, University of Puerto Rico, Mayaguez, Puerto Rico
Phytopathology 102:S4.108

Mango (*Mangifera indica* L.) is a tropical fruit tree native to southern Asia. Limited studies have been conducted on fungal pathogens of the inflorescences. During 2009, a survey was conducted at the mango germplasm collection located the University of Puerto Rico's Experiment Station in Juana Diaz. Diseased inflorescences from cultivars 'Haden' and 'Irwin' were superficially disinfected and plated onto acidified potato dextrose agar. A total of 205 fungal isolates were identified morphologically and by PCR amplification of the ITS region of the rDNA. *Albonectria* sp., *Alternaria* spp., *Curvularia pseudorobusta*, *Botryosphaeria* spp., *Cochliobolus lunatus*, *Colletotrichum gloeosporioides*, *Fusarium* spp., *Neofussicoccum* sp., *Nigrospora oryzae*, *Phoma* sp., and *Phomopsis* sp. were the most common fungi isolated. *Albonectria* sp. and *Botryosphaeria* spp. were the most pathogenic on 'Haden' causing 100% necrosis of the inflorescences 8 days after inoculation (DAI), while 'Irwin' had 17% necrosis. *Albonectria* sp. caused vascular discoloration and flower necrosis along the rachis. Inflorescences blight and rachis necrosis were observed when inoculated with *Botryosphaeria* spp. On 'Irwin' *Phomopsis* sp. and *Fusarium* sp. were the most pathogenic causing 100% of inflorescence rot and wilt at 5 and 8 DAI, respectively. On 'Haden', *Phomopsis* sp. was less virulent, causing 16% of inflorescence rot. At 8 DAI, *Phomopsis* sp. produced necrotic spots and flower abortion on both cultivars. On the rachis of 'Irwin', *Phoma* sp. caused cankers, *Fusarium* sp. caused rotting and wilting, and *Alternaria* sp. caused necrotic spots. The other fungi were not pathogenic, but the potential exists for pathogenicity to occur on other cultivars.

Regulation of glycohydrolase family proteins from *Xanthomonas citri* subsp. *citri* in xylan utilization during pathogenesis

D. SHANTHARAJ (1), G. V. Minsavage (1), G. Nong (2), V. Chow (2), J. F. Preston (2), J. B. Jones (1)
(1) Plant Pathology Department, University of Florida, Gainesville, FL, U.S.A.; (2) Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.108

Xanthomonas citri subsp. *citri* (*Xcc*), causal agent of citrus canker, secretes various enzymes which degrade plant cell wall components; importantly, xylanases that hydrolyze xylan. Regulation of different cell wall degrading enzymes during pathogenesis remains to be understood. Xylan is a polymer consisting of a linear backbone of 1,4-linked D-xylanopyranosyl residues and side chains of arabinose, glucuronic acid, or methylglucuronic acid. In this study, the role of xylanase as in virulence of *Xcc* 306 strain has been deciphered using the available genome sequence. The *Xcc* 306 genome contains one operon constituting *xyn10A*, *xyn10B*, and *xyn10C* genes that encode three endoxylanases of glycoside hydrolase family 10 (GH10) and a second operon with *ara43* gene encoding a putative GH43 α-L-arabinofuranosidase and an *agu67* gene encoding a GH67 α-glucuronidase. Regulation of the three GH families during xylan utilization was assessed by developing an *in vitro* xylanase activity assay and testing different combinations of deletion mutants for their overall effect. Similarly, contribution of these GH family genes towards pathogenesis was tested by quantifying *in planta* bacterial growth and differences in canker lesion development during the course of infection. Deletion mutants of GH10 genes showed a marginal reduction in lesion development and lower bacterial population compared to the wild type. The study reveals regulation of GH family proteins during *Xcc* pathogenesis.

Transcript profiling of CYP83B1 at different level of infection by *Alternaria brassicae* and *Alternaria brassicicola* in cauliflower

P. SHARMA (1), M. Sharma (1), S. Deep (1), D. Singh (1), D. Singh (1)
(1) Indian Agricultural Research Institute, New Delhi, India
Phytopathology 102:S4.108

CYP83B1, a CytochromeP450 member, a branch point enzyme connects metabolic flux of two important pathways Auxin and Glucosinolates biosynthesis. Necrotrophic fungi induce damage in the host and causes inability to down-regulate the auxin pathway thus increasing the plant susceptibility to pathogen attack. Significant reduction in radial growth pattern and sporulation count was seen >500 ppm auxin concentration in two isolates of *Alternaria brassicae* (CaAbD2) and *Alternaria brassicicola* (CaAbcR6) with respect to control. Expression profiling of CYP83B1 was performed using semi-quantitative RT-PCR of both isolates grown on supplemented media with IAA ranging from100 ppm-900 ppm. There was a marked increase in CYP83B1 expression at in *Alternaria brassicae* till 900 ppm. On the other hand decrease in expression was observed from 100 ppm to 800 ppm with a significant up regulation at 900 ppm of IAA concentration in *A. brassicicola*. As per biochemical estimation (HPLC) of IAA in infected two months old cauliflower leaves (Ab and Acola), a decreased trend of IAA was observed twenty days after infection compared to healthy leaf, indicating abiotic stress on the infected leaves. Varied gene expression of CYP83B1 was observed by quantitative Real time RT-PCR.

Reaction of lines of *Arabidopsis* and the Rapid Cycling Brassica Collection to Canadian pathotypes of *Plasmodiophora brassicae*

K. Sharma (1), B. D. Gossen (2), M. MCDONALD (1)
(1) Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada; (2) Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada
Phytopathology 102:S4.108

Clubroot, caused by *Plasmodiophora brassicae*, is a serious constraint to canola (*Brassica napus*) production in areas of the Canadian prairies. The objective of this study was to assess the reaction of lines of *Arabidopsis thaliana* and the Rapid Cycling Brassica Collection (RCBC) to the pathotypes of *P. brassicae* present in Canada. The clubroot reaction of 86 lines of *Arabidopsis* and 5 lines of RCBC (*B. carinata*, *B. juncea*, *B. napus*, *B. oleracea*, *B. rapa*) were evaluated for their reaction to pathotypes 2, 3, 5 and 6 (William's system). A highly susceptible *Brassica* vegetable (Shanghai pak choy cv. Mei Qing Choy, *B. rapa* var. *communis*) was included as a control. Seedlings were grown individually in soil-less mix, inoculated with 3 x 106 resting spores of *P. brassicae*, and maintained at 25°/20°C day/night. Seedlings were assessed for clubroot incidence and severity at 6 wks after inoculation using a 0–3 scale. In *Arabidopsis*, most of the lines were susceptible to each of the pathotypes and no line was resistant to all of the pathotypes assessed. Lines with a differential reaction to pathotype were generally moderately resistant, rather than immune. The RCBC lines displayed a strong differential response to the pathotypes. These results indicate that lines of *Arabidopsis* and RCBC may have potential for use in a new differential set to characterize Canadian pathotypes of *P. brassicae*.

Proficiency test results summary for the 2011 National Plant Protection Laboratory Accreditation Program (NPPLAP) citrus greening (huanglongbing) detection assay

P. SHIEL (1), V. Mavrodieva (2), G. Dennis (1)
(1) USDA APHIS PPQ CPHST, Raleigh, NC, U.S.A.; (2) USDA APHIS PPQ CPHST, Beltsville, MD, U.S.A.
Phytopathology 102:S4.108

The USDA-PPQ-CPHST NPPLAP has been providing proficiency test (PT) panels developed and validated for real-time PCR detection of citrus greening's causal agent '*Candidatus* Liberibacter asiaticus' (HLB) since 2007. NPPLAP engages diagnostic laboratories in the National Plant Diagnostic Network, individual State Departments of Agriculture, or within the USDA capable of providing testing services for USDA regulatory programs. Panel distribution date is chosen by the participants based on their scheduling needs and are allowed 3 weeks to complete testing and submit results. A participant test result macro analysis based on generated quantitative cycle time (C_q) values was assembled for the 2011 HLB season. Analyses included participant results submission (14 ± 7 days) and NPPLAP review time (14 ± 9 days), instrument inventory survey, and panel member performance. Panel members were analyzed for repeatability, reproducibility, and specificity. Coefficient of variance (CV) ranged between 0.6 and 11.3%, and specificity ranged between 93-100%. Results were reproducible between program C_q values and participant values as surveyed by Pearson's test ($p > .05$). Response times indicated that the allotted testing period was adequate, and the reviews were done in a timely manner. Macro analysis of the HLB season 2011 program resulted in confirming the PPQ approved HLB real time PCR assay was repeatable, reproducible, and specific for its intended targets when used by multiple operators in laboratories at a national scale.

Identification of multiple virulence QTL in *Pyrenophora teres* f. *teres* associated with net form net blotch in barley

R. A. SHJERVE (1), Z. Liu (1), J. D. Faris (2), J. B. Rasmussen (1), T. L. Friesen (2)
(1) North Dakota State University, Plant Pathology Department, Fargo, ND, U.S.A.; (2) Cereal Crops Unit, Northern Crops Science Laboratory, USDA-ARS, Fargo, ND, U.S.A.
Phytopathology 102:S4.108

The necrotrophic fungal pathogen *Pyrenophora teres* f. *teres* (Ptt) causes the foliar disease net form net blotch (NFNB) on barley (*Hordeum vulgare*).

Several host genes that confer susceptibility to Ptt have been identified. The pathogen likely produces multiple necrotrophic effectors (host-selective toxins) that induce effector-triggered susceptibility. Here, we investigated the genetics of virulence in the barley-Ptt pathosystem by using 118 progenies derived from a cross between the Ptt isolates 15A and 6A. The barley lines Rika, Kombar, Hazera, CI11458, TR306, PI356715, and PI399482, which differ in their reactions to 15A and 6A, were evaluated for NFNB caused by the 15A × 6A progeny. Genetic maps generated by using SSR and AFLP markers in the Ptt population were scanned for quantitative trait loci (QTL) associated with virulence in Ptt. Preliminary results indicated a single common QTL conditioning virulence on Rika, CI11458, TR306, and PI399482, and a different QTL conditioning virulence on PI356715. The virulence of the fungal population on Hazera and Kombar was nearly identical and controlled by two completely different loci in the Ptt genome. Therefore, a total of four virulence loci were identified in this pathogen population. It is likely that the same numbers of unique loci are also present in the host lines used in this study.

Genetic diversity and population biology of a global collection of phytopathogenic *Verticillium dahliae*

D. P. SHORT (1), S. Gurung (1), K. Maruthachalam (1), P. Inderbitzin (2), Z. Atallah (3), F. Nigro (4), S. Benlioglu (5), K. V. Subbarao (6)
(1) University of California-Davis, Salinas, CA, U.S.A.; (2) University of California-Davis, Davis, CA, U.S.A.; (3) Hartnell College, Salinas, CA, U.S.A.; (4) University of Bari, Bari, Italy; (5) Adnan Menderes University, Aydin, Turkey; (6) University of California-Davis, Plant Pathology, Davis, CA, U.S.A.
Phytopathology 102:S4.109

Verticillium dahliae is an important soilborne phytopathogen that causes severe wilt disease on a wide range of crops. *V. dahliae* populations may survive in soil for many years, due to the formation of resistant microsclerotia. Recent work has demonstrated that *V. dahliae* is seed transmitted in some crops, and that there is evidence of recent transcontinental movement of *V. dahliae* through international seed trade. Here we investigated the genetic diversity and population structure of a global collection *V. dahliae* isolated from a variety of plant hosts. A total of 1,400 isolates were assembled from several countries including the United States, Italy, Denmark, Turkey, China and Australia. These isolates were genotyped using 12 Simple Sequence Repeat (SSR) molecular markers in order to test for differentiation among strains of *V. dahliae* collected from various geographic regions, and to test the hypothesis of global gene flow. Additionally, we characterized the mating type idiomorphs of these isolates using a multiplex PCR assay. Preliminary analyses indicated overall low genetic diversity, as certain haplotypes were frequently observed, even among geographically distant regions. The *MAT-1-1* idiomorph was confirmed in over 90% of the isolates, consistent with previous reports of the frequency of this mating type in *V. dahliae*. More detailed results of these analyses will be presented.

Population structure of *Phytophthora colocasiae* assessed using single nucleotide polymorphism (SNP) markers

S. K. SHRESTHA (1), J. Mudge (2), N. A. Miller (2), K. Lamour (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.; (2) National Center for Genome Resources, Santa Fe, NM, U.S.A.
Phytopathology 102:S4.109

Taro Leaf Blight (TLB), caused by *Phytophthora colocasiae*, severely limits the production of Taro (*Colocasia esculenta*) at many locations worldwide. Our objectives are to develop co-dominant molecular markers for *P. colocasiae* and to characterize the population structure at key locations. During 2010 and 2011, over 400 isolates of *P. colocasiae* were collected from symptomatic Taro in Hawaii, Vietnam, and Hainan Island in Southern China. A focused next generation sequencing strategy (known as Restriction-Site Associated DNA (RAD)) was applied to two isolates from Vietnam to identify potential polymorphic SNP sites. The RAD analyses resulted in almost 9M Illumina reads that were then aligned to the *P. capsici* genome (a close relative). Roughly 15% of the reads were able to be unambiguously aligned revealing >1000 potential SNP sites. A subset of the putative polymorphic sites are currently being converted to high resolution DNA melting analysis (HR-DMA) SNP assays and the populations are being analyzed. Marker development and population structure will be discussed.

The type VI secretion system in *Pantoea ananatis* plays a role in bacterial competition

D. Shyntum (1), S. N. Venter (1), L. Moleleki (1), T. COUTINHO (1)
(1) University of Pretoria, Pretoria, Republic of South Africa
Phytopathology 102:S4.109

The newly described type VI secretion system (T6SS) has been shown to play roles in virulence, antivirulence, pathogenicity and inter- and intrabacterial competition. This secretion system is believed to release effectors extracellularly or directly into other bacteria using a syringe-like secretion system. The genomes of a number of *Pantoea ananatis* strains have been sequenced and up to three different transcriptional units encoding the T6SS have been found. In this study the role of the T6SS in inter- and intrabacterial competition was investigated. Fusion PCR and Lambda Red recombination systems were used to generate mutants for *hcp*, *impA* and *clpV* and entire cluster deletions. Each of these genes was cloned and expressed from their native promoters on a low copy number plasmid to generate respective mutant complementation strains. Interbacterial assays were done by mixing a 1:1 ration of *P. ananatis* (LMG2665) with fifty-five different bacterial species and strains. Fitness was estimated based on the number of colony forming units of the competitor recovered. Results from individual loci knockouts and complementation of individual genes showed that only one locus is involved in interbacterial competition. *P. ananatis* is able to outcompete *Escherichia coli*, *Salmonella typhimurium*, *Pectobacterium carotovorum* subsp. *Carotovorum*, *Pantoea stewartii* subsp. *indologenes* and three other strains of *P. ananatis*. This ability could bestow a selective advantage to *P. ananatis* in an ecological niche and appears to be conferred by the effectors released by the T6SS.

Functional characterization of quorum sensing systems in *Pantoea ananatis*

P. Sibanda (1), J. Theron (1), T. A. COUTINHO (1)
(1) University of Pretoria, Pretoria, Republic of South Africa
Phytopathology 102:S4.109

Pantoea ananatis is a member of the Enterobacteriaceae and is capable of causing disease symptoms in a number of economically important plant hosts that include *Eucalyptus*, corn and onion. Two *N*-acyl-homoserine lactones have been reported to be produced by *P. ananatis* and shown to play a role in the pathogenicity of this pathogen in onion. In this study, three quorum sensing systems, namely, EanI/R, the RhlI/R and the LuxS, were identified in the genome sequence of the *Eucalyptus* strain LMG20103. The role of these three systems in the virulence of this pathogen has not yet been elucidated. A lambda red protocol was followed in order to delete the quorum sensing systems in LMG20103. Three mutant strains, EanI$^-$EanR$^-$, RhlI$^-$RhlR$^-$ and LuxS$^-$ were produced. Growth analysis showed that the wild-type and mutant strains had similar growth rates in nutrient-rich LB medium and M9 minimal medium. Virulence assays were conducted on onion seedlings, shown previously to be susceptible to this strain, using a stab inoculating method. A hypersensitive response, necrosis at the site of inoculation, was evident in the seedlings inoculated with the EanI$^-$EanR$^-$ and RhlI$^-$RhlR$^-$ mutant strains three days post-inoculation. Seedlings inoculated with the LuxS$^-$ mutant showed no symptoms, whereas the wild-type strain produced typical symptoms. From these results it is evident that the EanI/R and RhlIR systems are required for this pathogen to successfully colonize host tissue. The LuxS system possibly regulates a phenotype, which is crucial for the virulence of *P. ananatis*. These results thus indicate that quorum sensing is required for virulence in this plant pathogen.

PCR assays for diagnosis of postharvest fruit rots and early detection of *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* in apple fruit

P. SIKDAR (1), P. Okubara (2), M. Mazzola (3), C. Xiao (4)
(1) Washington State University, Wenatchee, WA, U.S.A.; (2) USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, WA, U.S.A.; (3) USDA-ARS, Wenatchee, WA, U.S.A.; (4) USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA, U.S.A.
Phytopathology 102:S4.109

Speck rot caused by *Phacidiopycnis washingtonensis* and Sphaeropsis rot caused by *Sphaeropsis pyriputrescens* are two recently reported postharvest diseases of apple. Fruit infection by the pathogens occurs in the orchard, but symptoms develop after harvest and are similar to that of gray mold caused by *Botrytis cinerea*. Accurate diagnosis and detection of these diseases is important to disease inspection, particularly for fruit destined for export. The aim of this study was to develop PCR assays for disease diagnosis and early detection of latent infection in apple caused by *P. washingtonensis* and *S. pyriputrescens*. Species-specific primers based on rDNA-ITS region, that amplified only the target pathogens, but not non-target fungi commonly associated with apple fruit, were developed for PCR assays. Conventional and real-time PCR assays were developed and validated using decayed fruit resulting from artificial inoculation in comparison with isolation-based assays. For wound-inoculated fruit, pathogens identified using PCR assays were consistent with those used as the inoculant. Real-time PCR assays effectively

detected latent infections in symptomless stem-end and calyx-end tissues of fruit that were inoculated with the pathogens in the orchard during the growing season. The PCR assays provide rapid, accurate methods for diagnosis and early detection of these diseases.

Xanthomonas axonopodis pv. _vasculorum_ affecting susceptible cultivar of sugarcane in Mexico

H. V. SILVA-ROJAS (1), A. Rebollar-Alviter (2), A. M. Malpica-Gutierrez (3), A. L. Cortes-Cueto (3), A. Aguilar-Granados (4), C. R. Martinez-Gonzalez (5), E. N. Zambrano-Zepeda (6), E. Molina-Gayosso (6), S. Garcia-Morales (3)
(1) Colegio de Postgraduados, Edo De Mexico, Mexico; (2) Centro Regional Morelia/Universidad Autónoma Chapingo, Morelia, Michoacan, Mexico; (3) Colegio de Postgraduados, Texcoco, Mexico; (4) Direccion General de Sanidad Vegetal, Centro Nacional de Referencia, Mexico; (5) Universidad Nacional Autonoma de Mexico, Instituto de Biologia, Mexico; (6) Universidad Politecnica de Puebla, Puebla, Mexico
Phytopathology 102:S4.110

Sugarcane (*Saccharum* spp. Hybrids) is one of the most important industrial crops in Mexico by its yield in field and factory. However, during autumn 2011, red-orange streaks arising from leaf margin and developing downwards of the leaves with discoloration to pale-yellow were observed in ITV 92-1424 cultivar in Zacatepec, Morelos. In order to determine the causal agent of those symptoms, small portions of tissue were excised, disinfested and placed on Yeast Nutrient Broth (YNB), King's B and PDA media, later incubated at 28°C. After 72 h, a fungi colony was observed in all media. However, 5 days later a yellow bacteria layer growing below mycelia was detected. Thirty five bacteria strains showed a strong yellow color on YNB medium, and colonies with slime appearance developed. They were Gram negative and nonfluorescent on King's B medium. Molecular analysis based on the amplification and sequencing of 16S rDNA indicated that strains had an identity of 100% with *Xanthomonas axonopodis* pv. *vasculorum* (Xav). Results of the phylogenetic and bioinformatic analysis clustered those strains with GU969137 and GU969138 accessions belonged to Xav from Korea. Pathogenicity of six representative strains and controls was confirmed by inoculations into 3-week-old stems of ITV 92-1424 plantlets obtained from in vitro regenerated plants. All strains developed pale yellow streaks 5 days post-inoculation. In addition, mycelia of the fungus showing a red-orange coloration on PDA medium was identified through amplification of ITS of rDNA as *Bionectria ochroleuca* a fungi that grows above leaves streaks caused by Xav. These results will provide useful to establish disease a management program to reduce new infections.

AUDPC and AUDPS: What is the difference?

I. SIMKO (1), H. Piepho (2)
(1) USDA-ARS, Salinas, CA, U.S.A.; (2) Universität Hohenheim, Stuttgart, Germany
Phytopathology 102:S4.110

We have developed the formula termed the Area Under the Disease Progress Stairs (AUDPS) that combines repeated observations of the disease progress into a single value. The AUDPS has the same conceptual basis as the Area Under the Disease Progress Curve (AUDPC), however the two approaches differ in the weight that is given to the first and the last observations. While AUDPC gives less weight to the first and the last observations, AUDPS approach gives the two observations the same weight as to all other observations (if all observations are performed at regular intervals). Our empirical investigations indicate that this weighting scheme often improves the precision of disease assessment. Because of this difference, AUDPS scales linearly with the duration of the disease while AUDPC does not. Empirical evaluations suggest that AUDPS outperforms AUDPC in terms of precision in most of the tested trials when statistics from one-way ANOVA are compared.

Plastoquinone partitioning in the chloroplast affects disease resistance in apple

D. K. Singh (1), T. N. Laremore (2), P. B. Smith (2), S. N. Maximova (2), T. MCNELLIS (2)
(1) Cornell University, Ithaca, NY, U.S.A.; (2) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.110

Fibrillin4 (FBN4) is a protein component of plastoglobules, which are antioxidant-rich sub-compartments attached to the chloroplast thylakoid membranes. FBN4 is involved in plant biotic and abiotic stress resistance and is required for the accumulation of osmiophilic material inside plastoglobules. In this study, the contribution of FBN4 to plastoglobule lipid composition was examined using cultivated apple trees in which *FBN4* gene expression was knocked down using RNA interference. Chloroplasts and plastoglobules were isolated from leaves of wild-type and *fbn4* knock-down trees. Total lipids were extracted from chloroplasts and plastoglobules separately, and analyzed using liquid chromatography-mass spectrometry (LC-MS). Three lipids were consistently present at lower levels in the plastoglobules from *fbn4* knock-down apple leaves compared to the wild-type as determined by LC-MS multiple ion monitoring. One of these species had a molecular mass and fragmentation pattern that identified it as plastoquinone, a known major component of plastoglobules. Our results suggest that the partitioning of plastoquinone between the plastoglobules and the rest of the chloroplast is disrupted in *fbn4* knock-down leaves.

Recent studies on sweet orange scab (SOS) in Texas

M. SKARIA (1), A. Satpute (1), M. Kunta (1), J. daGraca (1), J. Perez (2), N. Malik (2)
(1) Texas A&M University-Kingsville, Weslaco, TX, U.S.A.; (2) USDA-ARS, Weslaco, TX, U.S.A.
Phytopathology 102:S4.110

Sweet orange scab (SOS) of citrus caused by fungus *Elsinoë australis* was detected for the first time in the United States on a lemon sample collected in Spring, TX in 2010. The sample was taken during a citrus commodity survey conducted by the Texas A & M University-Kingsville, Citrus Center, a project funded by the USDA-APHIS-PPQ. Polymerase chain reaction (PCR) was used to confirm the presence of *E. australis* from scab-like symptoms. Since then, PCR was used to test the presence of *E. australis* on various cultivars of citrus fruit at the TAMUK Citrus Center. USDA-APHIS-PPQ has now confirmed the presence of SOS in all citrus producing states except California. To date, 370 SOS suspected fruit were collected and 185 were confirmed PCR positive from several Texas counties, including Hidalgo, Cameron and Willacy. The symptomatology of SOS as observed has been consistent with the PCR results. Isolation of pure culture of *E. australis* and inoculation, re-isolation, followed by PCR and sequence data has fulfilled the Koch's postulates confirming its status as a new citrus pathogen in the US. Study of the pathogenicity was conducted with different citrus cultivars and detached leaf assays. To infect leaves, sporulation experiments were conducted. We have now perfected a system to readily produce conidia for artificial inoculation. As part of an effort to create a practical solution for the organic citrus packers, we studied the effect of heat under light and dark conditions. The results show that with the detached leaf assay, infections occur more readily under dark. Out of five separate studies, temperature regimes above 40°C seem to have an inhibitory effect. A study on changes in phenolics in SOS infected 'Rio Red' grapefruit showed that several phenolic compounds (eg. Caffeic acid, Luteoline-7-glycoside, Naringin, Naringenin, Apigenin-7 glycoside and Eriodictoyl) increase in the inoculated leaves compared to uninoculated leaves kept in the dark. In light, the effect of inoculation resulted in an increase of Naringin, Naringenin and Apigenin-7-glycoside. The levels of other polyphenols either were unchanged or decreased.

Characterization of recent clonal lineages of _Phytophthora infestans_ in the United States using microsatellite markers

I. M. SMALL (1), K. L. Myers (1), G. Danies (1), S. Guha Roy (2), K. Bekoscke (3), W. E. Fry (1)
(1) Department of Plant Pathology, Cornell University, Ithaca, NY, U.S.A.; (2) Department of Botany, West Bengal State University, Barasat, Kolkata, India; (3) St. John Fisher College, Rochester, NY, U.S.A.
Phytopathology 102:S4.110

Knowledge of the characteristics of *Phytophthora infestans* populations through phenotypic and genotypic characterization enables informed late blight disease-management. Simple Sequence Repeats (SSRs) are tandem repeats of 1-6 DNA base pairs that serve as highly versatile, PCR-based molecular markers. Because they are PCR-based, they require very small amounts of DNA. The objectives of this study were to i) determine whether SSR analysis could be used to group uncharacterized isolates into previously described genotypes; and ii) investigate the SSR profiles for isolates of *P. infestans*, from populations found in the US and Canada during 2009, 2010 and 2011. The use of SSR markers enabled the differentiation of all reference genotypes investigated in this study. Samples were received from 25 states and 2 Canadian Provinces. From these samples, the prevalent genotype found during 2009 and 2010 was US22, with a limited number of US8, US23, and US24 samples also identified. In 2011, US 22, US23 and US24 were found to be the dominant genotypes, with several US8 samples also identified. Considerable diversity was observed among the isolates of *P. infestans* examined, with several "rare" and as yet uncharacterized genotypes of *P. infestans* identified based on SSR results. SSR analysis has the potential to provide a rapid means to characterize *P. infestans* isolates into previously described clonal lineages, enabling informed late blight disease-management strategies.

Botryosphaeria stem blight of southern blueberries: Effect of temperature on infection and lesion development

B. J. SMITH (1), M. A. Miller-Butler (1)
(1) USDA-ARS, Thad Cochran Southern Horticultural Laboratory, Poplarville, MS, U.S.A.
Phytopathology 102:S4.111

Botryosphaeria stem blight is a destructive disease of rabbiteye (*Vaccinium ashei*) and highbush (*V. corymbosum*) blueberries in the southeastern United States. Historically this disease has been reported to be caused by the fungus *Botryosphaeria dothidea*. Recently other species in the *Botryosphaeriacea* family also have been identified as causal pathogens. The effect of temperature on infection of southern highbush (SHB) and rabbiteye (RE) blueberry cultivars by *B. dothidea* and other species was compared using a detached stem assay. Succulent, partially-hardened stems were wounded, inoculated with a mycelial block from 14 to 28-day-old cultures of the pathogens, and incubated for 14-22 days at 10, 15, 20, 25, 30, and 35°C. Lesion length was measured, and isolations were made from locations 10 mm above and 10 and 20 mm below the inoculation point on each stem. Main effects due to cultivar, species and temperature showed that stems of the RE cultivar, Climax, and the SHB cultivar, Star; those inoculated with *B. dothidea* and *Diplodia seriata*; and those incubated at 30 and 35°C had the greatest average lesion length and the highest recovery percentage of each inoculated species. The effect of temperature and culture medium on the growth and pycnidia production of the three species was also determined. Information gained from this study will be used to more effectively manage Botryosphaeria stem blight on blueberries.

A weather-based fungicide application advisor for control of black rot of grape in Oklahoma

D. L. SMITH (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.111

Black rot (caused by *Guignardia bidwellii*) is the most damaging disease of grapes grown in Oklahoma. To control black rot, as many as 10 fungicide sprays are applied during a single season. To reduce fungicide applications in Oklahoma, a weather-based fungicide application advisor was developed based on the black rot model by Spotts. The advisor uses the Oklahoma Mesonet for weather data inputs and output recommendations. The interactive interface is site-specific and provides a simple spray/no spray recommendation. Replicated field validation was conducted in 2009 and 2011. Fungicides were applied every 7 to 14 days (calendar) or using the advisor. In 2009, levels of fruit disease severity were not significantly different ($P>0.05$) for the advisory (5%) and calendar programs (8%), while the non-treated plots (55%) had significantly higher ($P<0.05$) levels of fruit disease. While not significantly different ($P>0.05$), average cluster weight was lower for plots not treated with fungicide versus the advisory and calendar programs. In 2011, fruit disease severity was significantly higher ($P<0.05$) in plots not treated with fungicide (23%) compared to the advisor (1.6%) and calendar (0.5%) programs, which were not different from each other ($P>0.05$). Yield was similar ($P>0.05$) for the calendar and advisor treatments but significantly lower in the non-treated plots ($P<0.05$). In all years, a minimum of 3 fewer fungicide sprays were made using the spray advisor.

Multisite validation of a weather-based fungicide application advisor for the control of dollar spot of creeping bentgrass

D. L. SMITH (1), J. Kerns (2), J. E. Kaminski (3), B. J. Horvath (4), M. Tomaso-Peterson (5)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) University of Wisconsin, Madison, WI, U.S.A.; (3) The Pennsylvania State University, University Park, PA, U.S.A.; (4) University of Tennessee, Knoxville, TN, U.S.A.; (5) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 102:S4.111

Dollar spot (caused by *Sclerotinia homoeocarpa*) is the most economically important turfgrass disease in North America. In the U.S., golf courses can spend 60-75% of their pesticide budget on dollar spot management. An improved fungicide application advisor would increase the accuracy of fungicide applications and potentially reduce input costs. Two newly developed prediction models were used in electronic advisories to control dollar spot of creeping bentgrass (*Agrostis stolonifera*) putting greens. Fungicides were applied in replicated trials at five locations according to a calendar program, or using advisory programs based on air temperature and relative humidity (AT+RH) or relative humidity (RH) alone. ZedX, Inc. supplied site-specific weather data (interpolated weather) and daily fungicide recommendations for both advisories. The AT+RH model provided control of dollar spot comparable ($P>0.05$) to the calendar program while providing an average reduction of one fungicide application per season. The RH model averaged a four-spray reduction versus the calendar program with significantly ($P<0.05$) improved control over not treating, but had a significantly ($P<0.05$) higher level of disease compared to the calendar program in two locations. The AT+RH advisory tended to over predict fungicide applications vs. the RH advisory, which tended to under predict fungicide application. Validation will continue in the 2012 season.

Seasonal variation in presence and abundance of inoculum of the *Heterobasidion* root disease pathogen in central Wisconsin

D. R. SMITH (1), J. Juzwik (2), G. R. Stanosz (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.; (2) USDA Forest Service, Northern Research Station, St. Paul, MN, U.S.A.
Phytopathology 102:S4.111

After infection of conifer stump surfaces following deposition of airborne basidiospores, the root disease pathogen *Heterobasidion irregulare* spreads through root grafts or by root contact to adjacent trees. Infection of fresh conifer stump surfaces resulting from felling is prevented, however, by borate application. Because the need for stump protection depends on inoculum availability, spore trapping was conducted from September 2009 through December 2011 in three infested plantations of predominantly red pine (*Pinus resinosa*) in central Wisconsin. At 2- (or sometimes 3-) week intervals, a semiselective medium in 9-cm-diameter Petri plates was exposed for 1 hour in daylight at each of four locations in each plantation. After 7-10 days incubation at 20°C plates were examined for the *Spiniger* asexual stage. Both presence and abundance of colonies of the pathogen were recorded. *H. irregulare* was detected during most of the two growing seasons, but colonies were most abundant during late summer, fall, and early winter. Relatively few colonies developed on medium exposed in winter during periods of deep snow and coldest temperatures, but colonies of the pathogen did develop occasionally on medium exposed at below 0°C. Definitive, biologically based guidelines for stump treatment require additional studies of seasonal factors influencing inoculum availability, *in situ* spore germination, infection, and establishment of the pathogen.

Functional analyses of parasitism genes of the root-knot nematode *Meloidogyne incognita* in *Arabidopsis thaliana*

A. D. SMITH (1), E. Davis (1), R. Hussey (2), T. Baum (3)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) University of Georgia, Athens, GA, U.S.A.; (3) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.111

The southern root-knot nematode, *Meloidogyne incognita*, is an important obligate plant parasite of multiple crops species with evolutionary adaptations such as a hollow, protrusible stylet and esophageal gland secretory cells that enable successful invasion and parasitism of host plant roots. Previous studies have identified more than fifty parasitism genes that encode proteins synthesized in root-knot nematode esophageal gland cells to be secreted from the stylet into plant tissues to promote parasitism. Many of the parasitism genes are pioneers without significant homology to genes currently listed in public databases. Functional analyses of three of the *M. incognita* pioneer parasitism genes designated 4D01, 5G05, and 35F03 are under investigation by overexpression in plant tissue and RNA interference (RNAi) gene silencing assays. The effects of constitutive expression of each parasitism gene product with and without the signal peptide are being analyzed in *Arabidopsis thaliana* plants. Changes in visible plant phenotype will be used as a measure of potential parasitism protein function in plant cells. Expression of double-stranded RNA to silence each *M. incognita* parasitism gene in transformed *A. thaliana* is providing a reciprocal way to analyze for potential RNAi effects on root-knot nematode parasitism of host roots. These strategies could provide knowledge about the function of root-knot nematode parasitism genes and identify parasitism gene products that are essential to host-parasite interaction.

New *Meliola* species on native Fabaceae from the Brazilian Cerrado

W. R. Soares (1), C. A. Inácio (1), M. D. Santos (1), J. C. DIANESE (1)
(1) Universidade de Brasília, Brasília, Brazil
Phytopathology 102:S4.111

Meliolaceae are authentic biotrophic fungi that infect epidermal and subepidermal leaf cells causing plant diseases designated as “black mildews”. Although disease losses in general are not significant, the potential for harm exists in the Tropics. Furthermore, the lack of information about these plant parasites present in the immense Brazilian savanna, the Cerrado, validates this research effort in a rich biome being extensively replaced by agriculture. In the family Fabaceae 164 meliolaceous species and 38 varieties are known. Five new species were identified on the following hosts that, as a group, appears associated with 13 species and 3 varieties of *Meliola*: *Albizia polyantha*, *Andira humilis*, *Erisoema congestum* and *Vignia* sp. Each fungal species was characterized based on the morphology of the ascomata,

ascospore dimension, shape and septation, hyphal branching, distribution of the phiallidic hyphopodes and appressoria on the superficial hyphae, and morphology of the mycelial setae with emphasis on their apices. A detailed description of the species allowed for a safe segregation of each one from the known taxa previously found on fabaceous hosts.

Molecular profiling of sequence data—A case study with 16S rRNA genes in *Betaproteobacteria*
S. SOBY (1)
(1) Midwestern University, Glendale, AZ, U.S.A.
Phytopathology 102:S4.112

Nucleotide sequencing is often coupled with computational methods for defining evolutionary relationships among bacteria. Genomic methods are important analytical tools, but have increased dependence on a few genes for classification of microbes, often without regard for functional constraints on the gene product. 16S rRNA gene sequences have been extensively employed for these purposes because alternating regions of high and low conservation provide convenient sequences for amplification and provide enough information for phylogenetic analysis. We have developed a method for comparing large numbers of orthologous gene sequences that can be used to identify regions under functional or structural constraints, and for quantifying variation within each structural region. Structural variations of large numbers of homologs are visualized as a 'spectrum' of base substitutions or indels at each base pair position. Combined with phylogenetic analysis, these gene spectra provide internal reliability for taxonomic assignments, and a clade-by-clade comparison of common structural features. The class Betaproteobacteria contains a number of plant and animal pathogens, including *Burkholderia*, *Ralstonia*, *Pandoraea*, *Bordetella*, *Chromobacterium*, and *Neisseria*. These genera are compared for variation with the 16S rRNA genes using a phylogenetic tree inferred using maximum likelihood and DNA spectrum analysis at hierarchical levels that range from gene copies within strains to class.

Profiling microbial communities in soils of SDS-infested sybean fields using next-generation sequencing
A. Y. SORUR (1), A. Warner (1), T. Reinhardt (1), M. Pfaff (1), J. P. Bond (1), L. Leonardo (2), D. Malvick (3), A. M. Fakhoury (1)
(1) Southern Illinois University, Carbondale, IL, U.S.A.; (2) Iowa State University, Ames, IA, U.S.A.; (3) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 102:S4.112

Sudden death syndrome (SDS) caused by *Fusarium virguliforme* is an endemic problem costing soybean farmers an average of 13.5% in yield losses annually. Despite the development of many management practices to control SDS, the disease continues to spread rapidly in soybean growing areas. The composition of the soil microbial populations contributes to the soil suppressiveness of soilborne pathogens. Determining those profiles, characterizing their role in suppressiveness of disease, and exploiting this information to design management recommendations to sustainably control SDS, is still challenging endeavor. Recent developments in next generation sequencing and in bioinformatics have made it possible to profile microbial communities in the soil and study their effect on soilborne pathogens. We have developed a strategy using next-generation (NGS) sequencing coupled with a denaturing gradient gelelectrophoresis (DGGE) of rDNA-amplified sequences. PCR-DGGE was useful toidentify markers that could be used to pre-screen soil samples. With the NGS, we used a two-locus barcode system to increase the discriminating power at the species level and to permit a higher-level phylogenetic analysis. Used together, NGS and PCR-DGGE provide a powerful tool to characterize the structure of the microbiota community structure in the soil and to study its effect on SDS incidence.

Determination of cape gooseberry (*Physalis peruviana*) plant growth regulators produced by rhizobacteria
C. Soto (1), J. Riaño (1), E. Coy (1), P. JIMÉNEZ (1)
(1) Universidad Militar Nueva Granada, Bogotá, Colombia
Phytopathology 102:S4.112

Cape goosberry is an economically important fruit crop in Colombia, mostly produced by small farmers. The most limiting pathogen for this fruit production is *Fusarium oxysporum* (>35%). In previous studies rhizobacteria, belonging to genera *Bacillus*, *Paenobacillus* and *Pseudomonas* resulted useful biocontrol agents of *F. oxysporum*. Based on that information 147 rhizobacteria were isolated from Cape gooseberry rhizosphere, and *in vitro* evaluated as inhibitors of *F. oxysporum* growth. A total of 4 bacteria strains that inhibited the fungus radial growth, above 30% inhibition, were selected. Half of these strains were identified as *Bacillus* sp. and the other half as *Pseudomonas* sp. During *in vitro* evaluation of their effect on Cape gooseberry seed germination, a noticeable increment of germination percentage, increased root growth and branching was found. Then, a medium-polar metabolic profiling was performed by liquid chromatography- mass spectrometry (HPLC-MS), the coumponds for each strain were identified using MassBank (High Quality Mass Spectral Database). Several aminoacids-conjugated auxins and cytokinins were identified; the presence of these compounds could explain the bacterial effect on seed germination rate and root growth. However, compounds that could be associated to the inhibitory effect of fungal growth were not clearly identified.

Western flower thrips (*Frankliniella occidentalis*) transmission of *Salmonella enterica* to crop plants
J. SOTO-ARIAS (1), R. Groves (2), J. Barak (1)
(1) Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, U.S.A.; (2) Department of Entomology, University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 102:S4.112

Recently, most foodborne illness outbreaks of salmonellosis have been caused by consumption of fresh produce. Crop plants become contaminated with *Salmonella enterica* pre-harvest; yet, the route(s) of contamination remains unclear. We hypothesize that plant-to-plant spread of *S. enterica* could occur via phytophagous insects. To test our hypothesis, we examined the transmission of *S. enterica* by a major pest of agronomic crops, Western flower thrips (*Frankliniella occidentalis*). Adult Westernflower thrips were fed *S. enterica* contaminated green beans, and subsequently, over 80% of the thrips tested positive for *S. enterica*. Five days after acquisition from contaminated beans, *S. enterica* was also isolated from 25% of the thrips demonstrating that *S. enterica* can persist on or in thrips for extended periods of time. Thus, thrips were capable of acquiring *S. enterica* from contaminated produce. Moreover, non-inoculated plants exposed to contaminated thrips subsequently became contaminated. Specifically, *S. enterica* was isolated from both tomato and lettuce plants in areas damaged by contaminated Western flower thrips. This is the first report of mechanical transmission of *S. enterica* by a phytophagous insect. Our data established that phytophagous insects may serve as a route of contamination for human enteric bacterial pathogens to fresh produce crops.

Progress of brown eye spot of coffee in three cropping systems
A. G. SOUZA (1), L. A. Maffia (1), E. S. Mizubuti (1), F. F. Silva (2), H. Teixeira (3)
(1) Universidade Federal de Viçosa/Departamento de Fitopatologia, Viçosa, MG, Brazil; (2) Universidade Federal de Viçosa/Departamento de Informática, Viçosa, MG, Brazil; (3) EPAMIG, Lavras, MG, Brazil
Phytopathology 102:S4.112

As the importance of brown eye spot of coffee (*Cercospora coffeicola*) is increasing in Brazil, we followed disease epidemics on three cropping systems: conventional (CC) and organic (OC) under full sun, and organic under shade (OS), in Ervália-MG, from November/2004 to October/2008. Twelve branches were marked in the lower, middle, and upper canopies, in each of ten plants/crop system. Monthly evaluations comprised: disease severity (SEV), disease incidence (INC), leaf fall (LF), and leaf setting (LS). Higher values of SEV, INC, LF, LS, area under disease progress curve, and maximum disease occurred in CC and on upper branches; lower values of all variables occurred in OS and on lower branches. Higher SEV and INC occurred in May-July, LF in July-September, and LS in October-January. Nonlinear sinusoidal model with ARMA errors described well the seasonal behavior of the disease along all years and cropping systems evaluated. Financial support: FAPEMIG and CNPq.

Use of Lamiaceae essential oils to control postharvest rots caused by *Botrytis cinerea* and *Penicillium expansum* on four cultivars of apple
D. Spadaro (1), G. Lopez (2), A. Garibaldi (2), M. GULINO (2)
(1) University of Torino, Grugliasco Torino, Italy; (2) Agroinnova-University of Torino, Grugliasco Torino, Italy
Phytopathology 102:S4.112

The most important postharvest diseases of apples include gray mold and blue mold rots. Essential oils have recently been identified as an alternative strategy to reduce the use of synthetic fungicides to control postharvest diseases of fruits and vegetables. The postharvest efficacy of the essential oils from basil, lavender, marjoram, oregano, peppermint, rosemary, sage, savory, thyme and wild mint was assessed on apples cvs Golden Delicious, Granny Smith, Red Chief and Royal Gala to control *Botrytis cinerea* and *Penicillium expansum*. Apples were artificially inoculated with a spore suspension of each pathogen. All the treated fruits were stored at 4°C. After 15 and 30 days, the diameter of the rot around each wound was measured. Results showed that, at the same concentration, treatments based on savory and thyme essential oils

were statistically more effective in controlling both pathogens than the other essential oils. Moreover, they were more effective on apples cvs Granny Smith and Red Chief than on cvs Golden Delicious and Royal Gala. Higher essential oil concentrations (10%) were sometimes associated to phytotoxicity symptoms. Apples cv Golden Delicious and cv Granny Smith were more susceptible to phytotoxicity. The efficacy of essential oil treatments and their phytotoxicity were partially cultivar-dependent, when applied on apples.

Preventing what ails rice with a strategic, statistical, prescriptive model system

A. H. SPARKS (1), A. Nelson (2), S. Savary (3)
(1) IRRI, Metro Manila, Philippines; (2) IRRI, Los Baños, Philippines; (3) INRA, Castanet Tolosan, France
Phytopathology 102:S4.113

Because many rice growers and their extension support systems are increasingly unable to diagnose crop health issues, it is important to find new ways to forward recommendations, which will be useful in reducing yield losses. We designed RICE-PRE as a system to predict the most likely crop health syndrome that may occur given the attributes of a production situation. A statistical analysis of survey data from 456 lowland rice farmers' fields in Asia indicated that despite a broad range of environments and highly diverse harmful agents, very strong statistical links exist between syndromes and the production situations. These production situations are then combined with a recently developed typology of rice ecologies, sets of agricultural objectives, and externalities (positive or negative) to create RICE-PRE, a set of prescriptions that include field operations and crop protection strategies aimed at minimizing yield losses. RICE-PRE is meant to be: strategic (before the season starts); based on strong statistical bases; and make use of prophylactic and preventive tools, especially resistant varieties. However, the use of preventive chemical protection is included when other options are unavailable and the risks involved are too high to be accepted. Results from the 2011 rainy season in the Philippines show that RICE-PRE increases yield over the control and farmers' practices, indicating that it is possible to design a simplified strategic recommendation system for farmers' use.

Identification of novel markers from whole genome sequences for phylogenetic analyses of oomycetes

C. F. SPIES (1), N. Rodrigue (1), B. Adhikari (2), J. P. Hamilton (2), C. Buell (2), H. Borhan (3), M. Links (3), N. Tisserat (4), C. Levesque (1)
(1) Agriculture and Agri-Food Canada, Ottawa, ON, Canada; (2) Michigan State University, East Lansing, MI, U.S.A.; (3) Agriculture and Agri-Food Canada, Saskatoon, SK, Canada; (4) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.113

The availability of whole genome sequences for an increasing number of oomycetes has opened up a wealth of genes for use in phylogenetics and taxonomical research. In light of the uncertain higher level taxonomic status of several key oomycete groups this study aimed to (i) identify genes in genome sequences of 16 stramenopile taxa that reflected the true phylogeny of oomycetes, (ii) develop primers for the amplification and sequencing of these genes in all oomycetes and (iii) validate the primers against a diverse range of oomycete taxa. A total of 888 single copy orthologous genes were identified from whole genome sequences of the 16 stramenopile taxa, aligned and concatenated into a single dataset of ca. 700 000 amino acids. This dataset was phylogenetically analyzed to obtain an accurate phylogeny (reference tree) of the represented taxa. Individual phylogenies of the 888 genes were compared to the reference tree to identify phylogenetically accurate genes. Out of the 888 genes, 26 genes gave phylogenies that were identical to the reference tree. Primer sets were designed for 12 of these and validated against 25 oomycete taxa. The amplified regions, being shorter than the actual genes, did not always produce phylogenies identical to the reference tree when considering only the initial 16 taxa. However, concatenated sets of different combinations of these genes produced trees topologically identical to the reference tree with good support for the main clades.

Spatial assessment of *Rhizoctonia solani* in fields undergoing rice and soybean rotations

T. SPURLOCK (1), C. Rothrock (1), W. Monfort (2)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Clemson University, Blackville, SC, U.S.A.
Phytopathology 102:S4.113

Rhizoctonia aerial blight of soybean is a single cycle disease caused by *Rhizoctonia solani* AG1-IA. This pathogen also causes sheath blight of rice. Many soybean fields in Arkansas are rotated with rice ensuring a source of inoculum each season. In 2009, soil assays using a toothpick baiting method and plant sampling on GPS positions in fields undergoing rice and soybean rotations was initiated. The objective was to test the hypothesis that early-season inoculum recovery should relate to seedling colonization by *R. solani* and subsequently relate to disease development. Disease assessments also were made on a spatial scale. Methodology was evaluated and refined each year to include more GPS positions on a smaller scale and controlling soil water for toothpick baiting to standardize saprophytic growth of the fungus. Soil assays and plant sampling in 2011 resulted in significant distribution of *R. solani* AG1-IA in two fields in eastern Arkansas. In both instances, *R. solani* was significantly dispersed using nearest neighbor statistics about a minimum enclosing rectangle (P<.0001). In a field near Hazen, levee maps indicated the levee position could be influencing the artificial nature of dispersion. In a field near Stuttgart, levee maps indicated the greatest inoculum potential lied in the lower elevations compared to higher elevations. Directional distribution ellipses for both distributions of *R. solani* AG1-IA indicated agreement with drainage in both fields. This spatial distribution was not associated with all AGs of *R. solani*. The spatial distribution of the early-season inoculum potential of *R. solani* AG1-IA reiterates the idea for rice that inoculum in the form of sclerotia and hyphae associated with crop residue may be floating and collecting at lower points within the levees. These data point to the influence of drainage on inoculum potential of the pathogen and suggest the potential for precision management of Rhizoctonia aerial blight of soybean through targeted within field scouting or fungicide sprays.

***Tomato spotted wilt virus* (TSWV)-resistant peanut genotypes and their interactions with thrips and TSWV**

R. SRINIVASAN (1), A. Shrestha (1), S. Sundaraj (1), A. Culbreath (1), H. Pappu (2), D. Riley (1)
(1) University of Georgia, Tifton, GA, U.S.A.; (2) Washington State University, Pullman, WA, U.S.A.
Phytopathology 102:S4.113

Thrips-transmitted *Tomato spotted wilt virus* (TSWV) is a major constraint to peanut production in the southeast since its introduction in the 1980s. At that time, most genotypes grown were very susceptible to TSWV. Over the last two decades, breeding efforts have resulted in the development of genotypes with high levels of field resistance. Currently, planting such genotypes is the main management tactic against TSWV. However, unlike TSWV resistant genotypes of tomato and pepper, genes conferring TSWV resistance have not been identified in peanut, and the mechanisms of resistance are unknown. The goal of this study was to evaluate the interactions between newly developed TSWV resistant peanut genotypes and thrips and TSWV. Four resistant genotypes and a susceptible genotype were selected. Thrips-mediated transmission experiments indicated that thrips transmitted TSWV to the susceptible genotype at a greater efficiency than resistant genotypes. All genotypes exhibited typical TSWV symptoms upon infection. Titer estimation using Real Time Reverse Transcriptase PCR (RT-RT-PCR) indicated that some resistant genotypes accumulated fewer or more N-gene copies of TSWV when compared with the susceptible genotype. Thrips development and reproduction did not differ drastically between susceptible and resistant genotypes. These results demonstrate that resistant peanut genotypes may be exhibiting tolerance than major gene-governed resistance to TSWV. N-gene sequencing of TSWV isolates from TSWV resistant and susceptible genotypes indicated no consistent differences between them, suggesting that the resistance exhibited by peanut genotypes might not exert a high selection pressure against TSWV.

Effect of phenolic compounds on reduction of growth and laccase *Botryosphaeria* spp.

P. SRIVASTAVA (1), P. C. Andersen (1), J. J. Marois (1), D. L. Wright (1), M. Srivastava (1)
(1) University of Florida, Quincy, FL, U.S.A.
Phytopathology 102:S4.113

Botryosphaeria spp. are ascomyceteous fungus that incite many diseases in economically important woody plant species. Four *Botryosphaeria* isolates, *B. rhodina* (*Lasiodiplodia theobromae*), *B. obtusa*, *B. dothidea* and *B. ribis* (*Neofusicoccum ribis*) were used in this study. *Botryosphaeria* spp. are ligninolytic and capable of growing in the presence of phenolic compounds and produce laccase. The effect of various phenolic compounds on ligninolytic enzymes was investigated in vitro as possible control of *Botryosphaeria* isolates. Ten naturally occurring phenolic compounds from plants were tested to find their effect on mycelium growth and the production of ligninolytic and pectinolytic enzymes. The concentration of phenolic compounds tested (1-25 mM) was found to be toxic to *Botryosphaeria* isolates resulting in reduced mycelium growth, biomass and the decrease of laccase production and pectinase activity. The effect of phenolic compounds *in vitro* varied with the *Botryosphaeria* isolates. Inhibition of mycelium growth was dose-dependent, and varied from 2-100% inhibition in the ten phenolic

compounds tested except syringic acid, which has no toxic effect on mycelium of *Botryosphaeria* isolates. A significant decrease in laccase production occurred when *Botryosphaeria* isolates were grown on phenolic compounds. Benzoic acid significantly inhibited pectinase activity in all isolates. The percent inhibition of pectinase activity in *B. dothidea* and *B. obtusa* was significantly increased in the presence of salicylic acid and syringic acid, respectively.

First report of downy mildew (*Peronospora parasitica*) on *Camelina* in Florida

P. SRIVASTAVA (1), H. M. Young (1), J. J. Marois (1), D. L. Wright (1), N. S. Dufualt (1), H. Dankers (1)
(1) University of Florida, Quincy, FL, U.S.A.
Phytopathology 102:S4.114

Camelina sativa (L.) Crantz, (Brassicaceae), is an annual oilseed species. It is grown as a forage and bio-fuel crop in Europe and North America. *Camelina* is an ideal low-input crop for bio-diesel production, due to its lower requirements for nitrogen fertilizer and pesticide requirements; *Camelina* production costs are substantially lower than many other oilseed crops such as rapeseed, corn, and soybean. It is an excellent rotation crop and can reduce disease, insect and weed pressure in wheat fields. During the spring of 2011, commercial and research plantings of *Camelina* cultivar "Calena" in Liberty and Gadsden counties in north Florida developed a foliar disease. A white downy mold was observed along the upper third of the plants on the upper stem internodes and on the developing seed. The affected stems exhibited a twisted growth. Conidiophores had main trunks with dichotomous branches terminating in slender curved tips. Conidia were ovoid. The foliar disease is identified as downy mildew caused by the fungus, *Peronospora parasitic* (Pers.) Tul. (*Hyaloperonospora parasitica* Constantinescu and Fatehi). It was not previously recorded in the Florida. This is the first documentation of *P. parasitica* on *C. sativa* in Florida.

In vitro and in vivo RNAi silencing of *Phytophthora capsici*

R. A. Stamler (1), N. P. Goldberg (1), S. Sanogo (1), J. J. RANDALL (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 102:S4.114

Phytophthora capsici is a soilborne oomycete plant pathogen that attacks a broad range of plant species including chile pepper. This pathogen infects all parts of the plant including roots, stems, leaves, and fruit. Effective management strategies are limited. This work investigates the possibility of using RNA interference (RNAi) gene silencing as a viable option for controlling diseases caused by *P. capsici*. Two genes, cdc14 and enolase, from *P. capsici* were isolated by PCR and cloned in various vectors to evaluate dsRNA production and silencing efficacy using in-vitro and in-vivo techniques. Electroporation of in-vitro dsRNA cdc14 into *P. capsici* resulted in a significant decrease of zoospore production. The ability of *P. capsici* hyphae to uptake of dsRNA was also evaluated. Using fluorescent microscopy and fluorescein-labeled dsRNA probes dsRNA uptake by *P. capsici* was visualized. *E. coli* and *Methylobacterium* sp. were subsequently engineered to produce dsRNA. The effectiveness of the bacterial lysate containing the dsRNA against *P. capsici* is being investigated. We are also evaluating plant infection by *P. capsici* in response to in-planta production of *P. capsici* cdc14 and enolase dsRNA. The ability of *P. capsici* to infect leaves is inhibited when plants are producing dsRNA, and zoospore production is also reduced approximately 46% and 35% in response to enolase and cdc14 dsRNA production in-planta, respectively.

Characterization of induced resistance in chile pepper against *Phytophthora capsici* following inoculation with nonhost *Phytophthora nicotianae*

R. A. STAMLER (1), N. P. Goldberg (1), S. Sanogo (1), J. J. Randall (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 102:S4.114

Phytophthora capsici (Leonian, 1922) is a pathogen that causes economic losses in many crops worldwide. In New Mexico (NM), losses in chile pepper (*Capsicum annuum*) due to *P. capsici* can exceed 50%, where it primarily causes root rot in saturated soils. The presence of both mating types and multiple races of *P. capsici* in NM results in a diverse pathogen population that makes management strategies difficult. Induced resistance against *P. capsici* was demonstrated using plant activators. However, microbial induced resistance against this pathogen has been little researched. In 2010, *Phytophthora nicotianae* (Breda de Haan, 1896) was identified for the first time in NM causing disease on onion (*Allium cepa*) and tomato (*Solanum lycopersicum*) but was found to be non-pathogenic on chile pepper. Results indicate that pre-inoculation of chile pepper with the non-pathogenic *P. nicotianae* limits systemic infection typically caused by *P. capsici* and reduces disease symptoms. A transgenic *P. capsici* reporter strain expressing the bacterial gene GUS, was inoculated onto chile pepper leaves treated or un-treated with *P. nicotianae*. Visualization of *P. capsici* through a colormetric GUS substrate showed that *P. nicotianae* treated plants had confined areas of GUS staining with limited *P. capsici* structures visible compared to untreated plants. Current work includes evaluation of induced resistance from non-host *Phytophthora* species that do not cause disease in crop systems.

Thermal adaptation in the fungal pathogen *Rhynchosporium commune*

T. STEFANSSON (1), Y. Willi (2), B. McDonald (1)
(1) ETH Zurich, IBZ, Plant Pathology, Zurich, Switzerland; (2) University of Neuchatel, Institute of Biology, Evolutionary Botany, Neuchatel, Switzerland
Phytopathology 102:S4.114

Despite the global agro-economic importance of the fungus *Rhynchosporium commune*, little is known about its evolutionary ecology, including adaptation to different thermal regimes across populations from climatically diverse locations. We conducted common garden experiments with 126 genetically distinct isolates from 9 field populations to measure variation in growth rates at 12°C, 18°C and 22°C. Populations from colder climates with higher temperature variation had higher growth rates at all three temperatures compared to populations from warmer more constant climates indicated by a positive correlation between variance in mean annual temperature and mean population growth rates ($r^2 = 0.86$, $p<0.003$). Population differentiation for growth rates (Q_{ST}) was significantly higher at 22°C than population differentiation at neutral microsatellite loci (G_{ST}) consistent with local adaptation for growth at higher temperatures. We found that *R. commune* has a high potential to rapidly adapt its growth rate to different thermal environments as heritability estimates ranged from 0.58 to 0.77. Our results suggest that this globally distributed pathogen has adapted to local climatic conditions, not through a shift in temperature optimum, but rather by acquiring generally fast growth in cooler, more variable climates or slow growth in warmer, more constant climates. This latter finding implies that there may be costs associated with fast growth under warm, constant climates.

Determining resistance conferred by *Wsm* loci to *Johnsongrass mosaic virus* (JGMV) and *Sorghum mosaic virus* (SrMV)

L. R. STEWART (1), M. W. Jones (1), M. Haque (2), M. G. Redinbaugh (1)
(1) USDA-ARS, Wooster, OH, U.S.A.; (2) Bangladesh Agricultural University, Mymensingh, Bangladesh
Phytopathology 102:S4.114

Maize dwarf mosaic disease is one of the most important viral diseases of maize throughout the world. It is caused by a set of related viruses in the family *Potyviridae*, genus *Potyvirus*, including *Maize dwarf mosaic virus* (MDMV), *Sugarcane mosaic virus* (SCMV), *Johnsongrass mosaic virus* (JGMV) and *Sorghum mosaic virus* (SrMV). Resistance to another member of the family *Potyviridae*, *Wheat streak mosaic virus* (WSMV), is conferred by three loci (*Wsm1*, *Wsm2*, *Wsm3*) from the maize inbred line Pa405. These loci also confer resistance to the potyviruses MDMV and SCMV, but it was not known whether they confer resistance to JGMV or SrMV. In this study, we tested near isogenic lines (NILs) carrying one or two of the *Wsm* loci introgressed into the susceptible line Oh28 and F1 progeny from NIL × Oh28 crosses for their response to JGMV and SrMV. Results of our greenhouse experiments indicated that *Wsm*1 provides resistance to both JGMV and SrMV but neither *Wsm*2 or *Wsm*3 alone provide resistance.

Validation of a unique sequence-based detection of plant pathogens using next-generation sequence data

A. STOBBE (1), U. K. Melcher (1), J. Fletcher (2), W. L. Schneider (3)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.; (3) USDA-ARS FDWSRU, Fort Detrick, MD, U.S.A.
Phytopathology 102:S4.114

With the arrival of next generation sequencing, sequence-based detection and diagnosis of pathogens has become a reality. A benefit of using next generation sequencing, the ability to generate several million bases of sequence data, is also a detriment to diagnosticians. Sifting through the tens of thousands of reads for those belonging to a pathogen is both computationally intensive and time consuming. In our previous work, we developed a theoretical method of sequence based detection in which raw sequence datasets are queried using designed pathogen-specific sequences (e-probes). The E-probe Detection Nucleic acid Assay (EDNA) has shown success in detection of pathogens in simulated 454 sequence datasets. In this work, we validated the previous simulated work. A 454 sequencing run of the extracted nucleic acid of a *Phaseolus vulgaris* (string bean) plant, which was infected with *Bean golden mosaic virus* (BGMV), contained 45,298 reads of sequence.

Using the EDNA method, we were able to quickly detect the presence of BGMV sequences. In contrast, the EDNA method was unable to detect *Spiroplasma citri* in a 454 sequencing run of the extracted nucleic acids of a *Daucus carota* (carrot) infected with *S. citri*. Further analysis of the sequence datasets revealed the proportion of pathogen reads for BGMV and *S. citri* to be 5% and 0.5% respectively, in agreement with previously determined limits of detection.

Designing and validation of e-probes to strain type *Plum pox virus*
A. STOBBE (1), U. K. Melcher (1), W. L. Schneider (2)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) USDA-ARS FDWSRU, Fort Detrick, MD, U.S.A.
Phytopathology 102:S4.115

The E-probe Detection of Nucleic acids Assay (EDNA) has shown that sequence based detection of pathogens is a useful method for diagnosis. The present work shows the ability to differentiate between strains of the potyvirus, *Plum pox virus* (PPV). The strains within the PPV genus include strains C, D, EA, M, W, and Rec (a common recombination of strains D and M). Using the EDNA method, strain-specific sequence e-probes were designed to detect strains C, D, EA, and W specifically. An additional e-probe set was designed to detect both the M and Rec strains. These e-probe sets were used as queries in a search of simulated next generation sequencing datasets. These sets include strains acquired from NCBI GenBank, as well as sequence information of four unknown isolates, including two recombinant strains. In the simulated studies, each set showed to be successful in determining the strain. In addition to simulated data, purified virus nucleic acids are to be sequenced using the 454 sequencing platform, and the datasets are to be subjected to the same analysis.

***Macrophomina phaseolina* (Tassi Goid.), cause of sugar beet charcoal root rot**
V. B. STOJŠIN (1), D. B. Budakov (1), F. F. Bagi (1), N. B. Đuragin (1), O. T. Neher (2)
(1) Faculty of Agriculture, Novi Sad, Serbia; (2) University of Idaho, Kimberly, ID, U.S.A.
Phytopathology 102:S4.115

Climate changes that bring harsh summers and severe droughts create favorable conditions for *M. phaseolina* since plants are stressed out due to extreme environmental conditions. Since sugar beet in Serbia is not irrigated, in years with severely dry and warm summers damages may be up to 100%. Primary symptoms are wilting and subsequently leaf decay which occur during mid and at the end of July. In the beginning, symptoms are visible on individual plants, which in time affect more plants and result in bare patches. In some localities, complete fields may be affected. During the mid and at the end of August, severely affected roots are completely rotten while leaves become dry. On the cross section, roots are spongy, dehydrated and at first yellow but with symptom progress the color turns into dark brown to black. Section between healthy and rotten root tissue is not clear. The color of the inner root tissue may vary, depending on sugar beet variety, environmental conditions and soil humidity, from lemon yellow, light to dark brown and black. Necrotic tissue may appear as dry or wet rot. Presence of microsclerotia on the surface and inside the root is not regular and therefore it is not reliable diagnostic symptom. This fungus creates sparse mycelium that after three days becomes black from abundant black microsclerotia.

Influence of tillage systems on *Rhizoctonia*-bacterial root rot complex in sugar beet
C. A. STRAUSBAUGH (1), I. A. Eujayl (1)
(1) USDA-ARS NWISRL, Kimberly, ID, U.S.A.
Phytopathology 102:S4.115

The *Rhizoctonia*-bacterial root rot complex in sugar beet caused by *Rhizoctonia solani* and *Leuconostoc mesenteroides* can cause significant yield losses. To investigate the impact of different tillage systems on this complex, field studies were conducted from 2009 to 2011. Split blocks with conventional and strip tillage as main plot treatments were arranged in a randomized complete block design with four replications. Within main plots, there were seven treatments (non-inoculated check and six *R. solani* AG-2-2 IIIB strains). Regardless of tillage, the roots responded in a similar manner for fungal rot (conventional 8% versus strip 7%), bacterial rot (26% versus 34%), total rot (33% versus 41%), neighboring roots infected (1.7 roots versus 1.5 roots), distance spread (157 mm versus 150 mm), and the number of dead plants (12% versus 14%). Based on these same variables, all 6 *R. solani* strains were pathogenic (significantly different from check; $P < 0.05$) and most responded in a similar manner, although differences were present at times. Strip tillage resulted in 6% more root yield in 2009 ($P = 0.087$), while conventional tillage resulted in 7% and 27% more root yield in 2010 ($P = 0.063$) and 2011 ($P = 0.012$), respectively. The tillage systems influenced disease variables in a similar manner, but more studies will be needed to determine their impact on yield.

Evaluation on perithecial development by *Fusarium graminearum* on the straws decomposed in different soils with different levels of temperature and moisture conditions
A. SUBEDI (1), K. L. Bowen (1), A. Hagan (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.115

In recent years wheat scab, caused by *Fusarium graminearum* and related *Fusarium* spp., has been reported at low incidence in northern but not southern Alabama. Plant residues of many cereal crops support overwintering of these fungi in the field. Decomposition of these crop residues might reduce perithecial development by the fungus. We hypothesize that higher annual rainfall and temperature cause greater residue decomposition and reduced fungal development in southern than in northern Alabama. In a preliminary study, a higher number of perithecia were produced on wheat straw incubated in soil with lower than higher soil moisture. In a follow-up study, wheat straw was incubated in three different soils with three soil moisture and three temperatures (20, 26 and 32°C) regimes. Sterilized, uniform straw pieces were buried in each soil sample and incubated. After one month, straws were removed, sterilized, placed on water agar, inoculated with a macroconidial suspension of *F. graminearum* and incubated at 26°C with diurnal light cycles. At 7 day intervals, numbers of perithecia were counted on each straw. Results showed significantly different numbers of perithecia on straw incubated in different soils. In non-sterile soils, straw incubated with lower soil moisture yielded higher numbers of perithecia. Temperature had no apparent effect on perithecia development.

Characterization of saprophytic bacteria that react with *Clavibacter michiganensis* subsp. *michiganensis* in seed health testing
P. SUDARSHANA (1), M. May (1), C. Kurowski (1), S. Thomas (1)
(1) Monsanto Vegetable Seeds, Woodland, CA, U.S.A.
Phytopathology 102:S4.115

Diagnosis of tomato canker involves examination of symptomatic plant tissue, testing with immunostrips, isolation of bacteria on selective media plates and PCR analysis. Saprophytic bacteria with similar colony morphology to *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) often result in false positive reactions with Cmm immunostrips and complicate the diagnostic process. In this study, eighty five tomato tissue samples asymptomatic to Cmm and positive by immunostrip testing were analyzed. Cmm-like bacterial colonies were isolated and characterized by conventional and realtime PCR and pathogenicity testing. All tested isolates were negative by pathogenicity assays and by the industry validated realtime PCR. However, some isolates resulted in false positive reactions using the published Cmm primers, CMM5/6. Sequence analysis of bacterial 16S rDNA fragments showed similarities to *Microbacterium paraoxydans* and *Ochrobactrum* sp. Developing specific and sensitive PCR primers by comparing the genome sequences of Cmm and saprophytic isolates will be discussed.

The impact of mixed-species cover crops on rhizosphere pathogens of organically managed tomato crops in New York, Ohio, and Maryland
C. F. SUMMERS (1), C. D. Smart (2), B. B. McSpadden Gardener (3), K. L. Everts (4), A. R. Dunn (2), S. Park (3)
(1) Cornell University-NYAES, Ithaca, NY, U.S.A.; (2) Cornell University-NYAES, Geneva, NY, U.S.A.; (3) Ohio State University, OARDC, Wooster, OH, U.S.A.; (4) University of Maryland, College Park, MD, U.S.A.
Phytopathology 102:S4.115

Mixed-species cover crops offer benefits beyond those of single-species cover crops, including a more optimized carbon to nitrogen ratio balance in the soil and more diversified microbial communities. Diverse microbial communities have been positively correlated to reduction in crop disease. The potential for reduction of disease and increase in yield provided by mixed-species cover crops in an organically-managed tomato production system was tested in New York, Ohio and Maryland using regionally-important cover crop treatments planted in randomized complete-block design. In addition to field experiments measuring disease incidence, we used macroarray analysis to test for the presence of forty fungal and oomycete tomato pathogens in rhizosphere DNA extracted from randomly-selected plants at 4-weeks post-transplant. Prevalent pathogens detected included *Alternaria alternata*, *Colletotrichum* spp., *Fusarium oxysporum*, *Fusarium solani*, *Phoma destructive*, *Rhizoctonia solani*, and *Septoria* sp. Statistical analyses of macroarray data from 2010 and 2011 will identify differences of the effects on rhizosphere pathogen populations conferred by mixed-species versus single-species cover crops, within and among the three regions. By integrating field data relating disease

incidence and yield with molecular data revealing pathogen populations and microbial diversity, we will identify the mixed-species cover crop most effective in reducing disease and increasing crop productivity.

WITHDRAWN

WITHDRAWN

Innate immunity: Perception and signalling induced by a bacterial microbe-associated molecular pattern (MAMP) in plants
T. Sundelin (1), G. Erbs (1), M. NEWMAN (1)
(1) University of Copenhagen, Frederiksberg, Denmark
Phytopathology 102:S4.116

We have established that lipopolysaccharide (LPS) has myriad effects in plants including the ability to prevent the HR induced by avirulent bacteria, priming of some plant defence responses and elicitation of others. However little is known about perception of LPS by plants or the associated signal transduction pathways that trigger LPS-induced plant disease resistance. We addressed this issue by analysing those sub-structures within LPS from the black rot pathogen *Xanthomonas campestris* that are required to trigger immune responses in *Arabidopsis* (*At*). We will present our new findings, that the *At* syntaxin PEN1 (AtSYP121) has a role in triggering immune responses in *At* in response to lipo-oligosaccharides (LOS; LOS is LPS without the O-antigen), but not in response to flagellin, another bacterial MAMP. Specifically we have found that PEN1 is required for PR1 gene induction and generation of reactive oxygen species (ROS) by LPS but not by other bacterial MAMPs. PEN1 is a SNARE protein that has been shown to be a component of the vesicle-targeting machinery involved in *At* resistance to *Blumeria graminis* f. sp. *hordei*. The involvement of SNAREs in contributing to fusion specificity is still debated, and our understanding of the regulatory role of PEN1 in fusion of intracellular transport vesicles with target membranes is still limited. Importantly our findings indicate that PEN1 may have roles in plant disease resistance that have not been appreciated thus far.

Suppression of cucumber powdery mildew by UV-B is affected by background light quality
A. SUTHAPARAN (1), A. Stensvand (2), K. A. Solhaug (2), S. Torre (1), K. Telfer (1), A. Ruud (1), L. Cadle-Davidson (3), L. Mortensen (1), D. M. Gadoury (4), R. C. Seem (4), H. R. Gislerod (1)
(1) Norwegian University of Life Sciences, Aas, Norway; (2) Bioforsk, Aas, Norway; (3) USDA-ARS, Grape Genetics Research Unit, Geneva, NY, U.S.A.; (4) New York State Agricultural Experiment Station, Geneva, NY, U.S.A.
Phytopathology 102:S4.116

Brief (5-10 min) exposure to UV-B radiation (280-300 nm) at 1 W m^2 suppressed powdery mildew (*Podosphaera xanthii*) on *Cucumis sativus*. The effect was enhanced by red light (600-660 nm), but offset by blue light (420-500 nm) and UV-A (300-420 nm). Compared to untreated controls, 2 h red light from specific light emitting diodes (LEDs) reduced disease severity by 25.4%, and by 98 % when combined with brief exposure to UV-B. We hypothesized that UV-B damaged fungal DNA, that blue light and UV-A were involved in DNA repair, and that red light exacerbated damage to DNA. We confirmed the expression of genes similar to phytochrome, cryptochrome, white collar, and photolyase (light-dependent DNA repair) using next generation sequencing of *Erysiphe necator*. Sequence and expression analysis of these light-responsive genes from *P. xanthii* will be reported. Our findings may explain how powdery mildews, which are principally external to the host and lack protective pigmentation, nonetheless thrive despite their lack of obvious protection from UV radiation. Our results suggest that efficacy of UV-B treatments against powdery mildews will be greatly enhanced by applying them during the night period, thereby circumventing the counteracting effects of blue light and UV-A, particularly when UV-B is used in combination with red light. Brief night exposure to UV-B, and/or inexpensive and specific LEDs may provide additional tools to suppress powdery mildews in diverse crops.

Induced resistance to pitch canker, caused by asymptomatic *Fusarium circinatum* infection, in seedlings of *Pinus radiata*
C. L. SWETT (1), T. R. Gordon (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.116

Fusarium circinatum causes pitch canker, a destructive disease of Monterey pines (*Pinus radiata*) in native forests in California. Pitch canker is characterized by branch and stem cankers in trees of all age classes. Seedlings often die soon after they are infected, but some remain symptomless. The objectives of this study were to assess the frequency of symptomless infection in pine seedlings and to determine if this serves to induce resistance to subsequent challenge with the pathogen. Symptomless infection was examined in native seedlings and under greenhouse conditions, wherein seedlings encountered the pathogen in soil or as they emerged through an infested litter layer. In litter layer trials, *Fusarium circinatum* caused up to 55% mortality within eight months following emergence, and up to 60% of seedlings were asymptomatically infected. In native stands, *F. circinatum* was recovered from approximately 70% of seedlings, all of which appeared healthy. In challenge inoculations, seedlings grown in soil with low and high infestation levels sustained stem lesions 43% and 63% shorter than controls, respectively. Similarly, seedlings exposed to infested litter had a 10% lower infection frequency and mean lesion length was reduced by 28%, relative to controls. These findings suggest that induced resistance can reduce the impact of disease on seedlings. If so, seedling survival may not depend solely on disease escape, but could also involve adaptive defenses.

Grasses as a new cryptic host of the pitch canker pathogen *Fusarium circinatum*
C. L. SWETT (1), M. Huang (1), T. R. Gordon (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.116

Fusarium circinatum causes pitch canker, a disease of pines characterized by resinous twig, branch and trunk cankers. No hosts outside the Pinaceae have been reported but the possibility that *F. circinatum* can infect grasses is suggested by ancestral relations with grass colonizing *Fuarium* species. The objectives of this study were to test for natural infection of grasses by *F. circinatum*, determine if *F. circinatum* can infect and sporulate on grasses

under controlled conditions and to characterize endophytic colonization in *Zea mays* (corn). In field surveys, *F. circinatum* was recovered at high frequency from the grass species *Festuca arundinaceae* and *Holcus lanatus*. Recovered isolates were pathogenic on pine, and were able to infect *F. arundinacea* and *H. lanatus* as well as four other California grass species. In field and greenhouse tests, *F. circinatum* colonized roots, stems and developing seed heads (ears) of corn from inoculated seeds, and also infected ears through silks and husk wounds. Histological studies show that the fungus colonizes epidermal and cortical tissue intercellularly. All inoculated plants remained symptomless, but the fungus sporulated on grass tissue once it began to senesce. These results suggest that grass species are alternate hosts for *F. circinatum*, constituting the first evidence for any host outside the Pinaceae. Studies to characterize the epidemiological impacts of grass infections are currently underway.

Epichloid endophytes of *Bromus laevipes*

G. SWOBODA (1), B. Hall (1), N. Charlton (1), M. Afkhami (2), S. Ghimire (3), K. Craven (1), C. Young (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.; (2) University of California-Davis, Davis, CA, U.S.A.; (3) RTI International, Research Triangle Park, NC, U.S.A.
Phytopathology 102:S4.117

Bromus laevipes is a native cool season bunchgrass found in California and Southern Oregon. The association of epichloid endophytes with *B. laevipes* can result in substantially greater fitness over plants that lack endophytes in some environments. To understand the selective advantage provided by the endophyte we have isolated epichloid endophytes from seeds of *B. laevipes* collected from northern and central California. A total of 58 isolates representing samples from 12 populations have been analyzed for morphological characteristics such as colony appearance, growth rate and conidiogenous cells. Genomic DNA was extracted from each isolate and PCR was used to determine gene diversity at the *EAS* (ergot alkaloids), *LOL* (lolines), *IDT* (indole-diterpenes) and *PER* (peramine) loci revealing four different chemotypes with at least two or more classes of alkaloid within each isolate. Heterogeneity of alkaloid gene profiles was found within two populations indicating that these two populations represented more than one endophyte. Molecular analyses reflected the morphological groupings based on analysis of conidiogenous cells, but more culture morphology variation was seen across samples with one of the chemotypes. Experiments are underway to reveal the phylogenetic progenitor of each isolate and functionality of each alkaloid loci. Preliminary data suggests that the isolates are interspecific hybrids that may have arisen from more than one independent hybridization event.

Development of a rapid molecular assay for the Ug99 race group of *Puccinia graminis*

L. J. SZABO (1)
(1) USDA-ARS, St. Paul, MN, U.S.A.
Phytopathology 102:S4.117

In the last decade a new group of highly virulent races of the wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici*, has caused epidemics in Northeast Africa and is a threat to wheat production worldwide. This race group (Ug99 RG) currently contains seven distinct members. A two-stage molecular diagnostic assay was developed using real-time polymerase chain reaction methodologies and fluorophore-labeled hydrolysis probes. Stage 1 uses a suite of four assays to identify samples belonging to the Ug99 RG. An internal control, based on the actin gene, is included to reduce false negatives. Only Ug99 RG samples are positive for all four of the assays. The stage 2 assay uses a suite of eight allele-specific SNP markers to predict the specific race phenotype of the sample within the Ug99 RG. A worldwide collection of over 400 samples has been used to validate this assay system. The assay performed reliably with a range of samples including pure fungal spores, infected plant tissue and ethanol-treated infected wheat stem tissue. This assay system provides a rapid and accurate method for diagnosis of Ug99 RG and will allow pathologists to monitor the movement this economically important group of races of the wheat stem rust fungus.

Overwintering onion thrips (*Thrips tabaci*) are a source of *Iris yellow spot virus* in Colorado

S. SZOSTEK (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.117

Iris yellow spot virus (IYSV) is a recurring problem in Colorado onion production. While several weed species have been described as alternate hosts for IYSV and likely act as green bridges for IYSV survival, the potential of the insect vector, *Thrips tabaci*, to overwinter and act as a source of inoculum has been little explored. Live thrips (adults and larvae) were recovered during the 2010-2011 and 2011-2012 winters from onion cull piles, onions left standing in the field, and *Malva neglecta* despite temperatures that fell below -17°C. IYSV was detected by RT-PCR in these thrips, indicating that IYSV infected thrips surviving the winter are a likely source of inoculum for the next growing season. Onion cull piles were constructed in October 2011. Temperature and thrips activity were monitored in subsequent months during fall and winter. Temperature changes in the cull piles were more gradual and less dramatic than those of the outside air. The internal temperature of cull piles is conducive to thrips survival, however, very few live thrips were found after the onset of onion decay within the cull piles. Thrips activity was monitored by sticky traps around the cull piles and thrips were active until early December 2011 when the outside temperature fell below -17°C. Monitoring will continue through the winter and into spring to determine when thrips resume activity and warrant pest management responses in nearby fields of new crop onions.

The dynamics of evolution in *Phytopththora infestans* as told by phylogeographical approaches

J. TABIMA (1), M. Mideros (1), A. Bernal (1), P. Jiménez (2), D. Riaño-Pachón (1), N. Grunwald (3), S. Restrepo (1)
(1) Universidad de Los Andes, Bogotá, Colombia; (2) Universidad Militar Nueva Granada, Bogotá, Colombia; (3) Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 102:S4.117

The plant pathogens dynamics of dispersion has always been a topic of interest for researchers, due to the impact of plant disease in a newly colonized site. History from the origin of the pathogen to the recent routes of dispersal, of these organisms, has been an unanswered question amongst research. One example of this, is the evolutionary history of *Phytophthora infestans*, the late blight disease agent of solanaceous plants. This study aim to combining new data analysis techniques, such as the use of six loci of nuclear and mitochondrial genes in a posterior probabilistic framework in order to reconstruct migration history. Thirty-seven *P. infestans* strains from around the world were used in this approach. Our results indicate rapid dispersal rates, concordant with the low genetic diversity of *P. infestans*, showing plausible migration due to human mediated practices, and *P. infestans* multiple dispersion from the Americas throughout the world in independent events.

Metagenomic screening for nonculturable oomycetes

J. TABIMA (1), J. Enciso (1), P. Jiménez (2), D. Riaño-Pachón (1), S. Restrepo (1)
(1) Universidad de Los Andes, Bogotá, Colombia; (2) Universidad Militar Nueva Granada, Bogotá, Colombia
Phytopathology 102:S4.117

Oomycetes is one of the most diverse lineages, which includes aquatic and terrestrial organisms, both of free-living and parasitic lifestyle. These organisms are of foremost importance in social, economic and health issues as several of them are recognized pathogens of economically important organisms. Since not all extant oomycetes can be cultured, current knowledge of this lineage remains incomplete. Finding of novel organisms is important, as it might contribute crucial information about some unknown pathogens as the case of fungi, where novel non cultivable lineages were recently reported. We propose the comparison of oomycetes sequences such as sets of classical genetic markers (SSU, ITS2) and 66 orthologous genes in several metagenomes of diverse ecosystems to establish whether uncultured lineages of this group are present in these environments in order to discover new species of this economically important group.

First detection of *Pseudomonas viridiflava*, the causal agent of blossom blight in apple by using specific designed primers

M. TAGHINASAB DARZI (1), M. Alimi (2)
(1) Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran; (2) Gorgan Branch, Islamic Azad University, Gorgan, Iran
Phytopathology 102:S4.117

During spring 2011, a newly occurring disease (blossom blight of apple) was observed in 4 to 5 years-old *Malus domestica* (cv. Mutsu) trees in Northern area of Iran. A bacterial population was repeatedly isolated from the infected plants. Koch's postulate (pathogenicity test) was fulfilled on potted plants under controlled environmental conditions (greenhouse). Based on morphological, physiological, biochemical and pathological tests, the causal agent was identified as *Pseudomonas viridiflava*. Polymerase chain reaction (PCR) identification of the bacterial isolates was done based on newly designed consensus primer pair (PsV-F and PsV-R). The consensus primers

were achieved by alignment of *P. viridiflava* 16S rRNA gene sequences available in nucleic acid data bank, National Centre for Biotechnology Information (NCBI) database. This primer set was successful for detection of *P. viridiflava* strains. Deoxyribonucleic acid (DNA) fragments amplified by this primer set gave a specific amplification band of ~180 bp. Sequencing was done directly (PCR products). Single bands of two isolates were extracted and sequenced for molecular characterization and compared with sequences available in Gene Bank (NCBI), using BLAST search tool. The results showed complete identity of isolates with those of the *P. viridiflava* strains in the databases. This to our knowledge is the first report of the occurrence of *P. viridiflava* on apple.

Identification of microorganisms associated with spear rot disease in Peruvian oil palm plantations

M. TALLEDO (1), R. Acuña (1), G. Huamani (2), A. Chigne (2), A. Trelles (2), E. Trinidad (2), J. Arevalo (1), Y. Montoya (1)
(1) Bio Links SA, Lima, Peru; (2) Palmas del Shanusi SA, Yurimaguas, Peru
Phytopathology 102:S4.118

In Peru the areas of oil palm plantations are increasing but concurrently are being affected by devastating diseases such as spear rot belonging to the complex of bud rot disease. The objective of this study was to identify the microorganisms infecting oil palms with spear rot in Peru. Sixty oil palm plants affected by spear rot were assessed. Ten different samples from each palm were collected to extract the DNA. PCR analyses were performed to detect fungi and bacteria by using universal primers for each group. In addition, specific primers to detect *Phytophthora*, *Pythium*, and *Fusarium* genus were included. The PCR products were separated by agarose gels, identified by DNA sequencing and using BLASTn. Likewise, microbiological cultures were also performed from several parts of the plant. *Fusarium oxysporum*, *F. equisety* and *F. sachary* were the species with the highest homology observed. This result was also confirmed by characterizing the microorganisms isolated by cultivation. *Phytium vexans* and *P. cucurbitacearum* were also identified. PCR products obtained by fungi universal primers showed only 77% of homology with *Phytophthora* the possible causal agent of bud rot disease in Colombia. The bacteria *Paenibacillus* sp. was identified by direct PCR amplification and by culture. The fact of knowing the microorganism associated with spear rot in oil palm is the first step to start with treatment tests to avoid the spread of the disease in oil palm plantations.

Early detection of airborne inoculum from wind-disseminated oomycetes

M. A. TANCOS (1), I. M. Small (1), W. E. Fry (1), C. D. Smart (2)
(1) Cornell University, Ithaca, NY, U.S.A.; (2) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.118

Aerially-dispersed oomycetes such as *Phytophthora infestans* (late blight of tomato and potato) and *Pseudoperonospora cubensis* (cucurbit downy mildew) present challenges to epidemiologists who try to model and predict these pathogens' complex dispersal patterns, population dynamics, and colonization rates. The ability of sporangia to survive wind dissemination and colonize fields in neighboring states stresses the importance of early detection. As a result, we have used an air-sampling spore trap that combines simplicity and efficacy in determining the epidemiology of late blight and cucurbit downy mildew. The solar-powered air sampler has two vertical spinning rods that can filter 62 liters of air/minute and collect wind dispersed spores within the area. Rather than staining, counting and differentiating spores based on morphology, we used the spore trap for total DNA extraction and polymerase chain reaction (PCR) assays. DNA-based assays allow for quick and reliable results with minimal training in oomycete identification/classification. Preliminary field trials were conducted in 2011 with sentinel plots located throughout two separate potato fields. The use of spore traps combined with PCR enabled the detection of airborne inoculum before symptoms appeared, with analogous results in cucurbit downy mildew field trials. These results suggest that combining air sampling spore traps with PCR can enable the detection and identification of *P. infestans* and/or *Ps. cubensis* sporangia prior to symptom development; thereby, allowing growers and extension educators to initiate preemptive control strategies.

Influence of watering on the dynamics of *Heterodera glycines* and *Fusarium virguliforme* interaction in soybean roots

N. TATALOVIC (1), G. L. Tylka (1), L. F. Leandro (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.118

Sudden death syndrome (SDS) of soybean, caused by *Fusarium virguliforme* (Fv), is an important soilborne disease in the US. An interaction between the soybean cyst nematode (SCN) and Fv is known to increase severity and cause earlier onset of SDS symptoms, but the mechanisms of this interaction are not known. A greenhouse experiment was conducted to compare root infection by both pathogens and to assess the influence of soil moisture on their interaction. There were four pathogen treatments – no pathogen, SCN alone, Fv alone, and co-inoculated, and two watering regimes - normal (NW) and reduced (RW). Plants were grown in either noninfested soil or SCN-infested soil for days 1 to 8 of the experiment, and then transplanted to noninfested soil or Fv-infested soil for days 9 to 17, after which the experiment was terminated. In the RW regime, Fv hyphae colonized mostly the root cortex of plants inoculated with Fv alone, whereas Fv hyphae predominantly colonized the vascular tissue in co-inoculated plants. In the NW regime, Fv occurred similarly in the cortex and vascular tissue in plants inoculated with Fv alone and in co-inoculated plants. SCN feeding sites, called syncytia, were found more frequently in the root cortex in co-inoculated plants (NW - 76%, RW – 63%), while syncytia were mostly found in the vascular tissue (NW – 84% and RW – 93%) in plants inoculated with SCN alone. Our data suggest that moisture highly affects SDS-SCN dynamics.

Identification of distinct functions of *Wheat streak mosaic virus* coat protein in virion assembly and virus movement

S. TATINENI (1), R. French (2)
(1) USDA-ARS, University of Nebraska, Lincoln, NE, U.S.A.; (2) USDA-ARS, Lincoln, NE, U.S.A.
Phytopathology 102:S4.118

Wheat streak mosaic virus (WSMV) is the type member of Tritimovirus genus of the family Potyviridae. The WSMV coat protein (CP) was subjected to point and deletion mutation analyses. WSMV mutants changing aspartic acid residues at amino acid (aa) positions 289, 290, 326, 333, and 334 to alanine elicited mild to moderate symptoms on wheat, but failed to infect SDp2 corn. Mutants with W165A, D216A, R237A, D282A, and D331A in CP did not infect wheat systemically, suggesting that these aa are required for virion assembly and/or virus transport. WSMV with a C-terminal 14 aa deletion infected wheat systemically with a slight delay in symptom onset. In contrast, deletion of 6-27 aa at the N-terminal region resulted in a fewer chlorotic streaks, suggesting that the N-terminal region of CP is required for efficient long-distance transport. Surprisingly, deletions consisting of three individual SGSGS amino acid repeats, all three SGSGS repeats (aa position 36-57), and 27 aa residues comprising 58-84 aa positions infected wheat similar to wild-type virus. WSMV with a deletion of 49 aa residues (aa positions 36-84) infected wheat systemically with slightly reduced symptoms. Taken together, our data suggest that the C-terminal 14 aa residues and 49 aa residues (position 36-84) are dispensable for virion assembly and virus transport in wheat. However, the N-terminal amino acids are required for efficient long-distance transport, but not for cell-to-cell movement of the virus.

Incidence and detection of *Peronospora variabilis* in quinoa seeds and plant tissue

A. L. TESTEN (1), J. B. Ochoa (2), G. Plata R. (3), P. A. Backman (1)
(1) The Pennsylvania State University, Department of Plant Pathology, University Park, PA, U.S.A.; (2) National Institute of Agricultural Research (INIAP) Santa Catalina Station, Quito, Ecuador; (3) Foundation for the Promotion and Investigation of Andean Products (PROINPA), Cochabamba, Bolivia
Phytopathology 102:S4.118

Quinoa (*Chenopodium quinoa*) is an increasingly important, emerging crop. Prized for its high nutrient content and tolerance to adverse abiotic conditions, quinoa is bred and researched as an alternative cropping system. Quinoa downy mildew, caused by *Peronospora variabilis*, is the key pest of quinoa, causing severe crop losses and is endemic in nearly all quinoa producing regions of the world. As quinoa production intensifies in South America and expands across the world, the ability to rapidly detect *P. variabilis* in quinoa seeds and plant tissue becomes an essential tool for management of quinoa downy mildew. Molecular methods were developed to assay 1,300-1,500 quinoa seeds simultaneously for the presence of *P. variabilis*. A sequencing-based survey of incidence of *P. variabilis* in imported and domestic quinoa seed lots detected the pathogen in 20 of 22 seed lots. *P. variabilis* specific primers were developed and amplified products were detected in 17 of 20 *P. variabilis* infested seed lots. These results indicate that viable quinoa seed sold for human consumption can unintentionally transfer exotic pathogens to points of consumption. Primers designed in this study can also be used to detect *P. variabilis* in plant tissue, which allows for rapid diagnosis of quinoa downy mildew, particularly in asymptomatic tissue. These molecular tools have applicability in the breeding of quinoa lines resistant to *P. variabilis* and in certification of *P. variabilis* free seed lots.

Examination of early plant gene expression changes in response to *Red clover necrotic mosaic virus* infection using a *Nicotiana benthamiana* microarray

P. THAMMARAT (1), T. L. Sit (1), S. A. Lommel (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.119

Plant viruses are minimalist pathogens that co-opt host processes to produce a successful infection. Many events and interactions during the critical early period where the virus reprograms plant physiological processes remain unknown. To elucidate these early events in the virus-host interaction, we utilized *Nicotiana benthamiana* (a model host for most plant viruses) and *Red clover necrotic mosaic virus* (RCNMV, a typical plus strand RNA plant virus). A custom microarray comprising 362,205 probes representing a total of 13,415 unigenes (equivalent to an estimated coverage of ~38% of the *N. benthamiana* transcriptome) was developed. Transcription profiles were analyzed at 2, 6, 12 and 24 hours post-inoculation (hpi). Microarray data was subjected to statistical analysis and 1,654 genes exhibited differential expression. The global snapshot of gene expression revealed that host genes are significantly down regulated at 2, 6 and 24 hpi and significantly up regulated at 12 hpi. This suggests that one infection cycle within the primary infected cell takes 12-24 hours. Viral infection affected the following key host pathways: metabolism, transport, membrane/cell wall-associated and defense. Select candidate genes were validated by qRT-PCR and are currently being functionally assayed for their effect(s) on RCNMV infection by both transient silencing and overexpression in *N. benthamiana* and these results will be presented.

Genetic variation of a novel ampelovirus in blackberry

T. THEKKE VEETIL (1), S. Sabanadzovic (2), K. E. Keller (3), R. R. Martin (3), I. E. Tzanetakis (1)
(1) Department of Plant Pathology, Division of Agriculture, University of Arkansas, Fayetteville, AR, U.S.A.; (2) Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.; (3) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 102:S4.119

A novel closterovirus was identified from cultivated and wild blackberries exhibiting symptoms of blackberry yellow vein disease. This study aimed to analyze the population structure of the virus in the United States. Genome structure and phylogenetic analysis indicate that the virus is closely related to *Grapevine leafroll associated virus*-3 and other subgroup II ampeloviruses (family *Closteroviridae*). In order to investigate the genetic variation among virus isolates, three areas were amplified and sequenced: polyprotein (region between methyl transferase and helicase domains), heat shock protein 70 homologue (HSP70h) and coat protein (CP); accounting for 3600 nt or about 20% of the virus genome. Comparisons of nucleotide and predicted amino acid sequences of isolates collected from Arkansas, Georgia, Mississippi, North and South Carolinas showed significant sequence diversity in the polyprotein (77-100%) and CP (86-100%) regions compared to HSP70h region (99-100%). Efforts are underway to develop efficient detection assays for the virus and identify potential vector(s).

Response of melon grafted on different cucurbit rootstocks to root-knot nematodes

J. A. THIES (1), J. J. Ariss (1), R. L. Hassell (2), A. Levi (1)
(1) U.S. Vegetable Laboratory, USDA-ARS, Charleston, SC, U.S.A.; (2) Clemson University, Charleston, SC, U.S.A.
Phytopathology 102:S4.119

Melon (*Cucumis melo*) is highly susceptible to root-knot nematodes (RKN) (*Meloidogyne incognita*, *M. javanica*, and *M. arenaria*) which cause severe root galling and fruit yield losses. Following the ban of methyl bromide, alternative technologies such as grafting are being considered for managing RKN in melons in the U.S. We evaluated response of 23 commercial cucurbit rootstocks and germplasm accessions representing pumpkin (*Cucurbita moschata*), hybrid squash (*C. moschata* x *C. maxima*), African horned cucumber (*Cucumis metulifer*), and melon in a greenhouse study. Melon, pumpkin, and squash exhibited the most severe root galling with root gall indices = 4.25 to 5.00 and egg mass indices = 3.79 to 4.88 on a 1 to 5 scale, where 1=no galling or egg masses and 5=greater than 80 percent roots affected. African horned cucumber exhibited the least root galling (2.62 to 3.26) and egg mass production (1.07 to 1.19). Several accessions representing the different cucurbit species were examined as rootstocks for grafted 'Athena' melon in a field infested with RKN (*M. incognita*) in Charleston, SC in 2008 and 2009. The *Cucurbita* spp. rootstocks and 'Dinero' melon rootstock supported the greatest RKN reproduction and African horned cucumber rootstocks had the lowest RKN reproduction. However, melon grafted on African horned cucumber produced intermediate yields (26.7-32.3 kg per plot of six grafted plants) compared with the high yields of melon grafted on the 'Strong Tosa' hybrid squash (*C. moschata* x *C. maxima*) rootstock (53.7 kg per plot).

Reduced sensitivity to propiconazole found in *Monilinia vaccinii-corymbosi* from lowbush blueberry fields in Maine

A. A. THOMPSON (1), S. L. Annis (1)
(1) University of Maine, Orono, ME, U.S.A.
Phytopathology 102:S4.119

Mummy berry disease, caused by the fungal pathogen *Monilinia vaccinii-corymbosi* (MVC), is a major disease affecting blueberry. In Maine and Canada, mummy berry disease is typically managed through 1 to 3 treatments of site-specific demethylation inhibiting (DMI) fungicides applied to lowbush blueberry (*Vaccinium angustifolium*) each crop year on a two-year crop cycle. This practice may increase the risk of MVC developing fungicide resistance. The sensitivity of 79 isolates of MVC from 3 types of fields: 4 conventionally managed, 3 organically managed and 1 unmanaged, was determined for two widely used DMI fungicides, propiconazole and fenbuconazole. Using the fungicide sensitivity data for the unmanaged area, a baseline EC_{50} (dose necessary to reduce mycelium growth by 50%) for each fungicide was established. The baseline EC_{50} was significantly higher for propiconazole (0.016 µg/mL) then fenbuconazole (0.00059 µg/ml). No significant differences in the fenbuconazole EC_{50} were found among management types. The propiconazole EC_{50} for conventionally managed fields (0.020 µg/mL) was significantly higher than for the unmanaged area (0.016 µg/mL). The propiconazole EC_{50} for organically managed fields (0.018 µg/mL) was not significantly different from that of the other field types. The propiconazole EC_{50} data suggest a decrease in sensitivity to propiconazole may be occurring in Maine, but no fungicide resistance has been seen in blueberry fields.

Identification of salicylic acid signaling networks in plant immunity

M. TIAN (1), C. von Dahl (1), P. Liu (1), G. Friso (2), K. van Wijk (2), D. F. Klessig (1)
(1) Boyce Thompson Institute for Plant Research, Ithaca, NY, U.S.A.; (2) Department of Plant Biology, Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.119

Salicylic acid (SA) is a signal molecule that plays central roles in multiple layers of plant immunity, including PAMP-triggered immunity (PTI), effector-triggered immunity (ETI) and systemic acquired resistance (SAR). Although a few direct protein targets of SA have been identified in plants, how SA is perceived and further activates downstream defense signaling remains largely unknown. Genome-wide identification of SA-binding proteins (SABPs) is likely an effective way to dissect the SA signaling mechanisms. Identifying additional SABPs has been very challenging since SA is a small molecule and likely regulates its effectors in a transient manner. Previous approaches are very time-consuming and not efficient for the identification of low-affinity and/or transient SA binders. Using SA analogs, two sensitive approaches have been developed to fish putative SABPs in *Arabidopsis* and validate their interactions with SA. These include an approach of photoaffinity labeling coupled with affinity chromatography, and a surface plasmon resonance (SPR)-based Biacore approach. With these approaches, proteins from multiple gene families that are involved in diverse cellular processes have been characterized as novel SABPs. The biological relevance of their associations with SA is being investigated. These newly developed approaches are promising to eventually illuminate the complex SA signaling networks.

In vitro activity of *Pseudomonas fluorescens* strain CL145A against phytopathogenic microorganisms

C. E. TODD (1), R. N. Asolkar (1), A. Cordova-Kreylos (1), P. G. Marrone (1)
(1) Marrone Bio Innovations, Davis, CA, U.S.A.
Phytopathology 102:S4.119

Marrone Bio Innovations has demonstrated that compounds derived from *Pseudomonas fluorescens* strain CL145A (PfCL145A), controls invasive quagga, and zebra mussels (US Patent Appln. Pub. No. 2010/0266717). During the course of studies with PfCL145A, inhibition was observed on agar plates where plant pathogens were grown alongside PfCL145A. It was hypothesized that PfCL145A produces metabolites that are responsible for in vitro phytopathogen inhibition. To test this hypothesis, the cell pellet from 10L spent fermentation broth was resuspensed in dilution buffer and extracted with Amberlite XAD-7 resin. The resin was eluted with acetone, after which the acetone was evaporated to obtain a crude extract. The crude extract was fractionated with C18 reverse-phase chromatography, and the active fractions were further purified via preparative HPLC to isolate and identify the com-

pounds responsible for activity. Crude extract and fractions were all tested for inhibitory activity against a panel of plant pathogens, including *Bacillus subtilis*, *Bacillus cereus*, *Xanthomonas campestris*, *Xanthomonas arboricola*, *Xanthomonas vesicatoria*, *Streptomyces scabiei*, *Erwinia carotovora* and *Botrytis cinerea*. Plant pathogens were plated on agar plates, and crude extract and fractions were loaded onto sterile filter paper discs that were subsequently placed on the agar plates. Activity was evaluated based on inhibition halos around the paper discs. Three fractions showed the best activity against the pathogen panel (fractions 2, 3 and 4). These active fractions were further analyzed for the presence of secondary metabolites using ESI-LCMS. Results indicate PfCL145A cells, extracts and fractions have good potential for biocontrol of fungal and bacterial plant pathogens.

Vegetable grafting: An alternative method for soil fumigation with methyl bromide—The Egyptian experience

A. TOHAMY (1), N. M. Abou-Zeid (1), M. S. Khalil (1)
(1) Plant Pathology Research Institute, Giza, Egypt
Phytopathology 102:S4.120

The phase-out of methyl bromide (MB) fumigation drives the search for alternative methods of soilborne pathogens control in vegetables. In Egypt, the plant pathologists found that soil solarization, biofungicides, basamid, metam sodium, in addition to their possible combinations as soil treatments provided good soilborne pest control if properly combined and integrated. Although alternative pesticides and other biological and physical soil treatments are being tested and developed, grafting with resistant rootstocks offers one of the best methods to avoid soilborne diseases. Three modern automated grafting greenhouses were established by UNIDO in three different sites resembling northern, southern and eastern governorates of vegetable production in Egypt. Five vegetable commodities were chosen for grafting trials, i.e., cherry tomatoes, pepper, cucumber, melon, and watermelon. Two grafting techniques were used: tube grafting was used for cherry tomato and pepper, while tongue grafting approach was used for grafting cucurbits. The percentage of grafting success was 95% in pepper and tomato, while it was 93% in case of cucurbits. Field and greenhouse observations provided excellent out-put of grafting in controlling soilborne pathogens of the tested commodities. In addition, grafting positively affected vegetative growth, fruit ripening date and quality, and provided higher yields of the grafted commodities when compared to the non-grafted ones. More information on the description of the automated grafting greenhouse, economic feasibility and the Egyptian experience in such grafting and other MB alternatives will be discussed.

Possible alternative mechanisms of azoxystrobin resistance in *Bipolaris* spp.

M. TOMASO-PETERSON (1)
(1) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 102:S4.120

Bipolaris leaf spot, caused by *Bipolaris* spp., is an important disease of ultradwarf bermudgrasses during spring and fall transition when plants are weak. Strobilurin fungicides are effective for controlling leaf spot; however, control failure using azoxystrobin has been mentioned by golf course superintendents in the Deep South. Twenty-five single spore isolates of *B. spicifera* were screened *in vitro* for azoxystrobin sensitivity at 0.031 and 8.0 µg/ml. All isolates displayed reduced sensitivity with relative growth ranging from 80 to 102% of the untreated control with a mean of 88% at 0.031 µg/ml azoxystrobin. Relative growth decreased 13% overall at 8.0 µg/ml compared to 0.031 µg/ml azoxystrobin with relative growth ranging from 64 to 92% of the untreated control and a mean of 78%. Nucleotide sequences of cytochrome b, including amino acid residues 129, 137, and 143 associated with strobilurin sensitivity, were obtained for *B. spicifera* isolates. Amino acids that confer sensitivity at these positions were retained. Alternative mechanisms of strobilurin resistance may exist within *B. spicifera* isolates. A N261D point mutation, structure change in the Rieske iron-sulfur protein, alternative respiration, or efflux transporters may account for the lack of azoxystrobin sensitivity *in vitro*. Future efforts will investigate these potential alternative mechanisms of resistance in *Bipolaris* spp.

Clues to the origins of individual lineages from the *Fusarium graminearum* species complex from genome-wide SNPs

C. TOOMAJIAN (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 102:S4.120

Isolates once classified into the single fungal plant pathogenic species *Fusarium graminearum* (*Fg*) have been shown to represent distinct phylogenetic species, but the functional differences and origins of the lineages in this species complex are not well understood. We aimed to discover sequence-based polymorphisms from the *Fg* complex to define genome regions with extreme levels of divergence between lineages. We created reduced-representation libraries from 24 isolates of the *Fg* complex by DNA size-selection after genomic restriction enzyme digest. Fragments from each library were tagged with a barcode, then pooled and sequenced on a single 454 GS-FLX run. Reads were mapped against the reference *Fg* genome, polymorphic sites identified using all reads, and genotypes of isolates defined after sorting reads by barcodes. We identified over 250,000 polymorphisms. After investigating the average level of sequence difference between pairs of isolates, we measured divergence levels between distinct lineages using sliding-windows to identify regions with extremes of divergence. We compared divergence levels to polymorphism within lineages to control for neutral mutation rate variation and classified polymorphisms based on genome annotation to test for the reduced efficacy of selection. Our results can help determine whether lineages have diverged in the absence gene flow, or whether gene flow continued across parts of the genome after an initial divergence of a few loci.

High-depth genome coverage of an unusual *Gibberella fujikuroi* species complex isolate that is cross-fertile with multiple species

C. TOOMAJIAN (1), M. Chiara (2), W. Strouts (1), D. Horner (2), A. Logrieco (3), G. Pesole (4), J. F. Leslie (1), J. Stack (1)
(1) Kansas State University, Manhattan, KS, U.S.A.; (2) Università Degli Studi di Milano, Milano, Italy; (3) Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, Bari, Italy; (4) Università Degli Studi di Bari, Bari, Italy
Phytopathology 102:S4.120

The study of natural plant pathogenic fungal isolates that are genetically intermediate between species may shed light on the frequency of interspecific hybridization and gene exchange in nature. Our goal was to perform an in-depth study of the genetic composition of such a potential natural hybrid between *Fusarium fujikuroi* (*Ff*) and *F. proliferatum* (*Fp*) collected from native prairie grass to test whether the isolate likely represents an interspecific hybridization event. Two genomic DNA libraries were sequenced with HiSeq 2000 for both the prairie sample and a representative *Ff* sample, and *de novo* assemblies were performed with the software Velvet. Megablast was used to identify likely homologous regions between these two samples and the published *F. verticillioides* (*Fv*) genome. Sequencing produced excellent depth of coverage for each sample, and *de novo* assembly and contig scaffolding resulted in N50s of 1 Mb and 180 kb for the prairie isolate and *Ff* sample, respectively. Scaffolds from each isolate were blasted against the *Fv* genome, and scaffolds from each of our sequenced strains blasting to the same *Fv* region were aligned. We used the extent and percent difference of well-aligned sequence to estimate divergence and investigated the distribution of divergences for evidence the prairie strain is a mosaic of *Ff* and *Fp* sequence. Our results have implications for the exchange of genes between related pathogen species on secondary hosts.

Novel broad-spectrum resistance to potato potyviruses

L. TORRANCE (1), G. Cowan (1), K. Mclean (1), A. Al-Abedy (1), S. MacFarlane (1), G. Bryan (1)
(1) The James Hutton Institute, Dundee, United Kingdom
Phytopathology 102:S4.120

Potyviruses are the most economically important virus diseases affecting potato production systems worldwide. The viruses are transmitted by many different species of aphid vectors and since the virus can be transmitted to the plants before the aphid is killed these virus diseases are inefficiently controlled by insecticides. New more severe recombinant strains of PVY are dominating potato production systems. Broad spectrum resistance to three potato infecting potyviruses has been found in germplasm derived from the *Solanum phureja* core collection held at The James Hutton Institute. Plants were challenged with the different viruses by mechanical inoculation, and resistant plants were either completely immune to *Potato virus Y* and *Potato virus A* (extreme resistance; ER) or infection was confined to the inoculated leaves (*Potato virus V*). Results of tests on seedlings derived from crosses between susceptible and resistant parents are compatible with the ER being conferred by a dominant major gene and preliminary mapping studies suggest that it is located in the middle of chromosome 9.

Transcriptome analysis reveals new insights into the *Colletotrichum graminicola*-maize anthracnose disease interaction

M. F. TORRES QUINTERO (1), E. A. Buiate (1), S. Amyotte (2), M. R. Thon (3), R. J. O'Connell (4), L. J. Vaillancourt (1)
(1) University of Kentucky, Lexington, KY, U.S.A.; (2) Univeristy of Ottawa, Ottawa, ON, Canada; (3) University of Salamanca, Salamanca, Spain; (4) Max Planck Institute, Cologne, Germany
Phytopathology 102:S4.120

Colletotrichum is a genus of hemibiotrophic fungal plant pathogens that cause anthracnose in a wide variety of crops. Biotrophic pathogens that colonize living host cells are known to manipulate host metabolism and suppress host defense responses and cell death. In contrast, necrotrophs that feed on dead host cells trigger plant defense responses, and/or secrete phytotoxins, to induce cell death. Biotrophs generally have fewer genes associated with secondary metabolism (SM) than necrotrophs. The genome sequence of *C. graminicola* encodes a very large number of genes associated with SM. A web-based software tool (SMURF), used to annotate putative secondary metabolite clusters (SMC), predicted more of these clusters in *C. graminicola* than in other closely related fungi. To better understand the role of SM and SMC in pathogenicity, we performed a transcriptome analysis (RNAseq) of *C. graminicola* at different stages of maize colonization. Our results show that SM genes are expressed at all stages of maize colonization analyzed in our study, with some clusters expressed early and others expressed late in the interaction. Preliminary analysis of maize genes expressed during the same stages of fungal colonization indicated that defense pathways are activated, rather than suppressed, very early in the interaction. Patterns of expression of maize invertases and, with one exception, of cell death inhibitors differed from those reported for biotrophic interactions. Overall, our results suggest that the hemibiotrophic *C. graminicola*-maize interaction resembles a necrotrophic interaction more than a biotrophic one during early stages of colonization.

Comparative analysis of methods to detect race 3 biovar 2 and native U.S. strains of *Ralstonia solanacearum*

T. M. TRAN (1), R. Kubota (2), A. M. Alvarez (2), C. Allen (1), A. Milling (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.; (2) University of Hawaii-Manoa, Honolulu, HI, U.S.A.
Phytopathology 102:S4.121

Ralstonia solanacearum strains cause bacterial wilt disease of many crops. Detecting and correctly identifying *R. solanacearum* in infected plants is important because the Race 3 biovar 2 (R3bv2) subgroup is a quarantine pathogen and a U.S. Select Agent, while the related sequevar 7 group is endemic to the southeastern U.S. Detection is complicated by frequent asymptomatic latent infections; uneven pathogen distribution within infected plants; occurence of aviable-but-not-culturable state; and biosecurity laws that restrict diagnosis of R3bv2 strains. Many detection assays have been developed, but their relativeutility was unknown. We therefore used immunostrip, direct plating, direct PCR, enrichment plus plating, enrichment plus PCR, Loop-mediated isothermal amplification (LAMP), and FTA cards to detect R3bv2 and sequevar 7 strains of *R. solanacearum* in geranium, tomato, and potato tissue in the lab and in naturally-infected tomato plants from the field. Methods were compared with respect to sensitivity, technical complexity, and cost.

Preliminary survey of blueberry yeasts and their potential for disease control

J. A. TRAQUAIR (1), P. D. Hildebrand (2), D. H. Langdon (3), G. J. Boland (3), S. Sabaratnam (4)
(1) Agriculture & Agri-Food Canada, London, ON, Canada; (2) Atlantic Food and Horticulture Research Center, Agriculture & Agri-Food Canada, Kentville, NS, Canada; (3) Environmental Biology Department, University of Guelph, Guelph, ON, Canada; (4) British Columbia Department of Agriculture, Abbotsford, BC, Canada
Phytopathology 102:S4.121

Blueberries are susceptible to several diseases caused by fungi that could be controlled biologically by application of microorganisms or by manipulation of epiphytic bacteria and fungi on leaves and fruit. Shoot blights caused by *Monilinia vaccinii-corymbosi* and *Botrytis cinerea* are serious lowbush blueberry problems in Canada during cold, wet spring periods. Yeasts washed from high- and lowbush blueberry leaves and fruit were identified using molecular methods based on sequences of D1 and D2 domains in large subunit ribosomal DNA. Selected isolates were screened *in vitro* for cold tolerance and for biocontrol potential using dual cultures with *B. cinerea*. Antagonistic isolates were screened on potted plants for suppression of Monilinia shoot blight of lowbush blueberry. A number of basidiomycetous yeasts with pink (*Rhodotorula graminis*, *R. fujisamensis*, *R. nothofagi*, *R. glutinis*, *R. mucilaginosa*, *R. sloofiae*, *Rhodosporidium habjevse*, *Sporidiobolus pararoseus*, *Sporobolomyces ruberrimus* and *S. gracilis*) and white (*Cryptococcus victoriae*, *C. arrabidensis*, *C. amylolyticus*, *C. saitoi*, *C. cellulolyticus*, *Kurtzmanomyces tardus* and *Bulleromyces albus*) colonies were found. More yeasts were found on fruit than on leaves. Pink yeasts predominated on highbush blueberry leaves at all localities and on fruit in Ontario and British Columbia. White yeasts predominated on fruit in Nova Scotia. Very few isolates grew on PDA at 10 °C but selected pink and white yeasts inhibited *B. cinerea* in dual cultures. Cold-tolerant *R. graminis* and *C. victoriae* isolates suppressed Monilinia blight of shoot buds by more than 50 % compared to untreated controls.

In vivo interaction studies of *Iris yellow spot virus* proteins using bimolecular fluorescence (BiFC) technique

D. TRIPATHI (1), M. Goodin (2), R. Dietzgen (3), H. Pappu (1)
(1) Washington State University, Pullman, WA, U.S.A.; (2) University of Kentucky, Lexington, KY, U.S.A.; (3) The University of Queensland, St. Lucia, QLD, Australia
Phytopathology 102:S4.121

Studies on *in vivo* self-interaction of viral proteins provide important information on virus replication in plants. The ability of viral nucleoprotein to form multimers has been suggested a common feature in plant viruses and a prerequisite for virus self-assembly. *Iris yellow spot virus* (IYSV, *Tospovirus*, *Bunyaviridae*) is an economically important viral pathogen of onion. As part of an ongoing project to better understand the IYSV-host interactions, the objective was to study the self-interaction of IYSV nucleoprotein (N) and its interaction with IYSV non-structural proteins (NSm and NSs) in the host plant *Nicotiana benthamiana*. IYSV genes were cloned into binary pSITE-BiFC vectors using the Gateway cloning technology. The expression clones were agroinfiltrated into marker *N. benthamiana* plants expressing auto fluorescent proteins (AFPs) in their nuclei. Expression of fusion proteins was confirmed by western blotting using anti-GFP antibodies. Confocal microscopy was performed to visualize the fluorescence of the expressed fusion proteins containing two complementary halves of AFPs. Preliminary results suggest the self-interaction of IYSV N protein and its interaction with IYSV NSm. Findings of this research will facilitate a better understanding of structure and function of IYSV proteins in infected host plants.

The poetry of phytopathology: Classical and modern verse inspired by plant disease

L. R. TRIPLETT (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.121

Plant diseases have served as inspiration for poets since the beginning of recorded history, providing powerful images of the long relationship between humans and plants. Some of these poems serve as an important historical record of plant disease in ancient times, or put a personal face on the devastating human consequences of catastrophic crop failures. Some poets view diseased plants as a metaphor for human weakness and mortality or a symbol of change, while others may admire disease symptoms as examples of nature's beauty. These poems illustrate the emotional human impact of plant disease, and they can serve as effective tools for engaging pupils or readers to consider the role of plant health in our lives. This poster discusses selected depictions of plant disease in published poetry, and provides references and descriptions of others for use in teaching and outreach.

Modulation of plant defense responses by salicylate hydroxylase of '*Candidatus* Liberibacter asiaticus' and its implication on canker pathogen *Xanthomonas citri* subsp. *citri* in huanglongbing-infected plants

P. TRIVEDI (1), N. Wang (2)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.121

Citrus huanglongbing (HLB) associated with pathogen '*Candidatus* Liberibacter asiaticus' (Las) is a devastating disease to US citrus industry. To gain knowledge on the mechanism(s) by which Las evades host defense responses we expressed salicylate hydroxylase (*sahA*) of Las in *Escherichia coli*. Our data indicate that Las encodes a functional salicylate hydroxylase, which converts salicylic acid (SA) into catechol, a product that does not induce resistance. To determine expression level of defense related genes after Las infection, *Xanthomonas axonopodis* pv. *citri* strain A^W (Xac A^W) was used to induce *PR-1* gene expression. The *PR-1* gene expression in Xac A^w challenged plants which were previously infected with Las was lower than Xac A^w challenged healthy plants. Using SA biosensor strain (*Acinetobacter* sp. ADPWH_lux), 4 fold reduction in SA accumulation was observed in the Las infected as compared to healthy plants. To understand the possible synergistic effect of the presence of Las on the citrus canker [caused by *X. citri* subsp. *citri* (Xcc)] we inoculated Xcc in Las infected and healthy grapefruit leaves. The population levels of Xcc were significantly higher during all the observation time points in Las infected as compared to healthy citrus. Modulation of SA production and regulation of defense related genes could be one of the mechanisms deployed by Las to evade plant defense

responses. The Las infected plants compromised with defense responses could further succumb to the infection by other pathogens.

Molecular identification of legume-infecting begomoviruses in Southeast Asia

W. TSAI (1), S. Shih (1), R. Safitri (2), B. Huyen (3), L. Kenyon (1)
(1) AVRDC, The World Vegetable Center, Tainan, Taiwan; (2) PT. East West Seed Indonesia, Purwakarta, Indonesia; (3) Plant Resources Centre, Hanoi, Vietnam
Phytopathology 102:S4.122

The legume-infecting begomoviruses are recognized as causing severe disease on legume crops primarily in Bangladesh, India and Pakistan in South Asia and occasionally in Thailand. However, in 2009, a high incidence of virus-like yellow mosaic symptoms was observed in legume crops in Java, Indonesia, and 20 symptomatic yard-long bean (*Vigna unguiculata* subsp. *sesquipedalis*) and five soybean (*Glycine max*) leaf samples were collected. Then, in 2011, yellow mosaic symptoms were wide-spread in mungbean (*Vigna radiata*) in Vietnam and 15 symptomatic samples were collected. All yard-long bean, soybean and mungbean samples tested positive for begomovirus infection by polymerase chain reaction, but none were positive for potyviruses or *Cucumber mosaic virus* by enzyme-linked immunosorbant assay. Based on comparison of the 1.5 kb begomoviral DNA-A nucleotide sequences, amplified by primer pair- PAL1v1978B/PAR1c715H, all the sequences from the 20 yard-long bean and five soybean samples from Indonesia had high identity (98.0 to 99.8%) and were considered to be the same begomovirus. All 15 mungbean samples from Vietnam were also considered to be infected by the same begomovirus because of the high nucleotide sequence identity (98.4 to 99.7%). The BLASTn and sequence comparisons showed the legume-infecting begomoviruses from Indonesia had greatest nucleotide sequence identity (96.1 to 97.9%) with *Mungbean yellow mosaic India virus* cowpea isolates from India, while the mungbean isolates from Vietnam had greatest nucleotide sequence identity (97.4 to 98.2%) with *Mungbean yellow mosaic virus* mungbean isolates from Cambodia. The results provide evidence for the emergence of legume-infecting begomoviruses in Southeast Asia.

Comparison of *Ralstonia solanacearum* strains isolated from tobacco and vegetable crops in North Carolina

H. TSENG (1), M. Katawczik (2), A. Mila (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) USDA-ARS, North Charleston, SC, U.S.A.
Phytopathology 102:S4.122

Ralstonia solanacearum (*Rs*), the causing agent of Bacterial wilt, is a devastating disease in tobacco and tomato growing regions of North Carolina. To better understand the diversity of the pathogen population in North Carolina, *Rs* strains were collected from symptomatic tobacco in 2008 and tomato in 2010. Phylogenetic study performed by *egl* sequencing, indicated that all strains were phylotype II with little variation in *egl* sequence. Pathogenicity tests were conducted using 4 to 6 weeks old tomato, pepper, and tobacco seedlings, inoculated with 5 ml of 10^8 CFU/ml bacterial suspensions and incubated at 30 °C, in a 12-hour light cycle. Results indicated that tobacco strains could infect all hosts. Tomato strains did not cause disease on tobacco plants except for strains collected from a tomato field where previously tobacco was planted for several years. Finally, the *avr*A gene from all strains was amplified using polymerase chain reaction with previous published primer settings. The 960-bp mutant *avr*A gene was found in all tobacco strains and the tomato strains that caused disease on tobacco whereas the wild-type 792-bp *avr*A gene was found in tomato strains that did not cause disease on tobacco. Given the wide incidence of Bacterial wilt on tobacco crops in North Carolina this study provides important information to tobacco and vegetable growers with regards to selecting fields for crop production based on previous crop history.

The sanitary effect of alkyl dimethyl benzyl ammonium chloride on bean pods infected by *Pseudomonas syringae* pv. *syringae*

K. M. TUBAJIKA (1)
(1) USDA APHIS PPQ CPHST, Raleigh, NC, U.S.A.
Phytopathology 102:S4.122

The ability of *Pseudomonas syringae* pv. *syringae* to attach and survive on bean pod surfaces is important for pathogen spread and disease control. The sanitary effect of alkyl dimethyl benzyl ammonium chloride (ADBAC) on beans infected by *Pseudomonas syringae* pv. *syringae* (*Pss*) was evaluated in comparison to quaternary ammonium (QA), chlorine, and sodium orthophenylphenate (SOPP) for inhibition and destruction of the pathogen propagule on fruit surfaces. Common green beans of equal dimensions were immersed in an inoculum containing *Pss* (10^8 CFU/ml) for 1 hr and stored at 20°C for up to 7 days. The inoculated beans were washed at 0, 24, 72, 120, and 168 hr with ADBAC (10, 100, and 1000 ppm), chlorine (150, 200 µg/ml), QA (500, 1000, and 2000 µg/ml) and SOPP (20,000 µg/ml) for 60 seconds. The incidence of *Pss* was determined by direct plating. A population of 5.6 log CFU/ml of *Pss* was recovered from the inoculated beans. No significant ($P < 0.05$) reduction of *Pss* was observed on beans stored at 20°C for up to 7 days, and the population was not reduced after washing with sterile distilled water. Washing bean pods with ADBAC was more effective than Chlorine in reducing *Pss* to 6.0 log CFU/ml. This research showed that destruction of *Pss* propagules from surface of plants can be selectively achieved with certain compounds and an effective fruit surface disinfection treatment should be incorporated in fruit sanaitazing protocols to achieve fruit surface decontamination.

Use of latent class analysis to estimate the sensitivities and specificities of diagnostic tests for *Squash vein yellowing virus* in cucurbits

W. TURECHEK (1), C. Webster (1), S. Kousik (2), S. Adkins (1)
(1) USDA-ARS USHRL, Fort Pierce, FL, U.S.A.; (2) USDA-ARS USVL, Charleston, SC, U.S.A.
Phytopathology 102:S4.122

Squash vein yellowing virus (SqVYV) infects numerous cucurbits and is cause of watermelon vine decline, a serious problem in Florida. Current methods for identification of SqVYV-infected plants are based on the polymerase chain reaction (RT-PCR), nucleic acid hybridization assays (NAHA), and visual symptoms. Latent class analysis was used to estimate the sensitivities and specificities of RT-PCR, NAHA, and visual symptoms as diagnostics for SqVYV and to determine whether their performances varied among tissue type (crown vs. vine tissue), where samples were taken along the vine relative to the crown, genus, and habitat (field- versus greenhouse-grown plants). Results showed that RT-PCR had the highest sensitivity (0.94) and specificity (0.98) of the three tests. NAHA had better sensitivity than symptoms for SqVYV detection (0.70 vs. 0.32), while symptoms were more specific than NAHA and a better indicator of non-infection (0.98 vs. 0.65). For the grouping variables, RT-PCR and NAHA had better sensitivity but poorer specificity for diagnosing SqVYV in crown tissue than vine tissue, whereas symptoms had very poor sensitivity but excellent specificity in both tissues. Test performance also varied with habitat and genus, but not with distance from the crown. The results given here provide quantitative measurements of test performance for a range of conditions, and provide the information needed to interpret test results when tests are used in combination for a diagnosis.

Influence of insecticide programs on *Tomato spotted wilt virus* in California processing tomatoes

T. A. TURINI (1), D. A. Rodriguez (1), M. Le Strange (2)
(1) University of California Agriculture and Natural Resources, Fresno, CA, U.S.A.; (2) University of California Agriculture and Natural Resources, Tulare, CA, U.S.A.
Phytopathology 102:S4.122

Since 2005, *Tomato spotted wilt virus* (TSWV) has had an annual economic impact on processing tomatoes in California. For control of thrips, which vector TSWV, insecticide use increased substantially since this virus became a persistent economic threat. To evaluate the impact of insecticide programs on TSWV in processing tomatoes, studies were conducted in Fresno County from 2009 to 2011. Processing tomato plants, cv. H8004, were transplanted in early- to mid-May and irrigated with drip tape buried at a depth of 25 cm. The experimental design was a split plot: Main plot treatments were drip-injected insecticides and subplot treatments were foliar applications. The drip treatment comparison was between thiamethoxam applied 3 weeks after transplanting with dinotefuran applied 7 weeks after transplanting, and an untreated control. Foliar treatments included 2-4 insecticide applications of rotations of spinetoram, dimethoate and methomyl (2009 only). Over all 3 years, drip treatments were similar to the untreated. Foliar applications lowered TSWV incidence by 32.8% in 2009 and 33.3% in 2011. However, in 2010, foliar treatments did not reduce TSWV. This may be due to higher regional TSWV levels in 2010 and more TSWV-infected thrips from outside of the trial area. There is no evidence that drip injected treatments were of benefit. However, foliar insecticide applications can be a useful tool for managing TSWV.

In vitro *Phakopsora pachyrhizi* isolate sensitivity to fungicides and effect of fungicide and timing of application on soybean rust severity

M. TWIZEYIMANA (1), G. L. Hartman (2)
(1) University of California-Riverside, Riverside, CA, U.S.A.; (2) USDA-ARS and Department of Crop Sciences, University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.122

Soybean rust, caused by *Phakopsora pachyrhizi*, is a devastating foliar disease of soybean that may cause significant yield losses if not managed by well-timed fungicide applications. To determine the effect of fungicide on *P. pachyrhizi*, urediniospores of different isolates collected in the U.S. were evaluated for fungicide sensitivity in vitro and the preventive and curative effects of selected fungicides representing the strobilurin and triazole fungicide classes were evaluated on detached leaves and in the greenhouse. Two fungicides, Quadris and Folicur were used to test fungicide sensitivity, whereas different concentrations of Quadris, Quilt and Folicur were used in the detached leaves and greenhouse experiments. Significant differences ($P < 0.05$) were observed among *P. pachyrhizi* isolates for fungicide sensitivity with EC50 ranging from 0.0025 to 0.0051 and 0.92 to 2.60 for Quadris and Folicur, respectively. The preventive and curative activity differed significantly ($P < 0.05$) among the fungicides used in both detached leaves and greenhouse experiments. For instance, no infection was observed on soybean leaves when inoculated immediately or 2 days after application of low or high concentrations of the three fungicides. Also, Quilt at 0.5 ppm, reduced rust infection by <80% when applied eight days after or before inoculation in both detached and greenhouse experiments and when applied 14 days before inoculation in the greenhouse. This study gives a clear understanding on how different isolates of *P. pachyrhizi* react to some commercial curative and preventative fungicides.

Comparative ability of some fungicides to manage the avocado branch canker (formerly Dothiorella canker) in California

M. TWIZEYIMANA (1), J. Mayorquin (1), D. H. Wang (1), F. Na (1), S. D. Akgul (2), A. Eskalen (1)
(1) Department of Plant Pathology and Microbiology, University of California, Riverside, CA, U.S.A.; (2) Manisa Viticulture Research Station, Turkish Department of Agriculture, Manisa, Turkey
Phytopathology 102:S4.123

California is the leading producer of avocado fruit in the United States. During the 2010/11 season, the avocado industry in California accounted for 126,500 tons valued approximately at $303 million. Members of the Botryosphaeriaceae family are known to cause branch cankers on avocado. Canker infections are initiated by spores entering the host plant through fresh wounds such as pruning wounds. The objective of this study was to evaluate the preventive ability of some commercial fungicides against infection by Botryosphaeriaceae fungi. Selected fungicides representing the strobilurin and triazole fungicide classes and a manufactured premix of strobilurin + triazole classes were applied within the labeled rates recommended by the manufacturer in the field. Prior to field experiments, many fungicides and combinations were screened in vitro, and only five fungicides: Cabrio 20 EG (Pyraclostrobin), Quash 50 WDG (Metconazole), Rally 40 WSP (Myclobutanil), Quilt Xcel (Propiconazole + Azoxystrobin) and Switch 62.5 WG (Fludioxonil + Cyprodinil) were selected. Significant differences ($P < 0.05$) were observed among fungicides; however, all fungi were similarly inhibited by each fungicide. Quilt Xcel had the highest percentage inhibition 44% and 58% in Trial 1 and 2, respectively though this level of inhibition was not significantly different ($P < 0.05$) from that of Quash 50 WDG. Rally 40 WSP had the lowest percentage of inhibition. Application of Quilt Xcel and Quash 50 WDG can play a key role in protecting California avocado against the Botryosphaeriaceae fungi especially when sprayed a day to one week after pruning.

WITHDRAWN

Effects of preconditioning cold treatments and incubation temperature on germination of ergot bodies of *Claviceps purpurea* from Kentucky bluegrass

S. UPPALA (1), B. Wu (1), S. C. Alderman (2)
(1) Oregon State University, Madras, OR, U.S.A.; (2) USDA-ARS NFSPRC, Corvallis, OR, U.S.A.
Phytopathology 102:S4.123

Claviceps purpurea is an important ovary-infecting pathogen that replaces seed with sclerotia in Kentucky bluegrass grown for seed. Grass seed contaminated with sclerotia is rejected or heavily discounted in value. Sclerotia overwinter in the soil and germinate in the spring to produce ascospores for infecting grass ovaries. To better understand ascospores production, the effects of pre-conditioning cold treatment and incubation temperature on germination of sclerotia were determined in controlled temperature studies. Sclerotia of *C. purpurea* collected from infected Kentucky bluegrass in 2011 were surface sterilized, rinsed with sterilized water, and placed on 7.5-mm-diameter polyurethane discs in foam cups. The sclerotia were kept at 4°C for 0, 2, 4, 6 or 8 weeks, and then incubated in darkness at constant 4, 15, 20, or 25°C, or in a growth chamber at 20/10°C (day/night; 12h day length). The sclerotia were sprayed with sterilized water twice a week. Meantime, germinated sclerotia were counted. No sclerotia germinated at 4°C. Four to six weeks of cold treatments followed with incubation at 15°C were optimal for germination. Some sclerotia germinated at 15°C without cold treatment, but germination was delayed compared with those subjected to a pre-conditioning cold period. The results from this study will be useful in prediction of ascospore production by *C. purpurea* and ultimately developing a model for predicting infection of grass flowers by the pathogen.

WITHDRAWN

Targeted lignin modification induces tolerance to soilborne fungal pathogens in alfalfa

S. UPPALAPATI (1), L. Gallego-Giraldo (1), Y. Ishiga (1), W. Li (1), L. W. Sumner (1), R. A. Dixon (1), K. S. Mysore (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.
Phytopathology 102:S4.123

Lignin modification benefits the biofuel, pulp, and forage industries through improving the accessibility of cell wall polysaccharides to chemical, microbial or enzymatic digestion. However, lignin reduction is suggested to negatively impact plant defense against pathogens, and down-regulation of cinnamate 4-hydroxylase (*C4H*), an early enzyme in the lignin pathway, resulted in increased susceptibility to fungal pathogens in alfalfa. Surprisingly, however, down-regulation of hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl

transferase (*HCT*), caffeoyl CoA 3-*O*-methyltransferase (*CCoAOMT*) and caffeic acid methyltransferase (*COMT*) resulted in increased tolerance with restricted growth of *Fusarium oxysporum* f. sp. *medicaginis* and *Phymatotrichopsis omnivora*. *HCT* down-regulated lines showed higher amount of tolerance than *CCoAOMT* and *COMT* down-regulated lines tested. Metabolite and gene expression profiling revealed that the induced tolerance in these lignin modified plants may result from increased accumulation and/or spillover of flux towards the (iso)flavonoid pathway. A continuous increase in accumulation of liquiritigenin, coumestrol, 7,4 dihydroxyflavone and medicarpin from 3 to 12 dpi with *F. oxysporum* were observed in lignin modified alfalfa roots when compared to the wild-type. Although none of these compounds completely inhibited the growth of *F. oxysporum*, *in vitro*, both 7,4 dihydroxyflavone and medicarpin caused 50-60% reduction in growth at 0.5 mM and 0.1 mM, respectively. These results demonstrate an additional commercial value of targeted lignin modification.

WITHDRAWN

WITHDRAWN

Pathogenic, mating type, and population structure of the blast pathogen from triticale

A. Urashima (1), N. TELLES (1)
(1) Universidade Federal de Sao Carlos, Araras, Brazil
Phytopathology 102:S4.124

After the outbreak of wheat blast in 1985, the area of triticale was increasing in Brazil because high yield was possible without fungicide. However, a first epidemics of blast on triticale occurred in 1995 and since then the need of chemicals has increased the cost of production and jeopardized its expansion. Blast diseases of rice and wheat have attracted more attention and little information is available on blast of oat, barley, rye and triticale. Therefore, this work aimed to characterize *Magnaporthe grisea* from triticale in terms of pathogenicity, mating type, and genetic diversity. The pathogenicity included host range of 20 triticale isolates on seven gramineous species and cross-inoculation of five isolates on rice, wheat, barley, and oat, all at seedling stage in four replications. Mating type employed 60 isolates from triticale and Bp3a (*MAT1-1*) from *Brachiaria plantaginea* and Br118.2a (*MAT1-2*) from wheat in three replications. For genetic analysis 60 triticale isolates were genotyped with 32 RAPD primers and clustered with UPGMA to generate a dendrogram of similarity. Triticale isolates infected triticale, barley, oat and wheat but not sorghum, millet or rice. On the other hand, barley, oat, wheat and rice isolates caused symptoms on triticale. Mating type was not determined because none crossed. Population structure showed that 59 triticale isolates formed a robust single cluster with more than 75% of similarity. The TtCb01-02 was the sole isolate that did not fit in this cluster and to cause disease on nine rice differentials.

Comparison of methods of DNA extraction for identification of *Leifsonia xyli* subsp. *xyli*: Feasibility for routine diagnosis in young plants in Brazil

A. URASHIMA (1), A. Zavaglia (1)
(1) Universidade Federal de Sao Carlos, Araras, Brazil
Phytopathology 102:S4.124

Mechanical harvest is getting widespread in sugarcane industry in Brazil. The main concern with this new reality is the dissemination of ratoon stunting disease (RSD), caused by *Leifsonia xyli* subsp. *xyli* (Lxx). A precise diagnosis of propagating materials is one major component of the RSD control. Laboratories in Brazil offer a RSD diagnostic service based on dot blot serology of xylem sap. Extraction of sap is not user-friendly for a large number of samples. Moreover, PCR-based analysis of RSD abounds and promises to be a viable alternative. Therefore, the present work aimed to investigate the effectiveness of PCR for RSD diagnosis for routine service, including young plants. DNA was extracted from xylem sap and internode tissue by five methods and PCR run with primers Cxx1/Cxx2. The precision of each method was measured by its sensitivity, specificity and accuracy. Subsequently, the best one was tested in five varieties with different titer of Lxx (four-month-old, first ratoon). PCR with CTAB DNA extraction from internode tissue had 100% of sensitivity, specificity and accuracy and surpassed dot blot, especially in young plants.

Development of a DNA macroarray for detection of fungal pathogens involved in the decline of young grapevines

J. R. URBEZ TORRES (1), P. Haag (2), D. T. O'Gorman (2)
(1) Pacific Agri-Food Research Centre, Summerland, BC, Canada; (2) Agriculture & Agri-Food Canada, Summerland, BC, Canada
Phytopathology 102:S4.124

Young vine decline (YVD), considered one of the most economically important diseases in the wine- and table-grape industries, causes grapevine dieback and eventual plant death in young vineyards (<6-year-old). Decline of young grapevines is primarily associated with Petri disease, caused by *Phaeomoniella chlamydospora* and several *Phaeoacremonium* species, and black-foot disease, caused by species in the genera *Campylocarpon*, *Cylindrocarpon* and *Ilyonectria*. Additionally, several species in the genus *Cadophora* have also been recently shown to be involved in YVD. Short DNA (16-25 bases long) oligonucleotide sequences (probes) from part of the beta-tubulin gene region BT2 were developed to specifically identify fungal species associated with YVD. The probes were attached to a nylon membrane by an amine modified linker arm and arranged in a precise pattern to form an array for detecting all possible fungal pathogens associated with YVD. Probe's specificity was determined by hybridizing pure cultures of the pathogens from different geographical grape-growing regions to the probes. Additionally, known ex-type specimens from the different genera associated

with YVD were also include as positive controls. The DNA array correctly identified over 60 different fungal species in the genera *Phaeomoniella*, *Phaeoacremonium*, *Campylocarpon*, *Cylindrocarpon*, *Ilyonectria*, and *Cadophora* including those showed to cause YVD symptoms. The array was validated by testing artificially inoculated grapevine cuttings as well as diseased field samples. The high specificity of this DNA array showed a promising detection system for accurate identification of YVD pathogens in a single test.

Rot disease of lettuce, cilantro, and chervil caused by *Plectosporium tabacinum*

T. USAMI (1), S. Morii (1), Y. Amemiya (1)
(1) Chiba Univ., Matsudo-city, Chiba, Japan
Phytopathology 102:S4.125

Rot diseases of hydroponically cultured leaf lettuce, cilantro (coriander), and chervil occurred in Chiba prefecture, Japan. These plants were cultured in the same nutrient solution. Stem bases of the plants were discolored and rotted soft. Vascular discoloration was also observed. Fungi that were similar in appearance were isolated from respective plants. When roots and lower stems of respective plants were soaked in spore suspensions of isolated fungi, the symptoms were observed. Therefore, we concluded that these fungal isolates are causal agents of the disease. The colonies of isolates were initially white, becoming cream-colored later. The colony surface appeared wet, with no aerial hypha. One-celled or two-celled spindle-shaped conidia were on a phialide and an adelophialide. Although the fungi grew well at 21–27°C, growth was suppressed remarkably at temperatures higher than 30°C. These morphological and cultural characters were consistent with *Plectosporium tabacinum*. The nucleotide sequences of rDNA-ITS regions of respective isolates were identical to that of *P. tabacinum*. This occurrence of the disease of each plant caused by *P. tabacinum* in Japan is the first described in the literature. Although *P. tabacinum* pathogenicity in lettuce has been reported in Italy, this report is the first in the world to describe disease effects on cilantro and chervil. This fungus appears to have a wide host range: *P. tabacinum* isolates were also pathogenic on basil and tomato.

Taxonomic reassessment of the ray blight pathogen of pyrethrum in Australia

N. VAGHEFI (1), S. J. Pethybridge (2), R. Ford (1), M. E. Nicolas (1), P. W. Crous (3), P. W. Taylor (1)
(1) Melbourne School of Land and Environment, the University of Melbourne, Melbourne, VIC, Australia; (2) Botanical Resources Australia - Agricultural Services Pty. Ltd., Ulverstone, Tasmania, Australia; (3) CBS Fungal Biodiversity Centre, Utrecht, Netherlands
Phytopathology 102:S4.125

Pyrethrum (*Tanacetum cinerariifolium*) is a perennial plant grown commercially for the extraction of the natural insecticide, pyrethrin, from its flowers. The Australian pyrethrum industry supplies more than 60% of the world's pyrethrin requirements. Ray blight is a major threat to pyrethrum production in Australia, capable of causing complete yield loss. The disease appeared in Tasmanian fields in 1995 and the causal agent was identified as *Phoma ligulicola* var. *inoxydabilis*. Further studies on the morphology and biology of the fungus revealed some divergence from the published descriptors of the type strains, which cast some doubts on its taxonomy. The aim of this study was to reassess the taxonomy of the pathogen. Australian and overseas isolates from pyrethrum and other *Asteraceae* were compared to the type strains of the ray blight pathogen based on morphological characters and multi gene phylogeny of LSU, ITS, TUB, ACT and EF sequences. Results showed that ray blight of *Asteraceae* is caused by multiple species of the genus *Stagonosporopsis*. The type strains of the ray blight pathogen, previously known as var. *inoxydabilis* and var. *ligulicola*, were elevated to species *S. inoxydabilis* and *S. chrysanthemi*, respectively. The pathogen associated with ray blight of pyrethrum in Australia was re-classified as a new species, *S. tanaceti*. This finding highlights the need for further studies into the biology of the pathogen since it may have quarantine and epidemiological implications for the industry.

DNA macroarray for the detection of fungal onion bulb rot pathogens

C. M. VAHLING-ARMSTRONG (1), J. L. Humann (1), S. Lupien (2), F. Dugan (2), L. J. du Toit (3), B. K. Schroeder (1)
(1) Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.; (3) Washington State University, Mount Vernon, WA, U.S.A.
Phytopathology 102:S4.125

Onion (*Allium cepa*) bulb rots in storage can be a major source of economic loss to the onion industry. Management of bulb rots is challenging because of the limited ability to rapidly and accurately diagnose the 25 potential bacterial and fungal pathogens that can cause bulb rots. This is compounded by the fact that infected bulbs typically do not show symptoms of infection prior to curing and storage, leaving growers vulnerable to losses after a majority of production costs (>$9,800/hectare) have been incurred. To address this, development of a DNA macroarray was initiated to identify and differentiate 13 filamentous fungi and 1 yeast known to cause onion bulb rots. The internal transcribed spacer (ITS) region of the rDNA was sequenced for >120 representative fungal isolates associated with onions, and bulb assays were performed to assess pathogenicity of the fungal isolates. Sequences of the isolates were aligned to reveal ITS regions unique to the pathogenic isolates of each species. Oligonucleotide probes were then designed to detect the unique DNA regions and rapidly identify fungal pathogens present in onion bulbs. Sensitivity of the DNA macroarray to the target pathogens increased when the primers were modified to amplify the ITS rDNA of target fungi without amplifying onion ITS rDNA. This macroarray will play a key role in the Onion ipmPIPE network, a platform integrating diagnostic tools, pest management, and marketing of *Allium* crops.

A novel method to monitor *Penicillium expansum*

R. VALDEBENITO-SANHUEZA (1)
(1) Proterra, Vacaria, Brazil
Phytopathology 102:S4.125

Penicillium expansum is the most common pathogen in the air and washing water in apple packing houses. This report describes a tool that can easily detect *P. expansum*. It consisted of covering 1/3 of glass microscope slide surface with acidified Martin agar (pH 4.5). The culture media on the microscope slide (MSMA) was further dehydrated in an oven at 30°C and stored in a sterile box. The MSMA was compared to the standard method used in Brazil to monitor *P. expansum*. In this method, the Martin agar media is placed in Petri dishes. In order to evaluate both methods, a set of 10 Petri dishes and 10 MSMA were exposed to air in storage for one hour. At the same time an aliquot of 0.1 ml of contaminated water distributed over Martin agar on Petri dishes while MSMA was dipped for 5 seconds in contaminated water. The MSMA were incubated for 3 days and the Petri dishes for 7 days at 20°C in the dark. The result showed that in the MSMA a threshold of about 5 colonies per slide represented a contamination level risk for apples compared to 100 colonies in the Petri dish method. The MSMA is an EMBRAPA patented product.

Organic chlorine to reduce apple rots caused by *Penicillium expansum*

R. VALDEBENITO-SANHUEZA (1), G. A. Meyer (2), M. C. Santos (3)
(1) Proterra, Vacaria, Brazil; (2) Proterra Research Center, Vacaria, Brazil; (3) Universidade de Caxias do Sul, Vacaria, Brazil
Phytopathology 102:S4.125

Penicillium expansum is the main target in apple sanitation in postharvest practices in Brazil. This works aims to evaluate organic chlorine disinfectants targeted to reduce apple rots in packinghouses. Organic disinfectants are supposed to have several advantages compared to the standard Sodium hypochlorite (SH). Two products containing clorhexidine digluconate (CD) and three with sodium dichloro isocyanurate (SDI), were evaluate in suppressing conidia germination. Conidia suspensions of *P. expansum* (103 con.mL^{1}) were treated with 6.25, 12.5 and 25 $\mu L.L^{1}$ of SDI and 50, 100 and 200 $\mu L.L^{2}$ of CD. Concentrations of 12.5 and 25 $\mu L.L^{1}$ of SDI were also compared with 200 and 300 $\mu L.L^{2}$ of CD and 25 and 300 $\mu L.L^{2}$ of sodium hypochloride (SH) under 6 °C and 18 °C. The number of colony forming units (CFU) on PDA was used for assessing treated and untreated samples of the conidia suspension. At the same time, a portion of the suspension was used to inoculate wounded apples. An apple rot control of 92 a 99% was obtained with 25 $\mu L.L^{1}$ of SDI, respectively. In contrast, the control obtained with CD was only 13 to 14 %. At the two higher doses all products reduced conidia survival and one of SDI's product was as efficient as SH in both temperatures, obtaining 100% of the conidia control.

Efficacy of fungicides and mixtures of fungicides for management of gladiolus rust in Mexico

A. J. VALENCIA-BOTÍN (1), J. W. Buck (2), S. Jeffers (3), C. L. Palmer (4)
(1) Univ. de Guadalajara, Guadalajara, Jalisco, Mexico; (2) University of Georgia, Griffin, GA, U.S.A.; (3) Clemson University, Clemson, SC, U.S.A.; (4) IR-4 Rutgers University, Princeton, NJ, U.S.A.
Phytopathology 102:S4.125

Gladiolus rust, caused by *Uromyces transversalis*, is widespread in Mexico and frequently affects commercial gladiolus fields. As part of an IR-4 project, two trials were conducted during Sep to Nov 2011 in Mexico —in Santa Isabel, Cholula Puebla and in Cuautla, Morelos— to evaluate the efficacy of five individual fungicides (cyproconazole, chlorothalonil , difenconazole,

epoxiconazole, flutolanil), a plant activator (acibenzolar-s-methyl [ASM]), and eight fungicide mixtures (epoxiconazole + chlorothalonil, epoxiconazole + azoxystrobin, fluoxastrobin + myclobutanil, fluoxastrobin + difenconazole, oxycarboxin + tebuconazole, trifloxystrobin + oxycarboxin, chlorothalonil + propiconazole, azoxystrobin + propiconazole) to manage gladiolus rust. Two non-treated control treatments were included: non-inoculated and inoculated. Applications began when plants had three full leaves, and fungicides were applied until run-off at 2-week intervals using a CO_2-backpack sprayer. Disease severity was recorded each week for 7 weeks after the first application. Chlorothalonil and ASM did not affect disease development, but all other treatments significantly ($P<0.05$) reduced disease severity. The two mixtures containing azoxystrobin completely prevented rust establishment. Based on these results, we confirmed that triazoles fungicides either alone or in combination with strobilurin fungicides were effective for managing gladiolus rust in Mexico.

Endornaviruses in common bean (*Phaseolus vulgaris*) germplasm

R. VALVERDE (1), R. Okada (2), S. Sabanadzovic (3), M. Pastor-Corrales (4), T. Fukuhara (2), H. Moriyama (2)
(1) Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.; (2) Laboratory of Molecular and Cellular Biology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan; (3) Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.; (4) Soybean Genomics and Improvement Laboratory, Beltsville Agricultural Research Center, Beltsville, MD, U.S.A.
Phytopathology 102:S4.126

Endornaviruses are dsRNA viruses that infect some members of the kingdoms Plantae, Fungi, and Chromista. These viruses have been reported infecting economically important crops. Although they do not cause symptoms, their overall effect to the host plant is unknown. An endornavirus has been reported in common bean (*Phaseolus vulgaris*) cv. Black Turtle Soup (BTS). The objective of this investigation was to clone and sequence the genome of this virus and determine its occurrence in a collection of 65 cultivars representing a broad spectrum of the common bean germplasm. During preliminary research, it was determined that two viral dsRNAs were present in BTS. dsRNAs from BTS were purified, cloned, and sequenced. Sequence analyses indicated that the dsRNAs infecting BTS consist of two distinct endornavirus species. The presence of dsRNAs in foliar tissues was determined by electrophoresis and RT-PCR. A duplex single tube RT-PCR protocol was developed for the simultaneous detection of the two viruses. The viruses occurred together in 38 cultivars and alone in 3 cultivars; 24 cultivars were virus-free. After screening many individual BTS plants, a virus-free plant was found. The progeny of this plant resulted in a virus-free BTS line. Preliminary visual inspections did not reveal phenotypic differences between virus-infected and virus-free plants. The results of this investigation will be useful to evaluate the effect of endornaviruses in common bean.

Water as a vehicle for transport of *Salmonella enterica* to tomato plants

A. H. VAN BRUGGEN (1), J. M. Cevallos-Cevallos (1), G. Gu (1), M. D. Danyluk (2), A. C. Wright (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.126

Several *Salmonella enterica* outbreaks have been associated with tomatoes. Yet, transmission of *Salmonella* via water to plants is poorly understood. *Salmonella* populations were monitored in 10 irrigation ponds for 1 year. Under controlled conditions, transmission of 2 gfp strains of *S. enterica* Typhimurium to tomato plants was investigated via splash dispersal and aerosolization in a rain simulator or leaf dipping in a suspension. Survival on leaf surfaces and inside inoculated and uninoculated leaves was monitored for 7-30 days on plants grown in organic or conventional soils. Internalization from leaves via stems in fruits and seeds was checked by confocal laser microscopy. *Salmonella* was isolated from each pond at some time, but there were seasonal differences dependent on oxygen content and temperature. Splash dispersal was enhanced when soil was covered by plastic compared to bare soil or natural mulch. It was greater for a *S.* Typhimurium strain without fimbriae than for one with fimbriae, especially when trichome density was low, but this was reversed for dispersal in aerosol. When a leaflet was dipped in *Salmonella* suspensions, both strains were internalized, but that with fimbriae survived better inside leaves and, in 1 experiment, was the only strain found in the phloem, fruits and seeds. Colonization was less in plants in organic than in conventional soils; fruit and seed infection was only observed for plants in conventional soil.

Comparative evaluation of the effect of plant products on the rhizosphere population of *Fusarium oxysporum* f. sp. *lycopersici* and the growth of tomato plants

G. C. VAN DER PUIJE (1), S. R. Gowen (2), A. N. Jama (2)
(1) University of Cape Coast, Cape Coast, Ghana; (2) Department of Agriculture, University of Reading, Reading, United Kingdom
Phytopathology 102:S4.126

A study was conducted at the glasshouse at the University of Reading, U. K. to compare the efficacy of aqueous extracts of *Icacina senegalensis* tuber (IcT), *Khaya senegalensis* bark (KhB) and *Azadirachta indica* leaves (NmL) using *Fusarium oxyporum* f. sp. *lycoperscici* (*Fol*) as the pathosystem. The extracts were prepared at 0, 50, 100, 150, and 200 g/L and applied to 3-week old tomato seedlings inoculated with *Fol*. The design was a randomised complete block factorial with 3 replications. KhB and NmL were significantly better than IcT at all the concentrations applied. KhB and NmL were similar at 50 to 150 g/L in reducing the rhizosphere population of *Fol*. Disease severity significantly reduced with increasing concentration except in IcT. Plant growth, measured as plant height, shoot weight and root weight, was severely reduced by IcT suggesting a phytotoxic effect. While there were no deaths in plants treated with KhB and NmL, mortality in plants treated with IcT at 50, 100, 150 and 200 g/L was, 11%, 30%, 33% and 50%, respectively. KhB and NmL were comparatively better than IcT in managing *Fol* and had a milder effect on plant growth. While plants can be a good source for managing plant diseases the study suggests the need for caution in their use.

Efficacy of resistance inducers against *Magnaporthe oryzae* causing blast disease of rice

Y. VARMA (1), S. P (2)
(1) Kerala Agricultural University, Calicut, Kerala, India; (2) Kerala Agricultural University, Thiruvananthapuram, Kerala, India
Phytopathology 102:S4.126

Blast disease of rice by *Magnaporthe oryzae* (Hebert) is the most destructive disease in almost 85 rice growing countries. IRRI reports more than 266,000 tons of rice lost in India every year. As a bio-control method, evaluation of five resistance inducers at three different concentrations *viz*., Salicylic acid, Benzoic acid, Naphthalene Acetic acid, Phosphoric acid (each @ 0.01%, 0.05%, 0.1%) and Benzothiadiazole (0.01%, 0.02%, 0.05%) with one fungicidal check, Tricyclazole 75%WP (0.06%) and one absolute control was done by a pot culture experiment during *kharif* 2008 at Regional Agricultural Research Station, Pattambi. Foliar application of resistance inducers were given on the seedlings 7th and 23rd day after transplanting. Two days after 1st application, plants were inoculated with (spore suspension of $1x10^6$ spores/ml) pathogen. Salicylic acid and Benzoic acid were the best performing resistance inducers with lowest disease incidence of 61.7% and 57.9% and lowest % of disease index of 6.31% and 6.74% compared to absolute control (95.76% DI and 78.31% PDI). In biometric observations *viz.*, height of the plant, number of total and productive tillers/hill, grain and straw weight/hill, the same resistance inducers performed better than control. The exogenous application of Salicylic acid or Benzoic acid (0.01%) has induced systemic resistance in crops and thus these inducers can be effective in bio-control methods.

Characterization of *salA*, *syrF*, and *syrG* regulatory networks involved in plant pathogenesis by *Pseudomonas syringae* pv. *syringae* B728a

V. L. VAUGHN (1), D. Gross (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 102:S4.126

Pseudomonas syringae pv. *syringae* B728a, causal agent of brown spot on bean, is an aggressive bacterial pathogen that utilizes extracellular signaling to initiate a lifestyle change from an epiphyte to a pathogen. LuxR regulatory proteins play an important role in the transcriptional regulation of a variety of biological processes involving two-component signaling, quorum sensing, and secondary metabolism. Analysis of the 6.09-Mb B728a genome identified 24 LuxR-like proteins, three of which are *salA*, *syrF*, and *syrG* located adjacent to the syringomycin gene cluster. Previous research established that mutants of *salA*, *syrF*, and *syrG* have an effect on virulence and syringomycin production. The role of *syrG* in the expression of syringomycin and other secondary metabolite genes remains unknown. Evidence showed that *syrG* is highly expressed in the apoplast; consequently, this study tested the hypothesis that *syrG* is a transcriptional regulator of secondary metabolite production that is activated in the apoplast. Deletion mutants of *salA*, *syrF*, and *syrG* in B728a were developed to define the regulatory networks controlled by these proteins. The utilization of qPCR analysis determined the influence of *syrG* on genes associated with epiphytic fitness and secondary metabolite production. By defining important components of the *syrG* regulon, current knowledge of the *salA*, *syrF*, and *syrG* regulatory networks and their role in plant pathogenesis was expanded.

Geographic and cultivar distribution of QoI-resistant *Alternaria alternata* isolates, causal agent of Alternaria brown spot on Florida tangerine hybrids

B. VEGA (1), M. M. Dewdney (2)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) University of Florida, CREC, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.127

Strobilurins are a commonly used fungicide class for *A. alternata* control in Florida, but there are increasing reports of control failure. To determine the sensitivity of the statewide *A. alternata* population, a survey was begun in 2010. More than 300 monoconidial isolates of *A. alternata* were tested from 28 citrus orchards with different QoI application histories in 7 counties. Sensitivity to azoxystrobin and pyraclostrobin was determined by RZ-based microtiter assay. A highly significant ($P < 0.001$) and strong correlation for fungicide cross-resistance was found between azoxystrobin and pyraclostrobin. Of the isolates tested, 63% were resistant to both fungicides. The EC_{50}'s for resistant isolates were greater than 5 µg/ml for azoxystrobin and 1 µg/ml for pyraclostrobin. The EC_{50}'s for sensitive isolates (37%) ranged from 0.016 to 0.696 µg/ml for azoxystrobin, and from 0.00345 to 0.0922 µg/ml for pyraclostrobin. The resistant phenotype was widespread among counties. Resistant isolates were recovered with higher frequency from the cultivar Minneola, followed by cultivars Murcott, Dancy and Orlando. Field disease severity was low in Sunburst and Orlando, moderate in Murcott and Minneola and high in Dancy and Lee. The presence of mixed sensitive and resistant populations also was observed in 13 of 28 citrus orchards. Molecular characterization of the cytochrome *b* gene will confirm if the resistant phenotype is associated with G143A substitution or an additional one.

A *Tobacco etch virus*-NW isolate overcomes two resistance genes in *Capsicum* sp.

N. Velasquez (1), J. F. MURPHY (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.127

Two important sources of pepper resistance, CA4 (*pvr1*) and Dempsey ($pvr1^2$), were evaluated for their response to inoculation with the Non-Wilting strain of *Tobacco etch virus* (TEV-NW, genus *Potyvirus*). Experiments were performed in a greenhouse with virus inoculations following standard procedures. Dempsey plants were virus-free; however, two CA4 plants had positive (but low) ELISA values with no apparent symptoms. When infected CA4 plants were used as inoculum, all newly inoculated CA4 plants had virus in non-inoculated leaves sooner with higher titers and developed systemic symptoms. This new NW isolate, referred to as NW-CA4, was tested further and shown to overcome the resistances expressed by both CA4 and Dempsey. The potyviral VPg is the viral determinant for infection of CA4 and Dempsey plants with the *pvr1* and $pvr1^2$ resistance genes encoding eIF4E. The VPg amino acid sequence for NW-CA4 was determined and compared with that of NW isolates. Substitutions were identified within regions of the VPg shown to interact with eIF4E resistance genes.

Comparison of nucleotide and amino acid sequences of three *Tobacco etch virus* strains

N. Velasquez (1), J. F. MURPHY (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.127

Genomic nucleotide sequences of three *Tobacco etch virus* strains (HAT, Mex 21 and N) were obtained by direct sequencing of PCR-amplified DNA of 13 fragments representing ~95% of each viral genome. Phylogenetic trees were constructed from the aligned nucleotide sequences and compared with other previously published TEV sequences (10 TEV strains total). All 10 TEV strains examined had 90.5-97.8% nucleotide sequence homology. The phylogenetic tree revealed 2 distinct genetic lineages among TEV sequences. Lineage 1 grouped strains Mex21, NW and N and lineage 2 grouped HAT with the other 6 TEV strains. Lineage 2 was further subdivided into two monophyletic clades. Amino acid comparison of the TEV strains showed sequence identities of 96.8-99.8%. The phylogenetic tree for amino acid sequences revealed 3 lineages. Lineage 1 grouped Mex21, N and NW, whereas HAT was grouped in lineage 2. Sequence comparisons of individual coding regions indicated the Nuclear Inclusion b region was much more conserved than the other nonstructural regions.

TAL effectors enhance virulence on diverse rice varieties when introduced individually into a TAL effector-deficient strain of *Xanthomonas oryzae*

V. VERDIER (1), L. Triplett (1), A. Hummel (2), R. Corral (1), A. Cernadas (2), A. Bogdanove (2), J. Leach (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.; (2) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.127

Most strains of *Xanthomonas oryzae*, the causal agents of bacterial leaf blight and bacterial leaf streak of rice, encode large numbers of TAL (Transcriptional Activator-Like) effectors. TAL effectors activate transcription of specific host genes, but the roles of individual TAL effectors in virulence in different host plant genetic backgrounds are poorly understood. We transformed the weakly virulent, TAL effector-free strain X11-5A of *X. oryzae* with individual TAL effector genes from more virulent strains to assess the virulence contributions of those TAL effectors in 21 diverse rice varieties. TAL effector genes that activate SWEET family sugar transporters increased X11-5A virulence on 13 of 21 varieties, to varying extents depending on the variety and the TAL effector gene. The previously identified transcriptional target for each effector was strongly activated by that TAL effector in all cultivars tested. These results confirm that SWEET-targeting TAL effectors are major contributors to pathogen virulence, yet reveals that their relative importance in virulence depends on host genetic background.

WITHDRAWN

Characterization of *Botryosphaeria dothidea* isolates causing postharvest decay on apple fruit

I. Vico (1), K. A. Peter (1), V. L. Gaskins (1), W. J. Janisieswicz (2), W. M. JURICK (3)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) USDA-ARS, AFRS, Kearneysville, WV, U.S.A.; (3) USDA-ARS, Food Quality Laboratory, Beltsville, MD, U.S.A.
Phytopathology 102:S4.127

Botryosphaeria dothidea has a worldwide distribution infecting species from over 80 genera of mainly woody plants. It is an important pathogen causing canker on limbs and branches of apple trees in orchards. Additionally, the fungus can cause white rot of apple fruit mostly preharvest but is also considered a postharvest pathogen. In this study, *B. dothidea* was isolated from decayed apple fruit collected in Pennsylvania cold storages. Postharvest fungicides were not applied to these apples before storage. Collected fruit exhibited large brown spots with an irregular margin. The decayed area expanded towards the core of the fruit and the infected tissue was soft. Obtained isolates proved to be pathogenic and differed in virulence on 'Golden Delicous' apple fruit. Fungal mycelia on PDA were initially white but turned either olive gray or black with a white margin after 7 days. Isolates were separated into groups based on their growth rate on PDA and virulence on apple fruit. Morphological characteristics were examined on V8 juice, oatmeal and water agar media, and virulence was tested on different fruit. The isolates were sensitive to the three commonly used postharvest fungicides. Molecular identification of the fungus was based on PCR amplification and sequencing of ribosomal DNA ITS region using ITS5 and ITS4 primers. Phylogenetic analysis of ITS sequences revealed several distinct clades within the group of isolates from apple fruit which were separate from 6 *B. dothidea* isolates from other hosts from around the world.

Genome organization and structure of a virus associated with cherry twisted leaf and apricot ring pox diseases
D. V. VILLAMOR (1), K. C. Eastwell (1)
(1) Washington State University, IAREC, Prosser, WA, U.S.A.
Phytopathology 102:S4.128

Cherry twisted leaf (CTL) and apricot ring pox (ARP) diseases are graft transmissible diseases assumed to be caused by closely related viruses. In a preliminary study, virus like sequences closely related to *Cherry green ring mottle virus* (CGRMV) and *Cherry necrotic rusty mottle virus* (CNRMV) were identified from known sources of CTL and ARP trees. Group specific primers designed from the 3'-terminus regions of CGRMV and CNRMV yielded amplification products of 1.1kbp from CTL and ARP source trees. Sequencing of representative clones generated two different sequences from each CTL and ARP source. One sequence from each source represented an isolate of CGRMV with shared amino acid (aa) sequence identities of 97% (CTL) and 98% (ARP) to the coat protein (CP) sequence of CGRMV. The sequences of the second virus from the CTL and ARP sources were closely related to CNRMV with aa identities of 80% and 79%, respectively, and 88% identical to each other. Primers designed from sequences obtained by 5'- and 3'-RACE allowed the complete 8.4 kb genome of the non-CGRMV from both CTL and ARP source trees to be cloned and sequenced. This virus is hereby named *Cherry twisted leaf associated virus* (CTLaV). Data on complete genomic sequences of both isolates of CTLaV will be presented.

Detection and quantification of trifloxystrobin-resistant *Venturia inaequalis* using allele-specific real-time PCR
S. M. VILLANI (1), K. D. Cox (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.128

Apple scab, caused by *Venturia inaequalis*, is an economically devastating disease of apple in the northeastern United States. The proliferation of resistance to DMI fungicides, combined with the limited release of novel chemistries with high levels of activity against apple scab has led to a greater reliance on QoI fungicides. Current techniques used to screen for QoI resistance in *V. inaequalis* provide only a qualitative analysis and fail to account for the heteroplasmic nature of the mitochondrial cytochrome B (*CYTB*) gene, the target site of QoI fungicides. To identify emerging QoI resistance in apple scab populations we quantified sensitive and resistant alleles in 31 monoconidial *V. inaequalis* isolates collected from 14 orchards in the northeastern US. Allele-specific PCR primers were designed to detect and quantify the relative abundance of the resistant A143 allele using allele-specific qPCR. The percentage of resistant alleles in monoconidial *V. inaequalis* isolates ranged from 0-100%. Isolates devoid of the resistant allele had % relative growth (%RG) values on trifloxystrobin-amended media ranging from 0-45%, while isolates in which only A143 was detected ranged from 81-135% RG. Nearly one third of isolates screened demonstrated heterogeneity of the *CYTB* gene having both resistant and sensitive alleles. For populations composed of such isolates, QoIs may still provide a high level of efficacy following application curtailment.

Characterizing the role of *CYP51A1* overexpression in myclobutanil- and difenoconazole-resistant *Venturia inaequalis*
S. M. VILLANI (1), J. Freier (2), K. D. Cox (1)
(1) Cornell University, Geneva, NY, U.S.A.; (2) Hobart and William Smith Colleges, Geneva, NY, U.S.A.
Phytopathology 102:S4.128

Due to their site-specific mode of action, DMI resistance in the apple scab pathogen *Venturia inaequalis* is well established in the northeastern US. Myclobutanil resistance in *V. inaequalis* has previously been attributed to *CYP51A1* overexpression and insertions upstream of the gene. The release of new DMI chemistries necessitates a reexamination of the role of *CYP51A1* overexpression in isolates demonstrating varying degrees of DMI resistance. *V. inaequalis* isolates from northeastern US apple orchards of varying sensitivity to myclobutanil and difenoconazole (0.1 µg/ml) were examined for *CYP51A1* upstream anomalies and expression of the gene. Amplification upstream of *CYP51A1* identified 2 novel insertions of 186 & 196 bp. In addition, the upstream regions of several isolates contained other insertions that have not been fully characterized. Within the various insertions, 11 promoters were identified but not associated with DMI sensitivity. Expression of *CYP51A1* was evaluated for all isolates using RT-qPCR. No relationship was found between myclobutanil sensitivity and expression of the *CYP51A1* gene, however there was a strong relationship between difenoconazole sensitivity and expression ($P < 0.0001$, $R^2 = 0.942$). The results suggest that cross resistance is not always observed within DMI fungicides and that overexpression of *CYP51A1* is one of several potential mechanisms of DMI fungicide resistance in *V. inaequalis*.

Bermudagrass putting greens: A closer look at the root of the problem
P. L. VINES (1)
(1) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 102:S4.128

The growth and quality of bermudagrass putting greens decline during the summer and early fall months in the Deep South. The root systems often appear blackened, rotted, or stunted. Dark, runner hyphae are typically associated with roots that appear asymptomatic. The focus of this study was to isolate and identify ectotrophic fungi from healthy roots. Roots from a TifDwarf bermudagrass putting green colonized with dark, runner hyphae were cut into 1-cm sections ($n = 200$), surface disinfested with a 0.6% NaOCl solution, rinsed three consecutive times with sterile-distilled water, plated on modified potato dextrose agar (PDA), and incubated for 7 days at room temperature under 24 hour fluorescent light. Hyphal tips were transferred to PDA. Genomic DNA was extracted from sterile pure cultures of isolated fungi. The internal transcribe spacer (ITS) regions of rDNA were amplified by PCR using ITS1 and ITS4 primers. Each of the sequences shared 94 to 95% similarity to other sequences found in the GenBank. Of the ectotrophic fungi isolated from bermudagrass roots, 63% were most similar to *Gaeumannomyces cylindrosporus*, 26% to *Magnaporthe poae*, 5% to *G. incrustans*, and 5% to *M. rhizophila*. Future research will focus on determining the role each of the fungi play in the decline of the root systems of bermudagrass putting greens.

A comparison of two isolation techniques for *Geosmithia morbida*, the causal agent of thousand cankers disease in black walnut
L. VITO (1), J. Grant (1), M. Windham (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 102:S4.128

Thousand cankers disease of black walnut is caused by the fungus *Geosmithia morbida* and is vectored by the beetle, *Pityophthorus juglandis*. *Geosmithia morbida* has killed thousands of black walnut trees in the western part of the United States where they are grown for landscape and nutmeat purposes. The disease was first confirmed in Tennessee (within the native range of black walnut) in August, 2010 and since then, a large number of limb samples from suspect trees have been submitted for fungal screening. The fungus may be isolated from cankers and/or beetles, and this study was conducted to determine which method was more efficient at isolating pure cultures. Beetles were individually processed by placing them in sterile micro-centrifuge tubes, rinsing them with 200µl sterile water for 1 min, then transferring the water to petri dishes containing 1/10 PDA. Cankers were processed by removing thin sections of suspect tissue with a scalpel and placing them directly onto the same medium. Samples were checked daily for emerging hyphae which were transferred to fresh medium. The cultures were confirmed to be *Geosmithia* by microscopic identification. Twelve cultures were obtained from 88 beetles (14%), whereas 28 cultures were obtained from 98 canker samples (29%). Direct plating of diseased tissue is about twice as efficient for obtaining fungal cultures as beetle rinsing, has fewer input requirements, and is more feasible at this time.

A multiplexed immunofluorescence method identifies *Phakopsora pachyrhizi* urediniospores and determines their viability
R. VITTAL (1), J. S. Haudenshield (2), G. L. Hartman (3)
(1) University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.; (2) USDA-ARS, Urbana, IL, U.S.A.; (3) USDA-ARS, University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.
Phytopathology 102:S4.128

Soybean rust, caused by *Phakopsora pachyrhizi*, was reported for the first time in Brazil in 2001 and the Continental United States of America in 2004, and since then, research on the disease and its pathogen has greatly increased. One area has focused on detecting urediniospores by microscopy, or molecular techniques but the methods cannot determine whether the spores are viable. We developed a method to detect viable *P. pachyrhizi* urediniospores using an immunofluorescent assay combined with propidium iodide (PI) staining. Antibodies reacted to *P. pachyrhizi* and did not react with other common soybean pathogens based on an indirect immunofluorescent assay. Two vital staining techniques were used to assess viability of *P. pachyrhizi* urediniospores: one combined carboxy fluorescein diacetate (CFDA) and PI, and the other utilized FUN 1. Using the CFDA-PI method, viable spores stained green with CFDA and non-viable spores counterstained red with PI. Using the FUN 1 method, intravacuolar structures were induced to form within metabolically active urediniospores, causing them to fluoresce bright red to reddish-orange, whereas dead spores had an extremely diffused faint-fluorescence. An immunofluorescence technique in combination with PI counterstaining was developed to specifically detect viable *P. pachyrhizi* urediniospores. The method is rapid and reliable with a potential for application in forecasting soybean rust based on the detection of viable urediniospores.

WITHDRAWN

Kasugamycin and kasugamycin-fungicide mixtures for managing bacterial spot of tomato

L. WADE (1), H. Forster (2), J. E. Adaskaveg (2)
(1) Arysta LifeScience, Roseville, CA, U.S.A.; (2) University of California, Riverside, CA, U.S.A.
Phytopathology 102:S4.129

Bacterial spot of tomatoes caused by four species of *Xanthomonas* can be a devastating disease resulting in plant defoliation and reduction of fruit quality and yield. The disease can be very difficult to manage because there are few effective registered treatments, tomatoes are often grown in regions where conditions favor infection, and copper-resistance is widespread in pathogen populations. Mature, fruit-bearing 'Roma' tomato plants (10 plants/3-m replication) were treated in the field with test materials using a mist-blower, air-dried, and then inoculated with a copper-sensitive strain of *X. vesicatoria* (10^7 cfu/ml and 0.5 L/rep) using the same equipment. Plots were watered for 5 h on three consecutive days using overhead sprinklers. After 3 weeks, severity of bacterial spot was rated on 30 randomly collected leaves/rep. Mancozeb (1200 g/Ha) was least effective; whereas kasugamycin (100 mg/L), fixed copper (500 g Mce/Ha), quinoxyfen (93 g/Ha) and mixtures of copper-mancozeb and kasugamycin-mancozeb effectively and similarly reduced disease severity from that of the untreated control. Kasugamycin-quinoxyfen was the most effective in these trials reducing severity by an average of 80%. Use of kasugamycin or any product will depend on integration of resistance management strategies including tank mixtures of different products with different modes of action and their rotation when multiple applications are needed for managing the disease under favorable environments.

WITHDRAWN

Normal destruction of cortical tissue in pine roots

C. H. WALKINSHAW (1), W. J. Otrosina (1)
(1) USDA Forest Service, Athens, GA, U.S.A.
Phytopathology 102:S4.129

Health of conifer roots is the subject of an increasing number of investigations in the southeast United States. Light microscopy of primary and secondary tissue was used to measure changes in conifer cortical cells in feeder roots. Death of cells in the xylem is a normal event in the anatomy of root development. These events are measured using three stain schedules and recording nine interacting traits. Growth of feeder roots in conifers involves a number of events that activate hydrolysis in cortical cells. The hypothesis is that death of cortical cells seems to be necessary for establishing xylem, phloem and other tissues such as resin ducts. Death of cortical tissue was distinct from death due to expanding necrosis in the secondary tissue. Primary tissue is largely replaced with tannins and over-sized parenchyma. This progress of necrosis in the secondary root cortex shows that feeder roots could be damaged in the process. This study adds to our knowledge of root pathology and could help explain possible decline in southern pine forests.

Breeding papaya on Guam for PRSV tolerance

G. WALL (1), A. Wiecko (1)
(1) University of Guam, Mangilao, Guam
Phytopathology 102:S4.129

PRSV is the most production-limiting disease of papaya in the Mariana Islands. Some years ago we observed a local papaya on Guam that was tolerant to PRSV and other important diseases during evaluation trials; only three entries survived to the end of the 2-year trial: Sun Up, Rainbow and Dagua Yellow. The first 2 (transgenic from Hawaii) tested PRV negative by ELISA, while Dagua Yellow tested positive. In spite of that, this papaya exhibited only mild virus symptoms and continued to produce fruit. For that reason we embarked in a breeding program to improve Dagua Yellow by eliminating the male character and also changing its flesh color from yellow to red (the local market favorite). We now have F_4 seed of PRSV-tolerant Dagua Yellow, Orange and Red.

Grapevines undergo varying shifts in secondary metabolic profiles when infected with *Xylella fastidiosa*

C. WALLIS (1), J. Chen (1)
(1) USDA-ARS, Parlier, CA, U.S.A.
Phytopathology 102:S4.129

Pierce's disease (PD) is a devastating disease of grapevine caused by the bacterial pathogen *Xylella fastidiosa* (Xf). Key to the development and optimization of PD-tolerant grape cultivars is improved understanding about how grapevines defend themselves against Xf. This study complements histological and molecular genetic studies by observing shifts in host metabolic profiles that occur when grapevines are infected with Xf. Infected grapevines initially possessed increased levels of specific defense-associated phenolic compounds in xylem sap, pulverized xylem tissues, and xylem cell walls. Of particular interest were observed increases in procyanidin and stilbenoid content, as both of these secondary metabolite classes were previously associated with grapevine tolerance to other pathogen infections. However, infected grapevines ceased to possess greater levels of these defense-related metabolites once PD symptoms were observed. Furthermore, infected grapevines with severe symptoms of PD (with greater than 50% of leaf tissue necrotic) had lower phenolic levels than non-infected controls. It was likely that severely disease plants were hampered in capacity to produce primary metabolite precursors necessary for phenolic compound production. Thus, a reduction in a group of compounds normally associated with defense against pathogens was observed in hosts expressing severe PD symptoms after a lengthy infection with Xf.

Large-scale shifts in potato (*Solanum tuberosum*) tuber physiology occur following infection by '*Candidatus* Liberibacter solanacearum'

C. WALLIS (1), A. Rashed (2), C. M. Rush (2)
(1) USDA-ARS, Parlier, CA, U.S.A.; (2) Texas AgriLife Research, Bushland, TX, U.S.A.
Phytopathology 102:S4.129

Zebra chip disease (ZC), putatively caused by '*Candidatus* Liberibacter solanacearum' (Lso), is an emerging threat to worldwide potato (*Solanum tuberosum*) production, as the disease renders infected tubers unmarketable due to increased browning symptoms when tubers are cut or fried. Potato tubers exhibiting zebra chip symptoms previously were characterized as possessing increased levels of phenolics and amino acids. Both phenolics and amino acids could result in increased ZC-characteristic non-enzymatic and enzymatic browning. However, the development of phenolics and amino acids

as an Lso infection progresses has not been documented. Likewise, increased sugar levels also could contribute to browning symptoms characteristic of ZC. Thus, changes in concentrations of tuber phenolics, amino acids, and reducing sugars were monitored over nine weeks after inoculation with Lso for both apical and terminal ends of potato tubers. This study also correlated levels of phenolics, amino acids, and sugars with zebra chip symptoms and Lso titers. Particular phenolics, amino acids, and sugars were more concentrated in tubers infected with Lso for longer durations. Terminal ends of tubers possessed greater phenolic and amino acid levels than apical ends. Levels of phenolics, most amino acids, fructose, and glucose were significantly positively associated with tuber symptoms. However, levels of these compounds were not significantly associated with Lso titers.

The influence of parasitic interactions on host gene silencing

E. WALSH (1)
(1) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 102:S4.130

Many of the plant pathogens responsible for reducing crop yields have a biotrophic lifestyle, yet much remains unclear about how these parasites succeed at draining resources from their host. One such parasite, root-knot nematode (RKN, *Meloidogyne* spp.), establishes intimate feeding sites (giant cells) within the roots of a variety of plant hosts. Microarray data generated from laser-captured giant cells in *Arabidopsis thaliana* roots suggest that the RKN infection process may be influencing mechanisms in the host's gene silencing pathways. Subsets of genes regulated by small RNAs, as well as the expression of several silencing machinery components, are upregulated during the nematode infection process. Preliminary results examining the effects of compromising these silencing pathways, suggest these components influence the host's susceptibility to RKN infection. Other lines of evidence suggesting disruption (or suppression) of these pathways have been generated using multiple transgenic tobacco lines expressing a silenced reporter gene. In addition to RKN, soybean cyst nematode (SCN, *Heterodera glycines*), and the parasitic plant dodder (*Cuscuta pentagonia*), all trigger restoration of the reporter gene during their biotrophic interactions. Confirmed involvement of host gene silencing with the nematode parasitism process would be noteworthy as it would heighten our understanding of the roles and connections between silencing and defense response pathways.

Stripe rust epidemics of wheat and barley and races of *Puccinia striiformis* identified in the United States in 2011

A. Wan (1), X. CHEN (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.130

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), was extremely severe in the Pacific Northwest and relatively low in the other regions of the US in 2011. Stripe rust of barley, *P. striiformis* f. sp. *hordei* (Psh), occurred in California, Idaho, and Washington at low levels. Stripe rust samples collected from 19 states were tested on 18 wheat and 12 barley differentials for identifying PSTv and PSH races, respectively. Eight previously existing Psh races were detected, of which PSH-33 (virulent on Topper and Abed Binder 12) was predominant (58%). A total of 35 PSTv races were detected including 10 new races. Among them, 33 races were detected in the western US and 10 in the eastern US. Race PSTv-11 (virulent to *Yr1*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrExp2*, and *YrTye*) had the highest frequency (34%), but was generally restricted in the western US. PSTv-37 (virulent to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*) was the most distributed race. Among 10 new races, PSTv-51 (virulent on 16 of the 18 single *Yr* gene lines used to differentiate Pst races) could be more dangerous if it becomes predominant. High virulence frequencies (>80%) were detected for *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr44*, and *YrExp2*; moderate (40-80%) for *Yr1*, *Yr43*, *YrTr1*, and *YrTye*; low (<10%) for *Yr10*, *Yr24*, *Yr32*, and *YrSP*; and none for *Yr5* and *Yr15*, indicating that these two genes are still effective against the Pst population.

Differential regulation of phenazine biosynthesis by RpeA and RpeB in *Pseudomonas chlororaphis* strain 30-84

D. WANG (1), J. Yu (1), L. S. Pierson III (1), E. A. Pierson (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 102:S4.130

RpeA is a two-component sensor protein that negatively controls biosynthesis of phenazines, which are required for biological control activity by *Pseudomonas chlororaphis* 30-84. In this study, we identified the cognate response regulator RpeB and investigated how RpeA and RpeB interact with the PhzR/PhzI quorum sensing system and other regulatory genes known to control phenazine production. In contrast to the *rpeA* mutant, quantitative real-time PCR revealed that expression of the phenazine biosynthetic genesas well as the *pip* and *phzR* regulatory genes were significantly reduced in an *rpeB* mutant, suggesting positive control of phenzaines by RpeB. Complementation assays showed that over-expression of *pip in trans* rescued phenazine production in an *rpeB* mutant; whereas multiple copies of *rpeB* genes were unable to restore phenazine production in a *pip* or *phzR* mutant. These results indicate that RpeA and RpeB differentially regulate phenazine production and act upstream of Pip and PhzR in the phenazine regulatory network. The differential regulatory functions for RpeA and RpeB also affected strain 30-84's capacity for fungal inhibition. Based on these results, a model is proposed to illustrate the relationship of RpeA/RpeB to other regulatory genes controlling phenazine biosynthesis in *P. chlororaphis* strain 30-84, a regulatory hierarchy that may be conserved in other pseudomonads and play a role in stress response.

Identification of blast resistance genes for managing rice blast disease

J. WANG (1), Y. Jia (2), J. Wen (1), W. Liu (1), J. Ren (1)
(1) Jilin Academy of Agricultural Sciences, Changchun, Peoples Republic of China; (2) USDA-ARS DBNRRC, Stuttgart, AR, U.S.A.
Phytopathology 102:S4.130

Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most devastating diseases worldwide. In the present study, an international set of monogenic differentials carrying 24 major blast resistance (*R*) genes (*Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita2*, *Piz*, *Piz-t*, *Pi1*, *Piz-5*, *Pi3*, *Pi5*, *Pi7*(t), *Pi9*, *Pil2*(t), *Pi11*(t), *Pi19*, and *Pi20*) and the recurrent parent LTH (Lijiangxintuanheigu) for these monogenic lines were evaluated with 44 blast isolates collected from the major rice areas in Jilin Province. Rice plants grown to the 3 to 4 leaf stage were inoculated with blast conidia suspensions at 5 X 10^5 conidia/ml under controlled environmental conditions. Six to 7 days after inoculation, disease reactions were evaluated. It was found that LTH was susceptible to all 44 isolates and all 24 monogenic lines were resistant to at least 4 isolates of *M. oryzae*. Among them, the percentage of isolates that demonstrated resistant reactions with the monogenic lines carrying *Pi9*, *Pi19*, *Piz*, *Piz-5*, *Piz-t*, *Pi12*(t), *Pi5*(t) and *Pik-h* were 94.2%, 84.1%, 81.8%, 81.8%, 79.5%, 72.7%, 68.2% and 68.2%, respectively. These results suggest that *Pi9*, *Pi19*, *Piz*, *Piz-5*, *Piz-t*, *Pi12*(t), *Pi5*(t) and *Pik-h* may be important for preventing blast disease in Jilin province. Progress on resistant spectra of these blast resistance genes will be presented.

Biological and molecular characterization of a distinct genetic variant of *Sweet potato virus G*

M. WANG (1), J. Abad (2), R. Li (1)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) USDA APHIS, Beltsville, MD, U.S.A.
Phytopathology 102:S4.130

A diseased sweet potato plant (WT325) originally collected from Taiwan induced vein chlorosis on *Ipomoea setosa* and vein banding, rugosity and leaf distortion on sweet potato pre-infected with *Sweet potato chlorotic stunt virus*, respectively, after grafting inoculation. A potyvirus was detected from the WT325 plant both by ELISA using the universal potyvirus group monoclonal antibody and by RT-PCR using group and subgroup degenerate primers. Sequence analysis of the amplicon showed that the virus isolated from WT325 shared nucleotide (nt) sequence identities of approximately 83-99% at the coat protein region with other SPVG isolates and was clustered with 16 Asian SPVG isolates in a distinct genetic group. The complete nucleotide sequence of SPVG-WT325 was determined to be 10,801 nt, excluding the 3'-poly(A) tail. The genomic organization and structure is identical to that of a North Carolina (NC) isolate of SPVG, containing a single large open reading frame with larger P1 and coat protein regions and a novel, unique ORF (PISPO) within the P1 region. Sequence comparisons show the WT325 and NC isolates showed identities of 78.6% at the complete genomic sequence and 87.6% at polyprotein sequence level, respectively. Genetic variation occurs mainly at the 3' one-third region of genome. The results reveal that WT325 represents a distinct genetic group of SPVG, and SPVG is also a component of synergistic disease complex of sweet potato.

***hmsF* Is a virulence factor of the citrus canker pathogen *Xanthomonas citri* subsp. *citri* 306**

J. WANG (1), Q. Yan (1), N. Wang (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.130

Xanthomonas citri subsp. *citri* is a Gram-negative aerobic bacterium that causes citrus canker, which is one of the most devastating diseases of most commercial citrus cultivars, responsible for significant losses worldwide. In our recent study, a *hmsF* (XAC1812) insertion mutant deficient in virulence was identified by screening the EZ-Tn5 mutant library *X. citri* subsp. *citri*.

The *hmsF* gene encodes a putative polysaccharide deacetylase, and the products of the *hms* operon are required for the biofilm formation of *Yersinia pestis*. It has been reported that the expression of this gene was regulated by QS and the HmsF protein has physical interaction with RpfG, which is a two component system response regulator and is responsible for signal perception and transduction system for DSF family signal-mediated quorum sensing in *X. citri* subsp. *citri*. The *hmsF* mutant was deficient in growth in host plant grapefruit. The reduced virulence of the *hmsF* mutant might result from its effect on tolerance of oxidative stress since the tolerance of the *hmsF* mutant toward H_2O_2 was reduced. During infection, *X. citri* subsp. *citri* needs to overcome the harmful effects of ROS, primarily H_2O_2, encountered at the site of invasion. How *hmsF* contributes to the virulence mechanism of *X. citri* subsp. *citri* is presented in this study.

Development of high-throughput assays for rapid and accurate 10-plex detection of citrus pathogens

J. WANG (1), G. Vidalakis (1), R. F. Lee (2), R. K. Yokomi (3)
(1) University of California-Riverside, Riverside, CA, U.S.A.; (2) National Clonal Germplasm Repository for Citrus & Dates, USDA-ARS, Riverside, CA, U.S.A.; (3) USDA-ARS, Parlier, CA, U.S.A.
Phytopathology 102:S4.131

The limitation to reliably detect and identify multiple plant pathogens simultaneously with present techniques, especially in woody perennial hosts, has led to the development of new molecular approaches. In this study, a Luminex-based system was developed that provided a robust and sensitive test for the simultaneous detection, identification, and quantification for nine citrus pathogen targets and a housekeeping citrus gene as an internal control. The protocols developed here is the first to successfully detect up to 10 targets in one multiplex assay. A procedure for high throughput robotic extraction and purification of nucleic acid targets, optimized for citrus tissues, was developed and used with the Luminex-based system to increase uniformity and cost effectiveness of the test. The assay is user friendly and can be applied for detection of endemic and/or exotic plant pathogens in quarantine or certification programs or monitoring services of plant health status and disease management programs.

Nutritional and environmental effects on germination and appressorium formation of *Guignardia citricarpa* conidia

N. WANG (1), M. M. Dewdney (2)
(1) CREC, University of Florida, Lake Alfred, FL, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.131

Guignardia citricarpa is an important fungal pathogen causing citrus black spot in many citrus-producing areas worldwide. To understand the requirements for conidial germination and appressorium formation (AF), the effects of nutrition, pH, temperature and incubation time were evaluated *in vitro*. Germination rarely occurred in sterile water (< 1%) whereas conidial germination and AF were favored in all tested citrus juice sources, especially in Valencia juice (> 85%; $P < 0.05$). Juice quality analysis did not show an association between oil and germination or AF and low Brix/acid ratio juices had lower germination and AF rates (< 50%, $P < 0.05$). The Valencia juice effect was concentration-dependent and maximum germination and AF rates were achieved in 1.5% juice. Conidia germinated only within the pH range of 3 to 6 with the optimum pH at 3.4. Most carbon, nitrogen or complex nutrient sources did not stimulate germination or AF, except 75 mM ammonium nitrate, ¾ strength PDB and 0.75% yeast extract ($P < 0.001$). Conidia germinated between 12 to 32°C with the optimum temperature at 24°C and no germination occurred at 36°C or below 8°C ($P < 0.001$). Incubation time of 2-3 h and 5-6 h at optimum conditions were required for germination and AF, respectively, and 18-24 h was required to reach peak germination. Taken together, these results provide further understanding of the biology and environmental conditions necessary for germination and AF of *G. citricarpa* conidia.

Detection and characterization of *Banana bract mosaic virus* in flowering ginger in Hawaii

I. Wang (1), D. Sether (1), W. Borth (1), M. Melzer (1), K. Dey (1), J. HU (1)
(1) University of Hawaii, Honolulu, HI, U.S.A.
Phytopathology 102:S4.131

In June 2009, flowering ginger plants, [*Alpinia purpurata* (Vieill.) K. Schum.] in Waimanalo, Hawaii were reported with symptoms that included severe mosaic on the leaves. Flowers displayed cupping and browning with reduced size and shortened shelf life. Symptomatic ginger plants were also identified at Lyon Arboretum, Honolulu, Hawaii. Double-stranded RNAs (dsRNAs) were isolated from pooled leaf samples collected from symptomatic plants at the two locations. Partial cloning and sequence analysis of approximately 6 kb of the dsRNA revealed 95%-98% nucleotide identity to sequences of the P1, HC-Pro, C1, 6K2, VpG , NIb, and CP genes and the 3'UTR region of *Banana bract mosaic virus* (BBrMV). A rapid, reliable, and robust reverse transcription PCR (RT-PCR) detection assay was developed for BBrMV. Samples collected from symptomatic and asymptomatic red and pink ginger plants from three nurseries in Waimanalo and from Lyon Arboretum were tested for BBrMV using this RT-PCR assay. Asymptomatic ginger consistently tested negative, whereas all 42 symptomatic red and pink ginger plants tested positive for BBrMV. RT-PCR amplicons were cloned into pGEM-T-Easy, sequenced, and found to be 99% identical to BBrMV. Preliminary evidence indicates that BBrMV can be acquired from infected ornamental ginger and transmitted to banana by *Pentalonia nigronervosa*. This raises the possibility that this virus, which is primarily found in banana, may also become established in Hawaii's valuable banana farms.

The orphan gene amyR, a homolog of *Escherichia coli* ybjN, is a negative regulator of amylovoran production in *Erwinia amylovora*

D. Wang (1), Y. ZHAO (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.131

We have previously reported the characterization of an orphan gene ybjN from *Escherichia coli*. In this study, we attempted to understand the role of amyR in *Erwinia amylovora*, a functionally conserved homolog of *E. coli* ybjN. As reported earlier, amylovoran production in the amyR knockout mutant is about ten-fold higher than that in the wild type (WT) strain. Interesting, when multicopy plasmid containing the amyR gene was introduced into the amyR mutant or WT strains, amylovoran production was strongly inhibited. Furthermore, amylovoran production was also suppressed in various amylovoran-over-producing mutants such as gacSA when containing multicopy of amyR gene. Consistent with amylovoran production, an inverse correlation was observed between amyR expression and expression of amylovoran biosynthetic genes. However, the amyR knockout mutant and over-expression strains both showed reduced levan production, another exopolysaccharide produced by *E. amylovora*. Virulence assays demonstrated that, while the amyR mutant was capable of inducing slightly severe disease than that of the WT strain, strains over-expressing amyR gene were completely abolished in causing disease on apple shoots and caused reduced disease on immature pear fruits. The regulon of amyR in *E. amylovora* was also determined using microarray. These results suggest that AmyR plays an important role in regulating exopolysaccharide production, and thus virulence in *E. amylovora*.

A Nep1-like fungal toxin targets a conserved ubiquitin-like protein in rice for necrotic cell death

Q. Wang (1), Z. Liu (1), Y. YANG (1)
(1) Penn State University, University Park, PA, U.S.A.
Phytopathology 102:S4.131

Necrosis and ethylene-inducing peptide1 (Nep1)-like proteins (NLPs) are virulent toxins commonly produced by many bacterial, oomycete and fungal pathogens. For example, rice blast fungus (*Magnaporthe oryzae*) contains a family of four MoNLPs that are capable of eliciting necrotic cell death in both dicots and monocots. To identify potential host cellular target(s) of NLP toxins, we have recently isolated a number of the MoNLP1-interacting rice proteins by the yeast two-hybrid screening. Among them is OsNPI1 (*Oryza sativa* NLP Interactor1), which is a ubiquitin-like protein highly conserved in eukaryotes. The interaction between MoNLP1 and OsNPI1 has been verified by in vitro protein pull-down assay and in vivo co-immunoprecipitation. The OsNPI protein is predominantly localized at the plasma membrane, but is also present in nucleus and cytoplasm. Quantitative RT-PCR shows that *OsNPI1* is constitutively expressed in rice plants and is not induced by the infection of *M. oryzae*. However, its protein level increases significantly in response to the fungal infection. Interestingly, suppression of *OsNPI1* by RNA interference in transgenic rice leads to necrotic lesion formation and reduced plant growth. In addition, knockout of the *NPI1* orthologue in *Arabidopsis* T-DNA mutants resulted in retarded plant growth and lethal phenotype. Preliminary results reveal that *OsNPI1* RNAi lines are more resistant to rice blast fungus whereas *OsNPI1* overexpression lines appear to be more susceptible to *M. oryzae* infection. Additional biochemical and functional analyses are being conducted to further characterize the MoNLP1-OsNPI1 interaction and to elucidate its underlying mechanism that mediates necrotic cell death and microbial pathogenesis.

Improved molecular detection of the soybean sudden death syndrome pathogen *Fusarium virguliforme* by real-time qPCR

J. WANG (1), J. L. Jacobs (1), M. I. Chilvers (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.131

Fusarium virguliforme is the causal pathogen of soybean sudden death syndrome (SDS), which can cause significant yield losses in the Midwest.

Traditional *F. virguliforme* detection techniques are labor intensive and lack sensitivity and specificity due to the slow growth and varied colony morphology of the pathogen. Current PCR based detection techniques for *F. virguliforme* are now known to lack sensitivity or specificity due to the recent taxonomic revision of the *Fusarium* spp. associated with SDS and root rot. A real-time qPCRassay to detect *F. virguliforme* was developed based on intergenic spacer region (IGS) of the ribosomal RNA repeats of *F. virguliforme*. IGS is multi-copy with moderate genetic variability, which greatly enhances the sensitivity of the assay over single copy genetic loci assays. The detection limit of our assayis: 100fg gDNA, 10 conidia/0.5g soil and 100 conidia/ 24h length spore trapping tape. The specificity of the assay was validated against four SDS causal *Fusarium* spp. and 32 other taxonomically related and/or commonly encountered fungal species. This assay has been tested on 11 symptomatic or asymptomatic soybean plants and detection results were confirmed by isolation of *F. virguliforme* from the roots. This assay will be a valuable diagnostic and research tool.

Development and characterization of microsatellite markers for soybean sudden death syndrome pathogen *Fusarium virguliforme*

J. WANG (1), J. L. Jacobs (1), M. I. Chilvers (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.132

Fusarium virguliforme is a soil-borne fungal pathogen and is the causal agent of soybean sudden death syndrome (SDS). SDS is a relatively new and devastating disease in the Midwest. Currently utilized DNA loci are not able to differentiate *F. virguliforme* isolates. Microsatellites or simple sequence repeats (SSRs) of one- to six-base DNA nucleotide motifs, often demonstrate polymorphism at the population level, are relatively inexpensive and highly informative. We developed a set of *F. virguliforme* microsatellite markers based on the *F. virguliforme* genome. *In silico* parameters utilized to search for the microsatellite loci were 20,14, 12, 7, 7, and 7 minimum repeats for mono-, di-, tri-, tetra-, penta- and hexa- motifs, respectively, 249 microsatellite loci qualified using these parameters. The program Primer3 was used to design primers in the flanking regions upstream and downstream (200nt) of the microsatellites, with an amplicon size range between 180 and 250bp, with these parameters primers for 108 microsatellite loci were developed. Of the 103 microsatellites tested against five isolates from four different geographic locations (MI, IL, IN and Argentina), 101 primer sets amplified PCR product and 18 (17.8%) of the microsatellite loci were found to be polymorphic. These markers will be useful for analysis of population genetic structure, movement, tracking introductions of *F. virguliforme*, and attempting to correlate genotypes with phenotypes.

Barberry does not play a role for stripe rust in the U.S. Pacific Northwest

M. Wang (1), A. Wan (1), X. CHEN (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.132

Common barberry (*Berberis vulgaris*) is a major alternate host for the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*, Pgt) and was recently found to be an alternate host for the stripe rust pathogen (*P. striiformis* f. sp. *tritici*, Pst). Although stripe rust is the most important disease and barberry grows in the US Pacific Northwest (PNW), their associations have not been found. To determine if *B. vulgaris* can be infected by Pst under the natural conditions in the region, aecia collected from barberry plants in the region were tested on wheat plants and with molecular markers in comparison with Pgt. Pgt was found from aecial samples from barberry and no Pst was found to be derived from sexual reproduction on barberry. Teliospores of Pgt from fields did not germinate until February and reached the peak (45%) in May. In contrast, Pst teliospores germinated under lab conditions when produced on wheat plants in the field in June and the germination rate was decreasing from 12% in December, 1% in February, and no germination in May as teliospores were degraded before May when barberry plants starts produce leaves. A time-series experiment showed that a minimum of 40 h of dew period at optimal 10°C is needed for Pst to infect barberry using teliospores as inoculum and infection increased to the peak when the dew period reached 93 h. These results indicate that the wheat stripe rust pathogen cannot infect barberry under the natural conditions in the US PNW.

Genetic characterization of virulence/avirulence genes of *Puccinia striiformis* f. sp. *tritici*

M. Wang (1), A. Wan (1), X. CHEN (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Pullman, WA, U.S.A.
Phytopathology 102:S4.132

Common barberry (*Berberis vulgaris*) was recently found as an alternate host for *Puccinia striiformis* f. sp. *tritici* (Pst), the causal agent of wheat stripe rust. Young barberry leaves were inoculated with teliospores produced from adult wheat plants of wheat infected with urediniospores from a single-uredium isolate (PST-127) under controlled conditions. Aeciospores produced on barberry leaves were inoculated on seedlings of wheat line 'Avocet Susceptible' (AvS). A total of 19 single-uredium isolates were obtained. The virulence and avirulence of the 19 isolates and the parental isolate were tested on wheat lines used as Pst race differentials or carrying a single Yr gene. Virulences to Yr1, Yr2, and Yr9 and avirulences to Yr5, Yr15, Yr24, Yr32, and YrSP were homozygous and virulences to other genes were segregating. Virulence or avirulence to other 14 Yr genes segregated at 1:1 ratio for a single locus (P, 0.05-0.89), except those to Yr17 and Yr43. Ten virulences (to Yr6, Yr7, Yr8, Yr19, YrUkn, Yr27, Yr43, Yr44, YrExp2, and YrTye) and four avirulences (to Yr10, Yr17, YrExp1, and YrTr1) appeared to be dominant. This is the first genetic study for characterizing Pst virulence and avirulence genes and demonstrating the gene-for-gene relationship for avirulence and resistance genes in the Pst-wheat pathosystem. More experiments are conducted to enlarge the segregating Pst population and mapping the segregating virulence/avirulence loci.

Temperature effect on appressorial formation of *Colletotrichum cereale* on detached and intact plant

Y. WANG (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.132

Turfgrass anthracnose, caused by *Colletotrichum cereale*, is a devastating disease on annual bluegrass (AB) and creeping bentgrass (CRB). Intensified putting green management has increased both incidence and severity of the disease worldwide, yet the biology of the pathosystem remains unclear. An experiment was designed to investigate the effects of temperature on *C. cereale* appressorial formation on two different hosts. Either intact plant or detatched three-week-old leaves of AB or CRB were inoculated with a conidial suspension ($2.5*10^6$ conidia/ml) of *C. cereale*. Inoculated leaves were incubated in dark growth chambers set at one of the following temperatures: 12 C, 18 C, 22 C, 26 C, 30 C and 34 C with high relative humidity (>95%). Twenty-five arbitrarily selected conidia were examined microscopically for appressorial production at different time points after inoculation. Appressoria were observed in each temperature treatment, but developed most rapidly between 22 C and 26 C. Appressorial occurrence was significantly hindered at 30 C and 34 C when compared to the other temperature treatments. The development on intact plant and detached leaves showed a similar pattern while a twelve-hour delay was seen on live plant. These results suggested that *C. cereale* may infect AB and CRB well prior to the onset of symptoms and defense activities are generated by intact host against fungal invasion.

Genetic relationships among subpopulations of competitive nonpathogenic strains of *Fusarium oxysporum* and *F. oxysporum* f. sp. *lycopersici*

M. R. WARR (1), T. A. Rush (1), R. W. Schneider (2)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.; (2) Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.132

Prior research showed that tomato roots are colonized by a wide variety of nonpathogenic strains of *Fusarium oxysporum*. Some of these nonpathogenic strains suppress infection (suppressive strains) by *F. oxysporum* f. sp. *lycopersici*, the tomato wilt pathogen, while others predispose tomato plants to infection (conducive strains) leading ultimately to more severe disease as compared to plants that were not co-inoculated. One of the questions arising from this work relates to the genetic relationships among these nonpathogenic subpopulations and their relationship to the pathogen. Previous work showed that there are separate subpopulations based upon vegetative compatibility tests, but these tests do not provide quantitative assessments of relatedness. Phylogenetic analyses were conducted using DNA sequences of the internal transcribed spacer region and the elongation factor-1 alpha gene. Results showed that these nonpathogenic strains were distinct from other GenBank accessions. Relationships between suppressive and conducive strains will be presented. Findings from this research will be useful in assessing soilborne populations of *F. oxysporum* for conducive and suppressive subpopulations and in finding ways to enhance disease suppressive strains.

Interaction of *Buchnera* GroEL from *Pentalonia nigronervosa* with *Banana bunchy top virus* (*Nanoviridae*)

S. WATANABE (1), A. Bressan (1)
(1) University of Hawaii, Honolulu, HI, U.S.A.
Phytopathology 102:S4.132

Circulative plant viruses, such as luteovirids and geminiviruses have been shown to specifically bind to GroEL proteins produced by endosymbiotic bacteria harbored within the hemipteran vectors. GroEL is a protein from the

chaperonin family, and is known to get involved in several biological processes, including protein folding and subunit assembly. It has been suggested that GroEL protects circulative viruses from the proteolytic degradation occurring in the vector's hemolymph. Similarly to luoteovirids and geminiviruses, *Banana bunchy top virus* (BBTV), a member of the family *Nanoviridae*, is transmitted in a persistent circulative manner, and can be detected in the hemolymph of the aphid vector, *Pentalonia nigronervosa.* To date, it is unknown if BBTV may interact with GroEL. In this study, we first localized bacterial endosymbionts within the bacteriomes of *P. nigronervosa*. Phylogenetic analysis of 16S rRNA and GroEL genes revealed this endosymbiont clustering into the *Buchnera* clade. Furthermore, a ~63 kDa protein was identified in western blotting assays in the hemolymph of *P. nigronervosa*. In vitro interaction assays were performed between *Buchnera* GroEL and BBTV by using dot-blot, far-western blotting, and Immunocapture PCR assays. However, we failed to obtain evidence for BBTV-GroEL interaction. Therefore, we suggest that unlike for other circulative viruses, BBTV might not interact with *Buchnera* GroEL in *P. nigronervosa*.

Aflatoxin management in corn with Afla-Guard

M. A. WEAVER (1), H. Abbas (2), G. Sciumbato (3), H. Pringle (3), T. Allen (3)
(1) USDA-ARS BCPRU, Stoneville, MS, U.S.A.; (2) USDA-ARS, Stoneville, MS, U.S.A.; (3) Mississippi State University, Stoneville, MS, U.S.A.
Phytopathology 102:S4.133

Aflatoxin contamination is a perennial threat to corn production in the southern United States. *Aspergillus flavus* is the predominant species associated with aflatoxin production; however, not all strains produce the toxin. Two non-aflatoxigenic strains of *A. flavus* were evaluated during 2011 at five field sites in the Mississippi Delta. Afla-Guard, a commercially available formulation of non-toxigenic *A. flavus* (NRRL 21882), was tested at three application (V10, VT and R2) timings and at two application rates (11 and 22 kg/ha) alongside untreated control plots and other plots treated with strain K49 (NRRL 30797), a Mississippi-native biocontrol strain of *A. flavus*. Minimum and maximum air temperatures were above average with less precipitation than average at Stoneville, MS in June and July. Three of the five sites had ≤ 5 ppb aflatoxin, so it was not possible to attribute any aflatoxin reduction to the biological control strain application. The remaining two sites, in contrast, were heavily contaminated with aflatoxin, with concentrations from individual plots between 30 to 540 ppb aflatoxin. Higher application rates (22 kg/ha) and early application (V10) were generally the most effective and strain K49, applied at V10 resulted in the lowest contamination concentrations, but the differences were not statistically significant.

Virus movement within grafted watermelon plants

C. G. WEBSTER (1), C. S. Kousik (2), R. L. Hassell (3), K. S. Ling (2), W. W. Turechek (1), S. Adkins (1)
(1) U.S. Horticultural Research Laboratory, USDA-ARS, Fort Pierce, FL, U.S.A.; (2) U.S. Vegetable Laboratory, USDA-ARS, Charleston, SC, U.S.A.; (3) Clemson University, CREC, Charleston, SC, U.S.A.
Phytopathology 102:S4.133

Watermelon production in Florida is impacted by several viruses including whitefly-transmitted *Squash vein yellowing virus* (SqVYV), *Cucurbit yellow stunting disorder virus* and *Cucurbit leaf crumple virus*, and aphid-transmitted *Papaya ringspot virus* type W (PRSV-W). While germplasm resistant to some of these viruses has been identified, it remains a challenge to transfer this resistance into commercial watermelon cultivars. Using resistant germplasm as a rootstock offers the potential for quickly providing resistance to viral diseases. Commercial seedless watermelon scions (cv. Tri-X 313) were grafted onto resistant rootstocks and independently inoculated with SqVYV or PRSV-W. Severity of virus symptoms was recorded and virus presence above and below the graft union was determined by serological methods and reverse transcription-polymerase chain reaction. Grafting on resistant rootstocks was not effective in preventing SqVYV-caused watermelon vine decline in 'Tri-X 313' scions. Movement of SqVYV and PRSV-W across the graft union and into most rootstocks was detected, overcoming resistance to both viruses. SqVYV titer in the 'Tri-X 313' scions was found to be similar in both non-grafted and grafted plants, but was lower for some resistant rootstocks. These results demonstrate the ability of viruses to move into resistant rootstocks of grafted plants suggesting that rootstock breeding strategies may need to take this into account.

Evaluating fumigation and at-plant treatments for the control of potato common scab in Wisconsin

B. J. WEBSTER (1), S. A. Jordan (1), A. J. Gevens (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.133

Potato common scab, caused by *Streptomyces scabies*, is a persistent soilborne disease that affects potatoes worldwide. Annually, Wisconsin growers are challenged to control common scab on susceptible varieties that are in market demand. Field trials in 2010 and 2011 in Antigo, Wisconsin evaluated the efficacy of fumigation and at-plant fungicides to control common scab on 'Yukon Gold.' Fumigation treatments included metam sodium and chloropicrin applied in fall prior to spring potato planting. Marketable tubers were assessed for disease severity with a scale of 0-3 with 0 = no disease, 1 = <10% surface area symptomatic, 2 = 10-25%, and 3 = >25%. In 2010, of the 6 biofungicides investigated, none provided significantly better common scab control than the untreated control. Although numerically, a low rate of the biofungicide containing *Bacillus subtilis* (32 fl oz/A) provided the best disease control. In 2011, treatments containing metam sodium, chloropicrin, PCNB, and elemental sulphur provided significantly less common scab than the untreated control. Metam sodium (applied at 40 gal/A) was the only treatment to have a statistically higher marketable yield (284.6 cwt/A) compared to the untreated control (216.3 cwt/A). Biofungicides provided variable results, with yield and quality benefits in 2010 and reduced disease control in 2011.

Thrips transmission of a tospovirus reassortant

C. G. WEBSTER (1), S. R. Reitz (2), G. Frantz (3), H. C. Mellinger (3), J. Funderburk (4), S. Adkins (1)
(1) U.S. Horticultural Research Laboratory, USDA-ARS, Fort Pierce, FL, U.S.A.; (2) Centre for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Tallahassee, FL, U.S.A.; (3) Glades Crop Care, Jupiter, FL, U.S.A.; (4) North Florida Research and Education Centre, University of Florida, Quincy, FL, U.S.A.
Phytopathology 102:S4.133

In late 2009 an M RNA reassortant of *Groundnut ringspot virus* (GRSV) and the closely related *Tomato chlorotic spot virus* (TCSV) was detected in tomato in south Florida. A subsequent increase in geographic and host ranges has been documented. Previous studies of the thrips vector specificities for the parental tospovirus species (GRSV and TCSV) are of limited use in predicting the vectors of the GRSV/TCSV reassortant because this is the first report of an interspecies tospovirus reassortant. Our initial work has shown that the reassortant can be acquired and transmitted by Western flower thrips (*Frankliniella occidentalis*), although at a low efficiency compared to *Tomato spotted wilt virus*. We are currently investigating the vectoring potential of several other important thrips species, including Florida flower thrips (*Frankliniella bispinosa*) and tobacco thrips (*Frankliniella fusca*). A detached leaf disc system has been used to examine the relative efficiency of these thrips species to acquire and transmit the reassortant. Acquisition of virus by thrips larvae and subsequent transmission to leaf discs was determined by serological and reverse transcription-polymerase chain reaction testing. Knowledge of the vectoring potential of these additional thrips species will benefit management strategies for this new tospovirus reassortant.

Three improved *Citrullus lanatus* var. *citroides* lines USVL246-FR2, USVL252-FR2, and USVL335-FR2, with resistance to *Fusarium oxysporum* f. sp. *niveum* race 2

W. WECHTER (1), C. Kousik (1), M. McMillan (1), M. Farnham (1), A. Levi (1)
(1) USDA-ARS, Charleston, SC, U.S.A.
Phytopathology 102:S4.133

Fusarium wilt (FW) is a major disease of watermelon in North America and around the world. Control of this disease is difficult because the soil-borne causal agent *Fusarium oxysporum* f. sp. *niveum* (Fon) produces chlamydospores that remain infectious in the soil for many years. Although various levels of resistance to Fon races 0 and 1 exist in watermelon cultivars, no resistance to race 2 or 3 has been reported. We have identified three *Citrullus lanatus* var. *citroides* plant introductions from the U.S. germplasm collection with high levels of resistance to Fon race 2. The most resistant individuals of each of these PI have been self-pollinated three times, with resistance screening and selection with each successive selfing. These improved lines USVL246-FR2, USVL252-FR2 and USVL335-FR2 have been extensively tested in greenhouse and field evaluations. The three lines have been found to be uniform, and exhibit a resistance phenotype significantly higher than that found in the watermelon cultivars Sugar Baby, Charleston Grey and Calhoun Grey, and have levels of resistance equal to or higher than the reported resistant lines, PI296341, PI271769, and 296341-FR. Data from greenhouse and field trials will be presented.

Field evaluation of foliar fungicides for control of northern corn leaf blight

J. D. WEEMS (1), K. A. Ames (1), C. A. Bradley (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.133

Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*, is a yield reducing foliar disease often controlled with foliar-applied fungicides. Field studies were conducted in 2010 and 2011 at Urbana, IL to evaluate fungicides and a non-treated control for their effect on NCLB. Plants were inoculated at the 4 leaf stage with an *E. turcicum* conidial suspension mixture of races 0 and 1. Fungicides were applied at tassel emergence in 2010 and two weeks after tassel emergence in 2011. The estimated percent leaf area affected by NCLB was recorded weekly for the leaf below the ear (LBE), the ear leaf (EL), and the leaf above the ear (LAE). Disease data were converted to percent disease control relative to the non-treated control and statistically analyzed. Ears were harvested from plots, average ear weights were calculated, and data were statistically analyzed. In 2010, most fungicides applied at higher rates provided significantly greater disease control than fungicides applied at lower rates on the EL and the LAE at 23 days after spraying (DAS) ($P = 0.0002$ and 0.0697, respectively) and on the LBE, EL, and LAE at 33 DAS ($P = 0.0009$, 0.0017, and 0.0527, respectively). In 2010, demethylation inhibitor fungicides provided greater disease control than quinone outside inhibitor fungicides on the EL and LAE at 23 DAS. No significant differences were observed among treatments for disease control in 2011 or average ear weight in 2010 and 2011.

Molecular identification of species and chemotypes of *Fusarium* causing head blight of wheat in Nebraska

S. Wegulo (1), A. PANTHI (1), H. Hallen-Adams (1)
(1) University of Nebraska, Lincoln, NE, U.S.A.
Phytopathology 102:S4.134

Fusarium graminearum and *F. culmorum* are two important fungal species that cause Fusarium head blight (FHB) in wheat. FHB reduces yield, lowers seed quality, and contaminates grain with mycotoxins. A collection of nearly 80 isolates from different regions of Nebraska causing FHB were sequenced for molecular identification. Nearly all isolates were identified as *F. graminearum* and one isolate was identified as *F. culmorum*. These *Fusarium* species both produce trichothecenes such as deoxynivalenol (DON), nivalenol (NIV), and acetylated derivatives such as 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON). To identify the chemotype of these isolates, a multiplexed PCR assay was carried out and results were obtained for 48 isolates. The 15-ADON chemotype predominates in Nebraska. This chemotype was identified in more than 95% of isolates examined, the remainder being 3-ADON. The NIV chemotype was not detected.

Effects of single and double infections of winter wheat by *Triticum mosaic virus* and *Wheat streak mosaic virus* on grain yield and yield components

S. Wegulo (1), E. BYAMUKAMA (1), S. Tatineni (2), G. L. Hein (1), R. A. Graybosch (3), P. S. Baenziger (1)
(1) University of Nebraska, Lincoln, NE, U.S.A.; (2) USDA-ARS, University of Nebraska, Lincoln, NE, U.S.A.; (3) USDA-ARS, Lincoln, NE, U.S.A.
Phytopathology 102:S4.134

Triticum mosaic virus (TriMV) is a mite-transmitted pathogen of wheat discovered in Kansas in 2006. It is commonly found co-infecting wheat with *Wheat streak mosaic virus* (WSMV) in the Great Plains region of the United States. In 2010-2011, a field experiment was carried out to determine the effects of single and double infection of winter wheat with TriMV and WSMV on grain yield and yield components. WSMV-susceptible cv. Millennium and WSMV-resistant cv. Mace were mechanically inoculated with TriMV, WSMV, TriMV+WSMV or sterile water (control) at the 2-leaf growth stage. In Mace, yield and yield components did not differ among treatments. In Millennium, grain yield was reduced by 96, 70, and 53%, in the TriMV+WSMV, TriMV, and WSMV treatments, respectively. Heads/m^2 were not significantly reduced by the WSMV treatment but were reduced by 88 and 20% in the TriMV+WSMV and TriMV treatments, respectively. 1000-kernel weight was reduced by 22% in the WSMV treatment and by up to 42% in the TriMV and TriMV+WSMV treatments. Kernels/head did not differ among the virus inoculation treatments but were significantly fewer than those in the control by up to 45%. These results indicate that grain yield loss is exacerbated by co-infection of winter wheat with TriMV and WSMV in the WSMV-susceptible Millennium but not in the WSMV-resistant Mace.

Fungicides do not reduce fruit rot following a simulated hail event

L. WELLS (1), P. McManus (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.134

Hail storms are a common occurrence in Wisconsin, with a few or many cranberry growers being affected every year. Growers usually apply fungicides immediately following hail storms to prevent fruit rot, despite the lack of research to support this practice. We conducted field trials in 2010 and 2011 to address the question of whether fungicide applications following a hail storm reduce fruit rot incidence (% rotten fruit), and if so, which fungicides are most effective. Hail damage was simulated by shooting pea gravel into cranberry beds, and the fungicides Abound (azoxystrobin) or Champion II (copper) were applied to fruit immediately following this damage. Fruit rot incidence was evaluated in late September and early October. The simulated hail damage increased fruit rot incidence ($p \leq 0.05$) compared with the non-damaged control in six of seven trials. Fungicides did not reduce fruit rot incidence ($p \geq 0.05$) in hail-treated plots compared to the non-treated control in six of seven trials. In a trial conducted on relatively immature berries, fruit rot incidence in hail-damaged plots treated with Abound was less ($p \leq 0.05$) than fruit rot incidence in hail-damaged plots treated with Champion II or no fungicide. Results suggest that if cranberries are damaged by hail, it is unlikely that an application of fungicide will reduce the amount of fruit rot at the time of harvest.

Genetic characterization of '*Candidatus* Liberibacter solanacearum' associated with zebra chip disease of potato in Washington and Idaho

A. WEN (1), C. Johnson (1), N. C. Gudmestad (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.134

Zebra chip (ZC) disease of potato was observed in commercial potato fields in the lower Columbia Basin of southern Washington and northern Oregon in August 2011. In September 2011, ZC disease of potato was observed in tubers in storage facilities in south central Idaho. In this study, a 16S qPCR assay (LsoF/HLBr/HLBp) was used to detect and quantify '*Candidatus* Liberibacter solanacearum' (Lso) in potato samples, and PCR assays using both SSR and biotyping primers were applied to further characterize the genetic variations of Lso. Thirty-nine of eighty potato cv. Umatilla Russet tubers and thirty-eight of fifty-two potato cv. Ranger Russet tubers from Washington potato storage facilities were found to harbor 5.86×10^2 to 3.13×10^7 and 4.84×10^2 to 8.30×10^6 genome of Lso per 100 mg of potato tuber tissue, respectively. From Idaho storage facilities, Lso titre in four potato cv. Ranger Russet tubers ranged from 1.46×10^5 to 1.76×10^6 genome of Lso per 100 mg of potato tuber tissue, and in 72 of 74 potato cv. Russet Burbank tubers ranged from 5.07×10^4 to 6.86×10^6 genome of Lso per 100 mg of potato tuber tissue. Genetic characterization revealed that the Lso associated with ZC disease of potato in Washington and Idaho was homogeneous, having only haplotype A or SSR type 1/ biotype 1. This suggests that the source of Lso of ZC disease in Washington and Idaho was from the same origin. Previous reports revealed that haplotype B Lso was detected in potato psyllid samples in Washington.

Suppression of soybean diseases through the use of cover crops

L. WEN (1), G. L. Hartman (2), D. M. Eastburn (1)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) USDA-ARS, University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.134

Suppressive soil is defined as a soil where pathogens do not become established, persist, or may become established but cause little or no disease. Some cover crops have been shown to increase soil suppressiveness. However, little research has been done to evaluate the use of cover crops to enhance soil suppressiveness for soybean diseases. Field experiments were conducted in four locations in Illinois in 2010-2011 to determine the effect of fall cover crops (mustard, annual rye, rape, and canola) on suppressing soilborne soybean diseases, changing microbial communities, and on crop yield. In plots at the University of Illinois, Champaign, that were infested with *Rhizoctonia solani*, soybean stands were significantly greater in plots that were previously planted to rye and rape, as compared to stands in plots planted to all other cover crops ($P<0.0001$). Cover crops did not affect soybean stand at the other three locations, but these locations were not intentionally infested with *R. solani*. Levels of sudden death syndrome of soybean, soybean cyst nematode populations, foliar diseases, and yield were not significantly affected by the cover crop treatments. Greenhouse bioassays are being conducted on soils collected from the cover crop treated plots to evaluate for differences in disease suppressiveness. DNA analysis is also being conducted to evaluate for differences in pathogen populations by QPCR and to assess differences in microbial community structures by ARISA.

Evaluation of pathogen and pest resistance in select commercial soybean cultivars from 1923 to 2007

E. D. WEST (1), C. R. Bowen (2), J. S. Haudenshield (1), G. L. Hartman (3)
(1) University of Illinois at Urbana-Champaign, Champaign, IL, U.S.A.; (2) USDA-ARS, Urbana, IL, U.S.A.; (3) USDA-ARS, University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.
Phytopathology 102:S4.134

Soybean is highly important to global food production both as protein component of feed for the livestock industry and for human food. We evaluated 40 cultivars in 2010 and 60 cultivars in 2011 at two locations for

disease and pest resistance. These cultivars were released as early as 1923 through cultivars released as late as 2007. The replicated field study was conducted for 2 years. During the first year one location was used for disease and pest evaluations while during the second year two locations were used. Negative correlations were found between date of cultivar release and disease susceptibility for bacterial diseases, pod and stem blight, and anthracnose. A strong correlation between disease incidence and rotation treatment (continuous corn followed by soybeans versus corn/soybean rotation) showed a greater difference for some diseases, such as brown spot and soybean cyst nematode, and less difference for other diseases, such as downy mildew. Based on this data, there are indications of increased disease in corn/soybean rotation plots than continuous corn followed by soybeans and in cultivars which had earlier release years compared to those released more recently.

Optimizing use of MCW-2 for management of root-knot nematode, *Meloidogyne javanica*, on tomatoes and cucumbers

B. B. WESTERDAHL (1), C. T. Schiller (2), C. A. Wilen (3)
(1) University of California, Davis, CA, U.S.A.; (2) Makhteshim Agan of North America, Raleigh, NC, U.S.A.; (3) University of California Cooperative Extension, San Diego, CA, U.S.A.
Phytopathology 102:S4.135

To evaluate the potential of the nematicide MCW-2 in a methyl bromide replacement program, its effectiveness in combination with herbicides and fungicides was evaluated in field trials on tomatoes (10 treatments) and on cucumbers (12 treatments). On each crop, a RCB trial with 5 replicates was conducted to evaluate effectiveness ($p=0.05$) compared to an untreated control (UC), a hand weeded control (HWC), and methyl bromide/chloropicrin (MBCP). Remaining treatments were combinations of MCW-2, 1,3-dichloropropene, chloropicrin (CP), metam potassium (MK), oxamyl, azoxystrobin, metalaxyl, halosulfuron-methyl, clethodim, metribuzin, and metolachlor. Nematode control was evaluated at 5-weeks after planting and at harvest. Weed evaluations were conducted 7-weeks after planting. In the tomato trial, all of the herbicides tested exhibited good weed control with some differences on particular weed species. MCW-2 at 2 and 4 kg ai/ha when used in combination with CP and MK were the only treatments to have greater yields than UC. MBCP and all treatments containing MCW-2 had lower root gall ratings (RG) at harvest than UC. At 5-weeks after planting, all cucumber treatments with a nematicide had a lower RG than UC. MBCP and HWC provided the best weed control, followed by treatments containing CP and MK. Heavy weed pressure complicated analysis of nematode control and yields at harvest.

Characterization and pathogenicity of *Rhizoctonia solani* isolates affecting potato in Idaho and Michigan, United States

P. WHARTON (1), J. Woodhall (2), J. Peters (2), W. Kirk (3)
(1) University of Idaho, Aberdeen, ID, U.S.A.; (2) The Food and Environment Research Agency, York, United Kingdom; (3) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.135

Rhizoctonia solani is an important pathogen of potato (*Solanum tuberosum*) causing both qualitative and quantitative losses. It has been associated with black scurf and stem canker. Isolates of the fungus are assigned to one of 13 known anastomosis groups (AGs), of which AG3 is most commonly associated with potato disease. In August 2011, diseased potato plants originating from Aberdeen, Kimberly Parma and Rupert, Idaho, and Three Rivers, Michigan were received for diagnosis. All samples displayed stem and stolon lesions typically associated with Rhizoctonia stem canker. The presence of *R. solani* was confirmed through isolation. AG was determined by sequencing the rDNA ITS region using primers ITS4 and ITS5. The resulting sequences of the rDNA ITS region of isolates were compared to those of other isolates present in sequence databases. The majority of isolates tested were AG3, two were between 97% and 100% identical to that of AG2-2IIIB isolates present in sequence databases and one was 99% identical to sequences of AG4 HG-II isolates in Genbank. Koch's postulates were confirmed for the AG2-2IIIB and AG4 HGII isolates. AG3 was the most common AG found in Idaho and Michigan. However, to our knowledge, this is the first report of AG2-2IIIB and AG4 HG-II causing disease on potatoes in the US. AG2-2IIIB and AG4 HG-II should now also be considered a potato pathogen in the US.

The use of volatile organic compounds to control *Colletotrichum coccodes* on potato in large-scale storage

P. Wharton (1), E. WOOD (1)
(1) University of Idaho, Aberdeen, ID, U.S.A.
Phytopathology 102:S4.135

Many naturally occurring plant volatiles are known for their anti-fungal properties. However, they have limited use because they diffuse rapidly after coming in contact with air. In an initial *in vitro* study, 2E-hexenal was shown to be fungi-toxic to *Colletotrichum coccodes* at a concentration of 5 ppm. In a further study, experiments were repeated using tubers naturally infected with *C. coccodes* instead of pathogen cultures. 2E-hexenal was shown to have fungi-static effects on these naturally infected tubers on a small scale. In the current study, a large scale *in vivo* trial was implemented using cv. Rosara tubers naturally infected with *C. coccodes*. Potatoes (23 kg) were placed into 190 L barrels and then exposed to a treatment of either 5, 7.5, or 10, ppm of 2E-hexenal in the sealed barrel for 96 hours at approximately 12 °C. The tubers were removed from the treatment barrels and placed into burlap sacks and stacked on a pallet in a humid cellar at approximately 12 °C for 5 months. In order to quantify the amount of *C. coccodes* present on the tubers, DNA extraction and qPCR were performed using tuber skin peels from each of the sample sets. Samples of 25 tubers from each replicate were collected prior to treatment, at 3 months post treatment, and at 5 months post treatment. Results are presented and discussed in relation to the future use of these volatile compounds in controlled atmosphere storage systems for the control of *C. coccodes* and other potato blemish diseases.

Toward general methods to identify and quantify partial resistance interacting with other plant attributes: An illustration in the case of rice sheath blight

L. WILLOCQUET (1), S. Srinivasachary (2), S. Savary (1)
(1) INRA, Castanet Tolosan, Cedex, France; (2) IRRI, Los Banos, Philippines
Phytopathology 102:S4.135

Host plant resistance in many pathosystems does not depend solely on resistance genes, but also on other genetic attributes, which will render a crop more, or less, vulnerable to disease. In such contexts, identifying and quantifying components of partial resistance is both difficult and critical for breeding. In the case of rice sheath blight, two main groups of mechanisms for partial resistance are hypothesized: disease escape, linked with morphological traits, and physiological resistance, i.e., induced or constitutive plant processes that decrease the infection and reproduction of the pathogen. Methods were developed to (1) measure physiological resistance, using detached tiller tests yielding lesion densities, sizes, and expansion, and (2) assess the overall partial resistance, using microfield experiments, where morphological characteristics, as well as disease intensification and spread were measured. Both types of experiments were conducted on a range of rice genotypes. The results indicate that disease escape represents an important component of partial resistance, which affects both disease intensification and disease spread, and is associated with morphological traits such as plant height. Variation in physiological resistance between rice genotypes was also detected with respect to the number of lesions, reflecting variation in infection efficiency. These results are discussed with respect to breeding strategies.

Effects of different foliar fungicides and application timings on *Stagonospora nodorum* leaf blotch in soft red winter wheat: A multistate study

K. T. Willyerd (1), C. A. Bradley (2), S. P. Conley (3), P. D. Esker (3), L. V. Madden (1), K. A. Wise (4), P. A. PAUL (1)
(1) Ohio State University, Wooster, OH, U.S.A.; (2) University of Illinois, Urbana, IL, U.S.A.; (3) University of Wisconsin-Madison, Madison, WI, U.S.A.; (4) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 102:S4.135

Fungicides are increasingly being used in soft red winter wheat (SRWW) in the absence of disease to "protect" or "boost" yields, as well as for their intended purpose of managing diseases such as *Stagonospora nodorum* leaf blotch (SLB). Questions have been raised regarding the biological, agronomical, and economical benefits of this practice. Plots of two SRWW cultivars, one resistant and the other susceptible to SLB, were planted in 6 unique environments (location x year), inoculated with a *S. nodorum* spore suspension, and then treated with fungicides. There were nine treatments consisting of two fungicides (Headline and Prosaro) applied either at manufacturer-recommended rates at Feekes 5, 8, or 10, or at half-rate at Feekes 5 followed by a second half-rate at Feekes 8, plus an untreated check. SLB severity and grain yield were determined. The magnitude and significance of the main and interaction effects varied among location-years, but very similar trends were observed for some responses. In general, a single application at Feekes 8 or 10 resulted in the lowest SLB severity, whereas applications made at Feekes 5 were the least effective, with levels of SLB comparable to that of the untreated check. Fungicide treatment generally resulted in higher grain yield than the untreated check; however, for any given fungicide, application timing had negligible effects on yield. Notable exceptions in both disease and yield responses to treatments were observed when SLB levels were extremely high or low. A cost-benefit assessment of the different fungicide programs will be presented.

Relative potential of major root-rot and bole-rot fungi to decay sapwood in landscape trees of southern temperate regions

A. WILSON (1)
(1) USDA Forest Service, Stoneville, MS, U.S.A.
Phytopathology 102:S4.136

The resistance of hardwood and conifer woods to decay by major indigenous rot fungi has a significant impact on management decisions for tree-planting selections after timber harvests or for replacing dead trees in municipal landscapes. Information on wood types of individual tree species that are resistant to decay by specific wood-rot fungi in particular regions or sites is useful for establishing tree-planting recommendations and hazard-rating systems for specific wood decay fungi at each location. Sapwood samples from nine tree species, including six temperate hardwood and three conifer species, were tested for resistance to decay by twelve major root-rot and bole-rot fungi. The fungi used for testing wood resistance to decay included *Armillaria gallica*, *A. mellea*, *A. ostoyae*, *A. tabescens*, *Daedalea quercina*, *Fomitopsis pinicola*, *Ganoderma lucidum*, *Heterobasidion annosum*, *Inonotus dryadeus*, *Laetiporus sulphureus*, *Phellinus pini*, and *Stereum hirsutum*. The decay fungi with the highest hazard rating, causing 7.4-9.5% weight loss over two years, were *Armillaria mellea*, *Ganoderma lucidum*, *Heterobasidion annosum*, and *Inonotus dryadeus*. Woods of conifer species generally exhibited greater resistance to decay than hardwood species. The order of decay resistance among hardwood species (most to least) followed the order: *Platanus occidentalis*, *Liquidambar styraciflua*, *Populus deltoides*, *Quercus lyrata*, *Fraxinus pennsylvanica*, and *Quercus nuttallii*.

Development of an electronic-nose technology for the rapid detection of agricultural pesticide residues

A. WILSON (1)
(1) USDA Forest Service, Stoneville, MS, U.S.A.
Phytopathology 102:S4.136

The detection and identification of pesticide residues in and on agricultural and landscape plants currently requires time-consuming and expensive chemical analyses of various types. This problem leads to delays in making many types of important crop-management decisions involving pesticides. Electronic-nose (e-nose) technologies provide a means for acquiring rapid identifications of pesticide residue types at relatively low cost by detection of headspace volatiles released from plant surfaces or extracted samples. Protocols and methods were developed using a conductive polymer (CP)-type e-nose device to obtain real-time determinations of various types of pesticide residues within 15 minutes. E-nose analysis did not provide highly accurate indications of residue concentrations since the method is only semi-quantitative. Unique electronic aroma-signature patterns were obtained for several classes of pesticides including fungicides, insecticides, herbicides, and other pesticide types. Recognition files, comprising aroma libraries of known pesticides, were developed for various types of pesticide-detection applications. Portable devices with smaller numbers of sensors in the sensor array provide a means for obtaining this data directly in the field. Some CP sensors in the array were highly sensitive to certain classes of pesticides and may be poisoned or inactivated if sample concentrations are too high.

Postharvest respiration rate and sucrose concentration of *Rhizoctonia*-infected sugar beet roots

C. E. WINDELS (1), J. R. Brantner (1), L. G. Campbell (2), K. K. Fugate (2)
(1) University of Minnesota, Northwest Research and Outreach Center, Crookston, MN, U.S.A.; (2) USDA-ARS, Northern Crop Science Laboratory, Fargo, ND, U.S.A.
Phytopathology 102:S4.136

Rhizoctonia crown and root rot (RCRR), caused by *Rhizoctonia solani* AG 2-2, is a widespread disease that reduces sugar beet root and sucrose yield prior to harvest and causes further losses by increasing respiration and sucrose losses during storage. Information on the respiration and sucrose concentration changes associated with different levels of severity of RCRR would allow sugar beet processors to determine when fields should be abandoned and manage piles to minimize sucrose losses during storage. In 2010 and 2011, roots in five RCRR categories (0-1, 2, 3, 4, and 5 = healthy to a few non-active lesions, <5, 6-25, 26-50, 51-75 % root rotted, respectively) were collected from plots inoculated with *R. solani*. Roots were washed, placed in perforated plastic bags, and stored at 4.4 °C and 90-95% R.H. Respiration rate was measured 30, 60, and 90 days after harvest (DAH) and sucrose concentration 30 and 90 DAH. At 30 DAH, respiration rates were 3.5, 3.4, 3.7, 4.4 and 6.3 and 3.5, 3.7, 3.7, 4.3 and 7.1 mg kg^{-1} h^{-1} in 2010 and 2011, respectively, for RCRR ratings of 0-1, 2, 3, 4 and 5, respectively. At 30 DAH, extractable sucrose concentrations were 154, 152, 148, 138 and 120 and 155, 147, 148, 133, and 107 kg Mg^{-1} in 2010 and 2011, respectively, for RCRR ratings of 0-1, 2, 3, 4 and 5, respectively. Overall, the impact of RCRR during storage was minimal at 30, 60, and 90 DAH for disease ratings of 0 to 3, but increased substantially at higher disease ratings.

Wide hybridisations for blackleg (*Leptosphaeria maculans*) resistance transfer into oilseed rape (*Brassica napus*)

H. WINTER (1), M. Mosch (1), F. Marthe (2), H. Peterka (2), O. Schrader (2), H. Budahn (2)
(1) Technische Universitaet Dresden, Department of Biology – Molecular Biotechnology, Dresden, Germany; (2) Institute for Breeding Research on Horticultural and Fruit Crops of Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, Quedlinburg, Germany
Phytopathology 102:S4.136

Blackleg caused by the ascomycete *Leptosphaeria maculans* (*Phoma lingam*) is the most significant disease affecting oilseed rape (*Brassica napus*) worldwide. Considering climate change, it is expected to become even more relevant in future. To widen the narrow base of oilseed rape resistance, offspring derived from somatic hybrids *B. oleracea* (+) *B. nigra* and *B. oleracea* (+) *B. carinata*, respectively, are currently characterised and developed towards the *B. napus* karyotype (genome AACC, 2n=38). The focus of this study is on blackleg resistance behaviour of selected selfing and backcross offspring produced using embryo rescue techniques. Adult plant resistant individuals of different generations, along with susceptible genotypes, were examined cytologically, e. g. by genomic *in situ* hybridisation (GISH). Furthermore, the most promising genotypes were self pollinated again and backcrossed with *B. napus* to obtain resistant plants with an AACC background. Moreover, a complete set of nine disomic *B. napus*-*Raphanus sativus* addition lines ($2n=38_{AACC}+2_{(R)}$), originally developed for nematode resistance transfer, has been examined in blackleg resistance tests. One of these lines showed adult plant resistance similar to *R. sativus*. GISH results are compared with those obtained earlier from blackleg resistant addition and putative recombination lines derived from interspecific, sexual hybrids between *B. napus* and *Sinapis arvensis*, *Coincya monensis* and *B. juncea*, respectively.

Environmental factors contributing to development of lettuce dieback disease and genomic characterization of *Lettuce necrotic stunt virus*

W. M. WINTERMANTEL (1), I. Simko (1)
(1) USDA-ARS, Salinas, CA, U.S.A.
Phytopathology 102:S4.136

The disease, lettuce dieback, causes severe losses for lettuce production in the western US and is caused by a group of tombusviruses, including both *Tomato bushy stunt virus* and the newly described *Lettuce necrotic stunt virus* (LNSV). Symptoms include yellowing, necrosis, stunting and death of plants, with losses ranging from a few plants to entire crops; however, incidence in a field can vary annually. The genome of LNSV was sequenced and has an organization typical of the genus, *Tombusvirus*. Much of the genome is most closely related to TBSV; however, the coat protein is closely related to that of *Moroccan pepper virus*, a partially characterized tombusvirus from Mediterranean regions. In order to identify factors contributing to variability in infection, soil analyses were conducted on adjacent fields with similar soil type, but differing for disease incidence. Experiments were conducted to elucidate environmental factors contributing to disease development, and identified moisture and salinity as significant factors contributing to infection. Similarly, efforts to induce disease under controlled conditions demonstrated that exposure to long days and high temperatures can also induce systemic infection of lettuce, but severity varied among specific treatments. These observations demonstrate that both exposure to the pathogen and specific environmental factors are required for development of dieback symptoms on lettuce.

Enhanced resistance to CYSDV in melon (*Cucumis melo* L.) and identification of significant reservoir hosts for virus transmission in the southwestern United States

W. M. WINTERMANTEL (1), J. D. McCreight (1)
(1) USDA-ARS, Salinas, CA, U.S.A.
Phytopathology 102:S4.136

Cucurbit yellow stunting disorder virus (CYSDV), which is transmitted by the sweet potato whitefly (*Bemisia tabaci* Gennadius) biotype B, emerged in the Southwest US in 2006. CYSDV can infect diverse regional weed and crop species, some of which serve as sources for virus transmission to melon. To determine the efficiency of recently identified hosts as reservoirs, CYSDV titer in source tissue as determined by qRT-PCR was compared with transmission rates to melon and squash. Bean, buffalo gourd, and lettuce had relatively high virus titers that resulted in a significant level of transmission. London rocket and Shepherd's purse had low levels of CYSDV and were poor sources for transmission. In efforts to identify strong sources of host plant resistance to CYSDV, field experiments were conducted in Imperial Valley,

CA. Melon PI 313970 exhibited high-level resistance to CYSDV in replicated field tests. Mean plant condition ratings of PI 313970 were significantly ($P_{0.05}$) better than those of the susceptible control, 'Top Mark.' Data from a cross with the CYSDV-resistant melon TGR-1551 indicated potential for significantly higher resistance than that exhibited by either resistance source alone. Although resistance to CYSDV may be increased with these sources combined, they must be used in combination with an active insecticide treatment program due to excessively high whitefly feeding pressure.

Characterization of ontogenic resistance to powdery mildew in hop cones

S. N. WOLFENBARGER (1), M. E. Nelson (2), G. G. Grove (2), J. L. Woods (3), D. H. Gent (4)
(1) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, U.S.A.; (2) Department of Plant Pathology, Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA, U.S.A.; (3) Department of Crop and Soil Science, Oregon State University, Corvallis, OR, U.S.A.; (4) USDA-ARS, Forage Seed and Cereal Research Unit, and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 102:S4.137

Powdery mildew of hop (caused by *Podosphaera macularis*) can result in considerable loss of cone yield and quality. While ontogenic resistance has been demonstrated in hop leaves it has not been characterized extensively in cones. Greenhouse and field produced cones inoculated with *P. macularis* in varying stages of development displayed progressive reductions in incidence or severity of powdery mildew with maturation. The most severe powdery mildew infection was seen at early stages of bloom and early developmental stages of cones. However, low levels of infection were possible throughout cone maturation. Field studies were conducted at two locations over three years to document yield and quality impact of powdery mildew when fungicide applications were ceased at varying points post-bloom. Fungicide applications applied during early stages of cone development were essential to minimize cone discoloration from powdery mildew and maximize bittering acid yield. Fungicides applied after this period only resulted in modest improvements in bittering acid yield, but improved cone color in two location-years. In two commercial fields in Oregon, two to three fungicide sprays targeting early stages of bloom provided disease control similar to six prophylactic sprays made throughout the season. Collectively, these studies indicate that partial ontogenic resistance occurs in cones, and targeting the early stages of cone development is important in managing powdery mildew.

Distribution of mating type of *Podosphaera macularis* in the Pacific Northwest

S. N. WOLFENBARGER (1), M. C. Twomey (1), D. H. Gent (2)
(1) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, U.S.A.; (2) USDA-ARS, Forage Seed and Cereal Research Unit, and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 102:S4.137

Sexual reproduction in Acomycetes is regulated by the mating-type locus, MAT1. Both idiomorphs, MAT1-1 and MAT1-2, of the mating type loci need to be present for sexual reproduction. Cleistothecia, the ascigerious stage of powdery mildew fungi, have not been observed in *Podosphaera macularis* (casual agent of hop powdery mildew) in the Pacific Northwest (PNW) but have been observed on hop in other populations. To characterize the frequency of mating type idiomorphs in *P. macularis* populations in the PNW, degenerate primers designed to the MAT1 loci of powdery mildew fungi were used to amplify and identify conserved mating type domains among isolates. Non-degenerate primers were then designed for both idiomorphs based on sequence data of isolates from the PNW and Europe. These primers were tested against a panel of other fungi commonly associated with hop to verify their specificity to *P. macularis*. Pairings with isolates from sexually recombining populations are underway to verify the robustness of the MAT1 PCR assays from *P. macularis*. A systematic survey of isolates across the PNW hop growing regions is underway to determine the prevalence of mating type idiomorphs in the pathogen population.

Potential of nematophagous fungi for control of the pale cyst nematode, *Globodera pallida*

J. Worapong (1), L. DANDURAND (1)
(1) University of Idaho, Moscow, ID, U.S.A.
Phytopathology 102:S4.137

Efficacy of four nematophagous fungi (*Paecilomyces lilacinus*, *Plectosphaerella cucumerina*, *Fusarium oxysporum*, *Fusarium solani*) and one bacterium (*Pseudomonas fluorescens*) for controlling the pale cyst nematode (PCN), *Globodera pallida*, was examined. Candidate organisms (10^8 cfu/g) were added to pasteurized sand one month prior to, and at the time of transplanting potato (Russet Burbank) tissue culture plantlets into 20-cm conetainers. Individual conetainers were infested with 10 PCN cysts one monrh prior to planting. After 8 wk, plants were removed and roots were weighed, stained with acid-fuschin, and the different life stages of the nematode (2nd and 3rd-stage larvae, adult females, adult males, and cysts) were counted. All of the tested organisms significantly reduced numbers of cysts formed, but did not significantly reduce numbers of nematodes in other stages. However, numerical distributions of the different life stages over time were significantly altered in the presence of the biocontrol agents compared to controls. With *F. oxysporum*, 51% of observed nematodes were males compared to 6% males for PCN alone. With *P. fluorescens*, 73% of observed nematodes were 3rd-stage juveniles compared to 26% for PCN alone. *P. lilacinus* appeared to delay maturity of PCN: 37% and 36%, respectively, of observed nematodes were 2nd- and 3rd-stage juveniles, whereas PCN alone had 12% 2nd-stage juveniles and 26% 3rd-stage juveniles. These effects on the PCN life cycle may have implications for disease development and management.

Potato zebra chip in the Pacific Northwest: Impact and probable psyllid source assessments

F. WORKNEH (1), M. Mirik (2), A. Rashed (1), P. B. Hamm (3), J. Ansley (2), C. M. Rush (1)
(1) Texas AgriLife Research, Bushland, TX, U.S.A.; (2) Texas AgriLife Research, Vernon, TX, U.S.A.; (3) Oregon State University, Hermiston, OR, U.S.A.
Phytopathology 102:S4.137

Since its first detection in south Texas in 2000, potato zebra chip (ZC), caused by '*Candidatus* Liberibacter solanacearum', which is vectored by the potato psyllid (*Bactericera cockerelli*), has spread into the central and southwestern US, causing substantial losses in production. In 2011, the disease was observed for the first time in the Pacific Northwest (PNW: ID, OR, and WA), triggering widespread concerns over its probable impact on the region, which accounts for the majority of potato production in the US. While the overall disease incidence across the region was low, harvested potatoes from some of the affected fields had up to 32% ZC incidence. Infra-red remote sensing of these fields, in which plants were classified as healthy or diseased, provided a significant correlation ($R^2 = 0.99$, $P = 0.002$) with ZC incidence in harvested tubers. However, remote-sensing overestimated ZC incidence when compared to the incidence in tubers, probably because of the presence of aerial plant symptoms similar to ZC that were caused by other factors. The source of potato psyllids that were responsible for the 2011 ZC outbreak in the PNW is unknown. However, potato psyllids overwinter in the southwestern US (TX, NM, AZ, and CA) and may migrate north in the spring each year. Air-parcel trajectory analysis for the 2011 season implicated Arizona and California as probable sources, but ruled out Texas and New Mexico.

Recent mortality episodes of *Populus tremuloides* and climate in North America

J. J. WORRALL (1), E. H. Hogg (2), G. E. Rehfeldt (3), A. Hamann (4), M. Michaelian (2), L. Gray (4)
(1) U.S. Forest Service, Gunnison, CO, U.S.A.; (2) Natural Resources Canada, Canadian Forest Service, Edmonton, AB, Canada; (3) U.S. Forest Service, Moscow, ID, U.S.A.; (4) University of Alberta, Department of Renewable Resources, Edmonton, AB, Canada
Phytopathology 102:S4.137

We review recent episodes of extensive crown thinning, dieback, and mortality in *Populus tremuloides* (aspen) in North America, and examine moisture stress, insect defoliation and other factors as potential causes. Attention is focused on the southern Rocky Mountains and Colorado plateau, the aspen parkland in western Canada, and the boreal shield of eastern Canada and the northern Lake States. Climate analysis shows that each of these regions experienced significant warming, coupled with exceptionally severe droughts over the past decade. These exceptional droughts were a major cause of crown dieback, mortality and growth reductions, especially in the drier regions of western North America. Other factors, notably defoliation by tent caterpillars and stem damage by fungi and insects also play a substantial role, and may amplify or prolong the impacts of drought on aspen. Regeneration potential is poor in some cases, raising concern that climatic drying could lead to widespread loss of aspen forest cover. Although aspen is widely distributed and successful in a broad array of habitats, in many parts of its range it appears to be quite sensitive to climatic fluctuations. Based on a newly developed range-wide climate profile, coupled with general circulation models used by IPCC, projections suggest that as the climates suited to the boreal forests shift northward and those of the subalpine forests are pushed upwards, suitable habitat for aspen will diminish.

Role of virus titer and aphid species population abundance in the spread of three potyviruses in Louisiana sweet potato fields

E. N. WOSULA (1), C. A. Clark (1), J. A. Davis (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.138

Sweet potato feathery mottle virus (SPFMV), *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2) are potyviruses non-persistently transmitted by various aphid species, including *Aphis gossypii* and *Myzus persicae*. Our objective was to determine how aphid populations and virus titers relate to spread of SPFMV, SPVG and SPV2 in Louisiana sweet potato fields. Aphids were trapped in four fields each year from March through September, 2009 to 2011. *Ipomoea setosa* sentinel plants for virus detection were placed in fields, those showing symptoms were tested for each potyvirus using NCM-ELISA. Leaf samples were collected from one field in 2010 and 2011 and virus titers were quantified using real time RT-PCR. SPFMV, SPVG, and SPV2 were found in 99, 11, and 3% of infected sentinel plants, respectively. The most dominant aphid species were *Rhopalosiphum padi* (33%) and *A. gossypii* (24%). Aphids were captured during the entire crop cycle, but virus infection of sentinel plants occurred mainly during the months of June to August (83-91% of the infected plants). Virus titers for SPFMV were greater in samples collected during June to August compared to other months. Significant aphid populations were present during April to June when there was very little virus infection of sentinel plants and virus titers were low in sweet potatoes, suggesting that low virus titers may reduce opportunities for aphids to acquire the viruses.

Multigene phylogeny reveals two new species-groups within *Alternaria*

J. WOUDENBERG (1), P. W. Crous (2), J. Z. Groenewald (2)
(1) CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands; (2) CBS Fungal Biodiversity Institute, Utrecht, Netherlands
Phytopathology 102:S4.138

Alternaria is an omnipresent fungal genus associated with a wide variety of substrates. Species of *Alternaria* are known as serious plant pathogens, causing major losses on a wide range of crops, and several taxa are also important postharvest pathogens. Molecular-based studies revealed that *Alternaria* species cluster in multiple distinct species-clades, forming a phylogenetic complex together with several other phaeodictyosporic genera. The molecular-based species-clades do not always correlate with the morphological species-groups based on characters of their conidia, branching patterns, and the nature of the apical conidial beaks. Currently six species-groups are recognised based on molecular phylogenies, which represent 55 *Alternaria* species. The *A. porri* and *A. sonchi* species-groups represent species with large-spored conidia, while the *A. alternata*, *A. radicina*, *A. brassicicola* and *A. infectoria* species-groups only harbour small-spored species. Sequence data of the 5.8S nrDNA with the two flanking internal transcribed spacers (ITS), the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and translation elongation factor-1 alpha (EF) gene region of ex-type and reference strains of *Alternaria* species described in the *Alternaria* identification manual reveal two new molecular species-groups, representing 13 *Alternaria* species. The *A. nobilis* species-group contains eight species that all occur on *Caryophyllaceae* and have beaked, ellipsoid to long-ovoid primary conidia. The *A. panax* species-group contains five species with branching chains of beaked conidia. Both species-groups contain large- and small-spored species.

Agronomic evaluation of soybean (*Glycine max* (L.) Merr.) recombinant inbred lines segregating for resistance to southern root-knot nematode (*Meloidogyne incognita*)

D. WRIGHT (1), J. Anderson (1), M. Reyes-Valdes (2), J. Bond (1), S. K. Kantartzi (1)
(1) Southern Illinois University, Carbondale, IL, U.S.A.; (2) Universidad Autónoma Agraria Antonio Narro, Saltillo, Mexico
Phytopathology 102:S4.138

One of the most economically important pathogens of US soybeans is the Southern Root Knot Nematode [(*Meloidogyne incognita*) (Kofoid and White) Chitwood] (Mi). Economically and environmentally sustainable control methods for this pathogen are limited to resistant varieties and non-host rotation. Evaluation and identifying resistance is highly important for plant breeding programs. The main objective of the present study was to screen within the greenhouse two $F_{5:7}$ recombinant inbred line (RIL) (n=96) from crosses between 'LS90-1920' or 'LS97-1610' (resistant parents) with 'Spencer' (susceptible parent) to identify sources of resistance for Mi. Additionally, the RILs were evaluated in two environments in southern Illinois (Harrisburg and Dowell) in 2011 for several agronomic characteristics including yield performance. Descriptive statistics, genotype x environment interaction, and broad-sense heritability were used to identify any major resistance genes. Additionally, correlation coefficients between Mi resistance and important agronomic traits such as lodging, pubescence, growth habit, plant height and yield were estimated. The results will be used for constructing mapping populations and accelerating selection practices.

Single nucleotide polymorphism study of recombinant inbred lines population for resistance to root-knot nematode (*Meloidogyne incognita*) in soybean (*Glycine max* (L.) Merr.)

D. W. WRIGHT (1), S. K. Kantartzi (2), K. Meksem (2)
(1) Southern Illinois University-Carbondale, Herscher, IL, U.S.A.; (2) Southern Illinois University, Carbondale, IL, U.S.A.
Phytopathology 102:S4.138

Soybeans [*Glycine max* (L.) Merr.] are susceptible to many pests including southern root-knot nematode (*Meloidogyne incognita*) (Mi). Mi can cause up to a 90% loss in yield and one of the most effective ways in controlling this high yield loss is to plant resistant varieties. Although traditional breeding continues to give resistant soybean varieties, molecular tools may help to accelerate progress. The objectives of the current study is to 1) screen within the greenhouse an F5:7 recombinant inbred line (RIL) population (n=96) from a cross between 'LS90-1920' (resistant parent) with 'Spencer' (susceptible parent) for resistance to Mi 2) genotype it with more than 5,000 single nucleotide polymorphism (SNP) markers and 3) reveal associations between phenotypic and molecular data using JMP® genomics. Statistical analyses of molecular and phenotypic data provide valuable information for soybean breeders developing new strategies for combining, pyramiding and mapping useful agronomic traits.

Genomic analysis of '*Candidatus* Liberibacter americanus' strain São Paulo

N. A. Wulff (1), S. ZHANG (2), E. C. Martins (1), D. Kumar (3), P. K. Chakrabarty (2), L. A. Fleites (2), J. M. Bové (4), D. W. Gabriel (2)
(1) Departamento Científico, Fundecitrus, Araraquara, SP, Brazil; (2) Plant Pathology Department, University of Florida, Gainesville, FL, U.S.A.; (3) Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL, U.S.A; (4) Université Victor Ségalen Bordeaux and INRA, Bordeaux, France
Phytopathology 102:S4.138

Citrus greening is a lethal disease of citrus caused by several species of '*Candidatus* Liberibacter' spp., which are psyllid transmitted, phloem limited, alpha proteobacteria. The complete circular genomic DNA sequence of '*Ca.* L. americanus' (Lam) strain "São Paulo" isolated from infected periwinkle (Vinca) in Brazil has been determined. The genome size is 1,195,199 bp. The average GC content is 31.12%. There are 1,071 genes, with 1,015 encoding predicted proteins, 9 encoding rRNA genes and 45 encoding tRNAs. Of the protein coding genes, 839 (78.34%) have a predicted function, while 176 (16.43%) have no predicted function. The overall gene organization and structure of the Lam genome is more similar to '*Ca*. L. solanacearum' (Lso) than to '*Ca*. L. asiaticus' (Las). There are 845 genes common to Lam, Lso and Las, 26 genes found in Lam and Lso but not Las, and only 6 genes common to Lam and Las but not found in Lso. As with Las, two circular phages were confirmed in Lam, with Lam-SC1 being 39,941 bp and Lam-SC2 being 16,398 bp in size. SC1 (and not SC2 as in Las) appears to replicate as a plasmid prophage and putative lysogenic conversion genes are found on this replicon---specifically peroxidases and adhesions. Las phages were found to become lytic in plant infections; Lam appears to have same potential, carrying a "suicide program" or lytic cycle genes. Experiments designed to exploit these genomics findings and determine what stresses can activate the Las lytic response are currently underway.

Comparison of putative secondary metabolite genes and gene clusters of *Colletotrichum graminicola* and *C. sublineolum*

K. V. XAVIER (1), M. F. Torres (1), E. A. Buiate (1), I. Gaffoor (2), S. Chopra (2), L. J. Vaillancourt (1)
(1) University of Kentucky, Lexington, KY, U.S.A.; (2) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.138

Colletotrichum graminicola and *C. sublineolum* cause anthracnose leaf blight and stalk rot in maize and sorghum respectively. The two closely related species appear to be host-specific, with *C. graminicola* infecting maize and *C. sublineolum* infecting sorghum. Host specificity in plant-pathogen interactions is often related to the presence or absence of pathogen virulence factors, including secondary metabolites (SM). The major classes of fungal SM include polyketides, peptides, terpenes, and indole alkaloids produced by polyketide synthases (PKS), non-ribosomal peptide syntheses (NRPSs), terpene synthases (TS), or dimethylallyl transferases (DMATs), respectively. We identified and compared genes that potentially encode proteins involved in

production of SM from the *C. graminicola* and *C. sublineolum* genomes. Putative PKS, NRPS, PKS-NRPS, TS, DMAT genes and surrounding gene clusters were identified by using the programs MCL, Ortho-MCL, and SMURF, followed by hand-annotation. *C. sublineolum* was predicted to have 9 DMATs, 46 PKSs, 11 NRPSs, 7 PKS-NRPSs, and 14 TSs. *C. graminicola* was predicted to encode 7 DMATs, 39 PKSs, 7 NRPSs, 7 PKS-NRPSs and 14 TSs. Phylogenetic analysis was performed to understand the evolutionary relationships among these gene families in the *Colletotrichum* genus.

Optimization of growing conditions to enhance phytotoxin production in cultural filtrates of *Fusarium virguliforme*, the causeof soybean sudden death syndrome

Y. XIANG (1), G. L. Hartman (2)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) USDA-ARS, Urbana, IL, U.S.A.
Phytopathology 102:S4.139

Fusarium virguliforme is the causal fungus of sudden death syndrome (SDS) and can causes significant yield losses under some environments. Foliar symptoms of SDS include interveinal chlorotic spots and interveinal necrosis. Cell-free toxic culture filtrates of *F. virguliforme* cause foliar symptoms on cuttings of soybean seedlings with stems immersed in culture filtrates as observed in whole plant inoculations. The objective of this study was to optimize the conditions to enhance the activity of cell-free toxic culture filtrates of *F. virguliforme*. Cell-free toxic culture filtrates of *F. virguliforme* from liquid broth cultures produced under various conditions were evaluated for intensity of foliar symptoms production on cuttings of soybean seedlings. Cell free culture filtrate from the fungus grown in dextrose soybean milk produced greater foliar disease rating on cuttings of soybean seedlings than when cutting were exposed to filtrates of the fungus grown in potato dextrose broth. Foliar symptoms on cuttings of soybean seedlings were more severe when incubated with the cell-free toxic filtrate at 15, 20, 25°C than at 30°C. Manipulation of fungal growing conditions in liquid culture and/or incubation conditions with cuttings of soybean seedlings with stems immersed in culture filtrates caused differences in SDS foliar symptom severity.

Pathogenic and genetic diversity among *Scleotium rolfsii* isolates in the southern United States

C. XIE (1), C. Huang (1), G. E. Vallad (1)
(1) University of Florida, Gulf Coast Research and Education Center, IFAS, Wimauma, FL, U.S.A.
Phytopathology 102:S4.139

Southern blight (caused by *Sclerotium rolfsii* Sacc.) is a serious fungal disease affecting diverse crops grown in tropical and subtropical regions of the world. The disease is becoming more problematic in vegetable production in the southern United States with changes in fumigation practices and the adoption of low-input production strategies. Eighty-four *S. rolfsii* isolates collected from several hosts, including peanut, pepper, and tomato, were assigned to 23 mycelial compatible groups (MCGs), of which 13 MCGs were exclusive to single hosts. Seven MCGs consisted of isolates originating from several hosts. Representative isolates from each MCG were tested for pathogenicity on peanut, pepper, and tomato plants in green house trials. While all isolates were pathogenic against the three hosts, significant differences in virulence were observed among isolates. As a group, isolates from peanut were statistically more virulent on pepper and peanut and produced significantly larger and fewer sclerotia than isolates from other hosts, with a positive correlation between virulence and sclerotia size. Preliminary cladistics based on sequence analysis of the ribosomal RNA internal transcribed spacer region from twelve isolates found that four out of six peanut isolates formed a single clade, suggesting that peanut isolates may genetically differ from those recovered from the other hosts.

Biological and molecular characterization of *Tomato chlorotic dwarf viroid* in Arizona

Z. XIONG (1), B. C. Wong (1), N. Yu (1), J. Cantúa (1), E. F. Allee (1), A. M. Cochran (1), S. A. Trinh (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.139

Tomato chlorotic dwarf viroid (TCDVd), a small, naked RNA pathogen, has caused significant losses in tomato productions, particularly in greenhouse tomato productions in Arizona. Due to the highly contagious nature of TCDVd and lack of tomato resistance, the viroid is managed mainly by exclusion and eradication. A key question that determines the outcomes of such management is the length of the incubation period, during which TCDVd accumulates in the infected plants to a level detectable by RT-PCR. To answer this question, nine tomato varieties were mechanically inoculated with TCDVd; and a one-step RT-PCR assay was developed to monitor the appearance of TCDVd in the inoculated plants for up to six weeks. TCDVd became detectable in most varieties at 17 dpi, but it was not detected in two varieties until one month after inoculation. Sequence analyses indicate that the Arizona TCDVd isolates formed its own phylogenetic clade together with a Mexican isolate and another Arizona isolate, suggesting an origin different from most other reported TCDVd isolates. Infectious cDNA clones of TCDVd were generated by cloning the dimeric forms of PCR-amplified TCDVd DNA. Transcripts synthesized from the clones were infectious on Roma VF and Mariachi tomatoes, and exhibited a similar incubation period. These results indicate that TCDVd has a long, host-dependent incubation period in tomatoes, which may present a challenge to the implementation of the exclusion and eradication management program for TCDVd.

Genetic analysis of antimicrobial activities of bacteria isolated from plant disease-suppressive niches

J. Xu (1), Y. Liu (2), P. Deng (3), S. Baird (3), S. LU (3)
(1) Institute of Food Quality and Safety Detection, Jiangsu Academy of Agricultural Sciences, Nanjing, Peoples Republic of China; (2) Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, Peoples Republic of China; (3) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 102:S4.139

Antagonistic bacteria are associated with disease suppressiveness in various plant pathosystems. The long-term goals of the research program are to characterize genes and compounds required for bacterial antimicrobial activities against plant pathogens and to develop biologically-based disease management strategies. More than thirty bacterial isolates, which possess significant antimicrobial activities against plant fungal and bacterial pathogens, have been isolated from various disease-suppressive niches, such as charcoal rot of soybean and fire blight of pear. The 16S rDNA analyses indicated that these bacterial isolates belong to the genera *Pseudomonas*, *Burkholderia*, *Bacillus*, *Enterobacter*, and *Streptomyces*. Currently genetic analyses of three isolates, *Pseudomonas kilonensis* JX22, *P. chlororaphis* YL1 and *P. vancouverensis* PD2, are underway. Mutants of each bacterial isolate, which are defective or significantly reduced in antimicrobial activities, have been obtained using a Tn*5* random mutagenesis. A few genes associated with antimicrobial activities have been partially cloned via a plasmid rescue procedure. Preliminary sequence analysis showed that these genes are associated with secretion and biosynthesis of antimicrobial compounds. The findings of the project will provide valuable insights to understand genetics and biochemistry of antimicrobial activities of the bacteria. (The first two authors contribute equally.)

WITHDRAWN

Differences in fruit surface chemistry in resistant and susceptible genotypes of peach fruit to the brown rot pathogen *Monilinia fructicola*

M. A. YAGHMOUR (1), J. H. Leveau (1), T. M. Gradziel (1), M. Lee (2), R. M. Bostock (1)
(1) University of California, Davis, CA, U.S.A.; (2) National Chung-Hsing University, Taichung, Taiwan
Phytopathology 102:S4.139

As peaches and nectarines mature they become increasingly susceptible to the brown rot pathogen, *Monilinia fructicola*. Associated with this increased

susceptibility are structural changes in the fruit surface, which includes thinning of the cuticle, and changes in fruit chemistry, such as production of sugars and a decline of antioxidant phenolic compounds. Also, there are significant differences among genotypes in their susceptibility to brown rot disease. The epiphytic bacterium, *Pantoea agglomerans*, engineered to express GFP in the presence of fructose, was used as a bioreporter to monitor changes in fructose on peach and nectarine fruit following different treatments. Comparison of brown rot resistant and susceptible genotypes at two maturity stages revealed significant differences in fructose availability among genotypes, as well as between intact fruit and fruit with micro-wounds. Development of a bioreporter to measure fruit surface redox state is in progress. These bioreporters will provide an important adjunct to traditional chemical methods to detect differences in surface chemistries that may influence infection and colonization by pathogens and fructoplane microbial communities.

The novel virulence-related gene *nlxA* in the lipopolysaccharide cluster of *Xanthomonas citri* subsp. *citri* is involved in the production of lipo-polysaccharide and extracellular polysaccharide, motility, biofilm formation, and stress resistance

Q. YAN (1), X. Hu (2), N. Wang (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) Zhejiang Sci-Tech University, Hangzhou, Peoples Republic of China
Phytopathology 102:S4.140

Lipopolysaccharide (LPS) is an important virulence factor of *Xanthomonas citri* subsp. *citri*, the causative agent of citrus canker disease. In this research, a novel gene designated as *nlxA* (Novel LPS cluster gene of *X. citri* subsp. *citri*) in the LPS cluster of *X. citri* subsp. *citri* 306 was characterized. Our results indicate that *nlxA* is required for O-polysaccharide biosynthesis by encoding a putative rhamnosyltransferase. This is supported by several lines of evidence. 1) NlxA shares 40.14% identity with WsaF which acts as a rhamnosyltransferase. 2) SDS-PAGE analysis showed that four bands of O-antigen part of LPS were missed in the LPS production of the *nlxA* mutant. This is also consistent with the previous report that the O-antigen moiety of LPS of *X. citri* subsp. *citri* is composed of a rhamnose homo-oligosaccharide. 3) Mutation of *nlxA* resulted in significant reduction in resistance of *X. citri* subsp. *citri* against different stresses including SDS, ploymyxin B, H_2O_2, phenol, CuSO4 and ZnSO4. Additionally, our results indicate that *nlxA* plays an important role in extracellular polysaccharide (EPS) production, biofilm formation, stress resistance, motility on semisolid plate, virulence and in planta growth in host plant grapefruit.

Isolation of *Phytophthora* and *Pythium* species from different depths of sediments in a runoff water sedimentation pond of eastern Virginia

X. YANG (1), C. Hong (1)
(1) Virginia Tech, Virginia Beach, VA, U.S.A.
Phytopathology 102:S4.140

Sedimentation pond is used to prevent pathogens and sediments in runoff water from returning to irrigation reservoirs. To maintain its holding capacity, regular excavation is required. The objective of this study was to determine the survival of *Phytophthora* and *Pythium* species in sediments. Triplicate samples of sediments were taken from each of five depths at 0, 0.8, 1.4, 1.8, and 2.4 m from the surface in a sedimentation pond of eastern Virginia in February, 2011. Each sample was air-dried then sieved through 35 and 200 meshes, resulting in subsamples 1 (particle> 500 µm), 2 (75-500 µm), and 3 (< 75 µm). Each subsample was plated in two Petri dishes with PARP and two with PARPH-V8 agar. A total 628 isolates were recovered with 80% from the top layer and 20% from the 0.8-m depth. DNA fingerprinting and sequence analysis of ribosomal DNA internal transcribed regions plus morphological examination were performed to determine the identities of individual isolates. Major plant pathogens identified include *P. pini*, *P. nicotianae*, *P. tropicalis*, *Py. acanthicum*, *Py. dissotocum*, *Py. irregulare*, *Py. monospermum*, and *Py. vexans*. All *Phytophthora* isolates were from the top layer and subsamples 1 and 2. *Pythium* isolates were recovered from both the top layer and at 0.8 m. The implications of these results are discussed.

First report of *Colletotrichum chlorophyti* and a new *Colletotrichum* species causing soybean anthracnose

H. YANG (1), J. S. Haudenshield (1), G. L. Hartman (2)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) USDA-ARS, University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.140

Colletotrichum species were isolated from soybean (*Glycine max*) petioles and stems in Illinois and identified by morphological characteristics and molecular analysis. Three types with curved spores and one type with straight spores were obtained. Multigene phylogenetic analysis was performed with partial sequences of rDNA internal transcribed spacers (ITS1, ITS2), cytochrome c oxidase subunit 1 (COX1), actin (ACT), beta-tubulin (TUB2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and histone H3 (HIS3) genes. The reconstructed phylogenetic trees showed that in additional to a clade of *C. truncatum* (the most common anthracnose pathogen of soybean) and a clade of *Glomerella glycines* (teleomorph of *C. destructivum* that has straight spores), one type was clustered with *C. chlorophyti* and another type formed a distinct lineage more related to *C. liriopes*, *C. spaethianum*, and *C. verruculosum*. Morphological observations matched the description of *C. chlorophyti* with the shapes and sizes of conidia and the formation of chlamydospores. Pathogenicity tests showed that the isolate of *C. chlorophyti* could cause soybean leaf necrosis but with reduced severity as compared to *C. truncatum*. *C. chlorophyti* was reported to infect *Chlorophytum* sp. and *Stylosanthes hamata* in India and Australia. This is the first report of *C. chlorophyti* causing soybean anthracnose in the USA. The unidentified *Colletotrichum* species caused few symptoms and its identification is underway.

Occurrence of soybean sudden death syndrome after adoption of Roundup Ready technology in North America

X. YANG (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.140

Sudden death syndrome (SDS) caused by *Fusarium virguliforme* is a major soybean disease in the North America. The occurrence of SDS in America was analyzed with time series data consisting of data from individual soybean production states. Prior to the adoption of Roundup Ready soybean technology, SDS was a minor disease with outbreaks limited to Delta area except for one outbreak in 1993, a year with a record flood. Increase in SDS damage occurred after introduction of RR soybeans in 1996. With adoption rates of RR soybean at 3, 12, and 37% of total US soybean planting acreages for the first three years of adoption, losses from SDS increased 10 folds. In years of severe outbreaks, the losses were 20 folds compared to losses in years prior to RR soybean adoption. There were two peaks of damage in the time series, one in 2000 and one in 2010. The susceptibility of RR soybeans to SDS was previously blamed for the first peak. The latest outbreaks were coincident with the use of higher rates of glyphosate that farmers use to manage glyphosate-resistant weeds. An early study on effects of herbicides (including glyphosate) on SDS had predicted that "the use of resistant cultivars is unlikely to counteract potentially negative impacts (plant or soil microflora-mediated) from excessive herbicide application".

WITHDRAWN

Loop-mediated amplification (LAMP) for specific detection of the tomato pathogen, *Clavibacter michiganensis* subsp. *michiganensis*

J. H. Yasuhara-Bell (1), A. M. ALVAREZ (2)
(1) University of Hawaii, Department of Plant and Environmental Protection Sciences (PEPS), Honolulu, HI, U.S.A.; (2) University of Hawaii, Honolulu, HI, U.S.A.
Phytopathology 102:S4.141

Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), is one of the most important bacterial diseases of tomato. Detection protocols for plants and seed are time consuming and expensive. Additionally, current molecular and immunological detection methods are prone to false results, especially when targeted against mobile pathogenicity genes. Loop-mediated AMPlification (LAMP) was used to detect *micA*, which encodes a type II lantibiotic, michiganin A, that acts on closely related subspecies of *Clavibacter*, making it ideal for specific detection of *Cmm*. A total of 404 bacterial strains (26 non-*Cmm* and 378 *Cmm*) were tested to determine assay specificity. Results were compared to genetic profiles based on the presence or absence of 7 genes (*dnaA*, *ppaJ*, *pat*-1, *chpC*, *tomA*, *ppaA* and *ppaC*), as established by PCR. *Cmm* strains produced 9 distinct profiles. The LAMP reaction proved to be a useful tool that identified *Cmm* strains regardless of their pathogenicity profiles, and it also discriminated between subspecies of *Clavibacter*. Non-pathogenic strains are often overlooked, even though co-infection between plasmid-free and plasmid-containing non-pathogens poses the possibility of restoration to full virulence. Therefore, this diagnostic test will provide an easy one-step test to definitively determine the presence of *Cmm* on tomato, saving time and money, and preventing potential outbreaks.

Natural suppression of Rhizoctonia root rot by soil microbial communities in wheat from a *Rhizoctonia* decline site

C. Yin (1), S. Hulbert (1), K. L. Schroeder (1), O. Mavrodi (1), D. Mavrodi (1), W. Schillinger (2), T. C. PAULITZ (3)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) Department of Crop and Soil Sciences, Washington State University, Pullman, WA, U.S.A.; (3) USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, WA, U.S.A.
Phytopathology 102:S4.141

Rhizoctonia root rot, caused by *Rhizoctonia solani* AG-8, limits yield in direct-seeded wheat, which develops distinct patches of stunted plants. At a cropping systems study near Ritzville, WA with continuously cropped wheat, bare patch reached a peak after 5 years of direct-seeding and then declined by year 11. From replicated plots, bacterial communities from bulk soil and rhizospheres of plants from inside, outside, and recovered patches, were analyzed by pyrosequencing with primers designed to the 16S rDNA. A total of 56,102 high-quality reads were generated in 2008 and 2010 field samples. *Mesorhizobium*, *Burkholderia*, *Dyella* and *Acidobacteria* Gp3 and Gp7 showed a trend with higher frequencies in the rhizosphere of healthy plants outside of the patches and from recovered patches, compared to diseased plants from inside patches. Oxalobacteriaceae (*Herbaspirillum* and *Massilia*), *Chitinophaga*, *Flavobacterium*, *Pedobacter* and *Enterobacteriaceae* were higher in the rhizosphere of diseased plants from inside patches. For selected taxa, pyrosequencing results were validated by real-time quantitative PCR. Furthermore, the shifts of some microbial communities in the rhizosphere over time were duplicated with cycling experiments in the greenhouse, with successive plantings of wheat in *Rhizoctonia*-inoculated soil. *Chryseobacterium soldanellicola* and *Pseudomonas* sp. were isolated from the rhizosphere of plants inside patches and exhibited significant antagonism against AG-8 in vitro in dual culture experiments and in greenhouse tests. In conclusion, this study identified novel bacterial taxa which respond to conditions affecting bare patch symptoms and may be involved in suppression of Rhizoctonia root rot.

Identification of putative virulent factors of '*Candidatus* Liberibacter asiaticus', the pathogen associated with citrus greening (huanglongbing)

X. YING (1), N. Wang (2)
(1) Citrus Research and Education Center, Lake Alfred, FL, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.141

Citrus greening or huanglongbing (HLB) is a devastating disease of citrus, and poses a major threat to the citrus industry in the United States. '*Candidatus* Liberibacter asiaticus' has been known to be associated with HLB in the United States. Unsuccessful attempts to culture '*Ca*. L. asiaticus' have notably hampered efforts to understand its biology and pathogenesis mechanism despite some limited progresses in culturing. In order to identify the putative virulence factors, we expressed putative virulent factors in *Nicotiana benthamiana* and *Arabidopsis*. Totally 24 putative virulent factors are being tested with most of them containing signal peptides. By transient expression of the candidates using TMV vector in *N. benthamiana*, we can screen the genes influencing plant development and morphology. Meanwhile, transformation of candidate genes into *N. benthamiana* and *Arabidopsis* driven by 35S promoter and phloem specific promoter respectively will further verify the function of putative virulence factors. Identification and characterization of the various virulence factors in '*Ca*. L. asiaticus' will advance the understanding of the biology and pathogenicity of the pathogen.

Estimation of incidence and spatial temporal distribution of citrus stubborn disease

R. YOKOMI (1), M. Sisterson (2)
(1) USDA-ARS PWA, Parlier, CA, U.S.A.; (2) USDA-ARS PWA, SJVASC, CDPG, Parlier, CA, U.S.A.
Phytopathology 102:S4.141

Citrus stubborn disease (CSD) is caused by *Spiroplasma citri*, a culturable wall-less prokaryote. The pathogen is graft-transmissible and vectored by the beet leafhopper (BLH). The objective of this study was to determine incidence and spread of *S. citri* in two sweet orange citrus groves in the San Joaquin Valley (SJV). The Ducor plot, with 20-y old trees, is an area with low overwintering populations of BLH; the Huron plot, with 10-y-old trees, is adjacent to a preferred habitat of overwintering BLHs. Each plot had 3 replicates consisting of trees in 16 rows x 16 trees, (768 trees total). DNA was extracted and pooled from 3 fruit columellae/tree and tested by qPCR assay for presence of *S. citri* DNA sequences. Spatial and temporal distribution over a 3-year period showed a slow annual spread of *S. citri* regardless of disease incidence. Disease incidence in Ducor went from 20.1 to 22.3% (2.2% increase), whereas disease incidence in Huron went from 3.1 to 3.9% (0.8% increase). Infected trees were distributed randomly in both plots, suggesting only primary spread was occurring. D-vac samples taken in and around the plots showed BLHs were absent from citrus foliage but present on weed hosts (e.g. Russian thistle, mustard). These data confirm grower observations that *S. citri* spread in citrus is low and originate from sources outside of the citrus grove. Therefore, disease management by weed control and prudent replacement of severely affected trees can be effective.

Image analysis of peroxidase localization on the cross section prepared from Japanese birch plantlet No.8 infected with *Inonotus obliquus* IO-U1 strain

S. YOKOTA (1), C. Ri (1), H. Suzuki (1), A. Yoshinaga (2), H. Kamitakahara (2), F. Ishiguri (1), K. Iizuka (1), N. Yoshizawa (1)
(1) Utsunomiya University, Utsunomiya, Tochigi, Japan; (2) Kyoto University, Kyoto, Japan
Phytopathology 102:S4.141

Inonotus obliquus is a canker-rot fungus of birch trees. We have found previously that peroxidase has important roles at the early defense mechanisms against the fungal infection in birch plantlet. In this study, cross sections were prepared from the stem of *Betula platyphylla* var. *japonica* plantlet No.8, which was infected with *I. obliquus* IO-U1 strain and further cultured for 2, 10, and 30 days. Then image analysis of peroxidase localization was performed on the sections with using matrix-assisted laser desorption/ionization/time-of-flight mass spectrometry (MALDI/TOF/MS) imaging system. Horseradish peroxidase (Sigma) was first analyzed with MALDI-TOF-MS (autoflex III, Bruker Daltonics (BD)) as a standard, and the following characteristic MS peaks were detected: *m*/*z* 23,000±1,000, 33,000±1,000, 40,000±1,000, and 62,000±1,000. Imaging analysis was carried out with using these MS peaks and fleximaging software (BD). As the results, it was found that peroxidase was localized near cambium and xylem part in the sections from the all infected plantlets. The results of the present study were in good accordance with those of our previous study where the staining for peroxidase activity was applied for the sections from the infected birch plantlets.

Role of phenazine structural derivatives in fungal inhibition and biofilm formation

J. YU (1), J. Levy (2), D. Wang (1), L. S. Pierson (3), E. A. Pierson (3)
(1) Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, U.S.A.; (2) Department of Horticultural Sciences, Texas A&M University, College Station, TX, U.S.A.; (3) Department of Plant Pathology and Microbiology, Department of Horticultural Sciences, Texas A&M University, College Station, TX, U.S.A.
Phytopathology 102:S4.141

Phenazine (PZ) production by the biological control strain *Pseudomonas chlororaphis* 30-84 is the primary mechanism responsible for pathogen inhibition and rhizosphere competence. *P. chlororaphis* 30-84 produces three PZ derivatives, phenazine-1-carboxylic acid (PCA), 2-hydroxy-phenazine-1-carboxylic acid (2OHPCA), and a small amount of 2-hydroxy-phenazine.

Adjacent to the phenazine biosynthetic genes is *phzO*, which encodes an enzyme responsible for the conversion of PCA to 2OHPCA. Many *Pseudomonas* strains have genes or combinations of genes such as *phzH*, *phzM*, *phzS*, and *phzMS*, which similarly encode enzymes responsible for the conversion of PCA into other phenazines e.g. phenazine-1-carboxamide, 5-methoxy-phenazine-1-carboxylic acid, 1-hydroxy-phenazine, and pyocyanin, respectively. We constructed isogenic derivatives of strain 30-84PCA, which produces only PCA, by introducing either *phzO*, *phzS*, *phzM*, *phzH*, or *phzMS in trans* on a stable plasmid under the control of the 30-84 phenazine biosynthesis promoter. Modification of the mixture of phenazines produced by these isogenic derivatives significantly affected their capacity and specificity to inhibit fungal plant pathogens. For example, relative to the wild type, the isogenic derivatives differed in their ability to inhibit a variety of pathogens including *Gaeumannomyces graminis*, *Pythium ultimum*, *Botrytis cinerea*, *Cochliobolus heterostrophus*, *Sclerotinia sclerotiorum*, *S. minor*, and *Alternaria brassicae*. These isogenic derivatives also differed in their ability to form biofilms *in vitro* and colonize roots. Although there was some variability in biofilm formation among derivatives and in different media, those that produced altered phenazine mixtures typically produced less biofilm than wild type in both minimal media and smaller populations on wheat roots.

Reevaluation of *Alternaria panax* associated with leaf spot and blight of araliaceous plants

S. YU (1), J. Deng (1), N. C. Paul (1)
(1) Chungnam National University, Daejeon, South Korea
Phytopathology 102:S4.142

In the present study genetic diversity among 35 isolates of *Alternaria* from ginseng and six other hosts of Araliaceae was determined using the rDNA sequence analysis of internal transcribed spacer (ITS), *Alternaria* allergen (Alt a1), elongation factor-1 alpha (EF-1 alpha), two different regions of betatubulin (BT1 and BT2), glyceraldehydes-3-phosphate dehydrogenase (gpd) and RNA polymerase II (RPB2) genes and amplified fragment length polymorphisms (AFLPs) analysis. Sequences of ITS rDNA were invariant among the isolates. However, the isolates of *Alternaria* were divided into three genetic groups (group A, B and C) based on phylogenetic analysis of sequences of Alt a1, EF-1 alpha, BT1, BT2, gpd and RPB2 genes and AFLP analysis. The isolates belong to the group C was all from ginseng, while the isolates of the group A and B were from other araliaceae plants. Morphologically, colony features such as pigment production and conidial morphology were different among isolates of the groups. Reverse color of the colonies of the group A isolates was reddish brown, while that of the group B isolates was yellow and group C isolates was black. Conidia of the group B isolates were larger and more exuberant hyperplasia than those of the group A and C isolates. Pathogecity tests revealed that group A and B isolates induced severe symptoms on detached leaves of *Aralia elata* and *Dendropanax morbifera*, while group C isolates induced very weak or no symptoms. On ginseng leaves, group C isolates produced larger lesions than group A and B isolates. Based on molecular characteristics, morphology and pathogenicity tests, three genetic groups of *Alternaria* should be considered as three different species, and group A and B are considered to be the candidates of new species.

New species of Tubakia leaf spot, *Tubakia koreanum* sp. nov., on *Quercus* spp. from South Korea

H. YUN (1), Y. Kim (1)
(1) Seoul National University, Seoul, South Korea
Phytopathology 102:S4.142

It has been known that Tubakia leaf spot on oak trees (*Quercus* spp.) is commonly caused by *Tubakia dryina*. However, a recent phylogenetic analysis shows its causal pathogen composed of *Tubakia dryina* species complex, which that can be discriminated into several lineages. In this study, one of lineages from *T. dryina* species complex, named as Korean Tubakia, that was collected from the leaves of *Quercus mongolica* and *Q. serrata* in Chungnam province and Gangwon province, Korea during the fall of 2009 and 2011 was examined for the species identification based on its cultural and morphological characteristics and analysis of gene sequences in ITS (internal transcribe spacer) and LSU (large subunit, 28S) regions of rDNA, which was compared with other *Tubakia* species. The Korean Tubakia produced the same leaf spot symptoms on oak tree leaves as the other *Tubakia* species. However, its colony color was brown but not whitish gray as in *T. dryina*. Its spore sizes were bigger than *T. dryina*. Analysis of ITS and LSU rDNA sequences showed that it belong to a monophyletic lineage independent off other *Tubakia* species. It is suggested that the Korean Tubakia is a new species different biologically and genetically from the other *Tuabkia* species causing leaf spots on oak trees. Therefore, this species is here named as *Tubakia koreanum* sp. nov.

Changes in importance of corn diseases in the past two decades in the U.S. Corn Belt

M. L. ZACCARON (1), T. L. Bruns (1), X. Li (1), S. O. Mallowa (1), N. A. Abdelsamad (1), E. Whigham (1), X. B. Yang (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.142

Corn is one of the most important crops for food. In 2011, 91 million acres of corn were planted in the US yielding over 12 billion bushels. Over 60 infectious corn diseases have been reported in the US and their importance related to production and research changes over time. To determine the importance of individual diseases in plant pathological research in the last two decades, we analyzed information on corn diseases in the US using published literature. Importance of individual diseases for each period of five years was determined quantitatively and ranked according to the number of papers published in major journals for plant pathology. In the early 1990's, ear rot diseases ranked as the most important with northern leaf blight second, while in the late 1990's ear rot and Stewart's wilt were the top two diseases. During 2000 to 2005, corn disease caused by *Fusarium verticillioides* ranked in first place, followed by Stewart's wilt. During the2005-2010 period, Steart's wilt ranked in the first, followed by southern leaf blight and common rust in second, additionally the bacterial disease Goss's wilt appeared in the top 5. Our findings suggest the increase of importance of bacterial diseases like Stewart's wilt and Goss's wilt in the US in the past two decades, while the importance of ear rot diseases appears to have relatively declined over the past decade. The effect of delay in publication time relative to occurrence will be discussed.

Small RNA chaperone Hfq and Hfq-regulated small RNAs RyhA and RprA are important virulence regulators in *Erwinia amylovora*

Q. ZENG (1), R. R. McNally (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.142

Hfq is a global small RNA chaperone that interacts with Hfq-regulated small RNAs (sRNAs) and controls the translation efficiency of many genes with diverse functions. In this work, we studied the genetic regulation of Hfq and Hfq-regulated sRNAs in controlling bacterial virulence in the gram negative fire blight pathogen *Erwinia amylovora* (Ea1189). An Ea1189 Δhfq mutant was significantly reduced in virulence in both apple shoot and immature pear models. In addition, 10 putative sRNAs were identified in Ea1189 using a bioinformatics approach, and the expression of five of them was confirmed to be regulated by Hfq when cultured in hrp-inducing minimal medium. Deletion analyses of the 10 putative sRNA-encoding genes identified two sRNAs, RyhA and RprA, as important regulators of virulence along with Hfq. Our results showed that Hfq positively controls the effector translocation of the type III secretion system (T3SS) while RprA and RyhA positively regulate the expression of the T3SS effector DspE, probably in a post-transcriptional manner. In addition, RyhA and Hfq also up-regulate other virulence determinants of Ea1189 including amylovoran production and cell motility, but down-regulate biofilm formation. Our findings suggest a critical function of sRNA chaperone Hfq and sRNAs RyhA and RprA in the virulence regulation of *E. amylovora*.

Function, evolution, and interaction of the coupled genes responsible for the *Pik-h* encoded blast resistance of rice

C. Zhai (1), L. Hua (1), N. Yao (2), F. Lin (1), Y. Zhang (1), Z. Liu (2), Z. Dong (1), L. Wang (1), L. Wang (1), Q. PAN (1)
(1) South China Agricultural University, Guangzhou, Guangdong, Peoples Republic of China; (2) Sun Yat-sen University, Guangzhou, Guangdong, Peoples Republic of China
Phytopathology 102:S4.142

Pik-h, which is an allele of Pik, confers resistance against certain races of rice blast. Its positional cloning showed that it comprises a pair of NBS-LRR genes, Pikh-1 and Pikh-2. The allele is distinguishable from other known blast resistance genes on the basis of key variable nucleotides, and SNP diagnosis among the five rice populations implies that it appears to be the most recently evolved of the set of Pik alleles. Comparisons between the sequences of Pik-h and other Pik alleles showed that the functional K haplotype exists as two sub-haplotypes, which both evolved prior to the domestication of rice. While Pikh-1 appears to be constitutively transcribed, the transcript abundance of Pikh-2 responds to pathogen challenge, suggesting that while Pikh-1 may well be involved in elicitor recognition, Pikh-2 is more likely to be responsible for downstream signalling. In vitro, the CC domain of Pikh-1 was shown interact directly with both AvrPik-h and Pikh-2. Transient expression assays demonstrated that Pikh-2 mediates the initiation of the defence response. In the proposed Pik-h resistance pathway, it is suggested that Pikh-1 acts as an adaptor between AvrPik-h and Pikh-2, while Pikh-2 transduces the signal to trigger Pik-h-specific resistance.

Chemotherapy for citrus huanglongbing disease in the field

M. Zhang (1), C. A. POWELL (2), Y. Guo (2), Y. Duan (3)
(1) University of Florida, Fort Pierce, FL, U.S.A.; (2) Indian River Research and Education Center, IFAS, University of Florida, Fort Pierce, FL, U.S.A.; (3) U.S. Horticultural Lab, USDA-ARS, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.143

Citrus Huanglongbing is serious disease of citrus worldwide, caused by the phloem-residing bacteria, '*Candidatus* Liberibacter'. Several antibiotics and their combinations have been effective in eliminating or suppressing the HLB bacteria in the greenhouse. Sixty HLB-affected 6-year-old citrus trees 10 cm in diameter at the USHRL farm in Fort Pierce, FL, were injected with 100 ml of each of the antibiotics and their combinations (PS: 5.0 g/tree penicillin and 0.5 g/tree streptomycin; KO: 1.0 g/tree kasugamycin and 2.0 g/tree oxytetracycline; and CK: water as control) using an Avo-Ject syringe injector in August 2010. The tapered tip was firmly fitted into a 19/64-in (7.5-mm) diameter hole, 3 cm deep, drilled into the tree. Treatments were repeated a total of seven times once every 2 months. Before treatment, 30 HLB-affected leaf samples per tree were taken from three positions around the canopy of the treated trees for qPCR assay at 2-month intervals for 14 months. HLB bacterial titers increased by more than 40-fold in the CK, 8-fold in KO- and 11-fold in PS- treatment in December 2010, and decreased by more than 13-fold in the CK, 259-fold in the KO- and 97-fold in the PS- treatment in April, 2011. Antibiotic combinations significantly suppressed the HLB bacteria in the field in 4 month post treatment. When the antibiotic treatments ceased in August 2011, the Las bacterial titers increased in the HLB-affected citrus in the KO-treatment and the CK in Oct. 2011, and remained at the lowest in the PS treatment. The results on the bacterial diversity of the antibiotic treatment are still being processed.

WITHDRAWN

Rapid and simple detection of *Plum pox virus* by recombinase polymerase amplification

S. ZHANG (1), R. Bohannon (1), P. Russell (1), N. McOwen (1), S. Bohannon (1), A. Vrient (1), C. Sutula (1)
(1) Agdia Inc., Elkhart, IN, U.S.A.
Phytopathology 102:S4.143

Plum pox virus (PPV), a member of the genus *Potyvirus* in the family *Potyviridae*, causes the most destructive viral disease known as plum pox or Sharka disease of stone fruit species including apricot, cherry, peach and plum. Detection of PPV as an important regulatory pathogen is thus of critical importance to quarantine and eradication of the disease. Agdia has recently developed an isothermal detection of PPV using recombinase polymerase amplification (RPA) in addition to Agdia's current ELISA and RT-PCR tests for PPV. In the RPA assay, PPV RNA-specific DNA amplicons were produced at a constant temperature without thermal cycling and the results were recorded with a portable fluorescence reader in real-time or on immunostrips. The whole test from sample preparation was completed under 40°C in as little as 30 minutes. Five strains (PPV-C, PPV-D, PPV-EA, PPV-M or PPV-W) of PPV were tested in tobacco and several stone fruit species and all the five PPV strains were detected in those PPV-infected plants but not in the control healthy plants. The detection sensitivity was determined using PPV-infected plant total RNA or *in vitro* transcribed PPV RNA and compared with that of ELISA. Our results demonstrated that the detection of PPV by RPA is not only rapid and simple but also highly specific and sensitive.

Comparison of quinone outside inhibitor fungicide-resistant and -sensitive isolates of *Cercospora sojina*

G. R. ZHANG (1), C. A. Bradley (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.143

In 2010 and 2011, isolates of *Cercospora sojina*, the causal agent of frogeye leaf spot (FLS) of soybean, were reported to be resistant to quinone outside inhibitor (QoI) fungicides. To better understand the fitness of QoI fungicide resistance, *C. sojina* isolates sensitive and resistant to QoI fungicides were compared in laboratory and greenhouse studies. The laboratory study compared sporulation and mycelial growth of QoI resistant and sensitive *C. sojina* isolates on agar. No differences in morphology or sporulation were observed among isolates on agar, but QoI resistant isolates had greater radial growth after 12 days. In the greenhouse study, conidia of QoI resistant and sensitive isolates were inoculated onto leaves of the FLS-susceptible cultivar Blackhawk and the FLS-resistant cultivar Davis. On cv. Blackhawk, QoI resistant isolates caused greater disease severity than sensitive isolates 7 to 8 days after inoculation, but no differences were observed after 9 days. On cv. Davis, QoI resistant isolates caused greater disease severity 8 to 14 days after inoculation. The preliminary results of these studies indicate that QoI resistant *C. sojina* isolates may have a competitive advantage over sensitive isolates for some of the characteristics evaluated in our studies, but additional research, including field trials, is needed.

The *i*PhyClassifier II: An update to the online tool for phytoplasma identification and classification

Y. ZHAO (1), I. Lee (1), W. Wei (1), J. Shao (1), X. Suo (1), R. E. Davis (1)
(1) USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD, U.S.A.
Phytopathology 102:S4.143

Inhabiting sieve elements of affected plants and transmitted by phloem-feeding insects, phytoplasmas are a group of cell wall-less bacteria responsible for numerous plant diseases. Since phytoplasmas cannot be cultured *in vitro*, phenotypic characters suitable for conventional microbial characterization remain inaccessible. To date, identification of phytoplasmas has been relied on molecular analysis of genes with different degrees of sequence conservation. Two years ago, we launched an Internet-based research tool, the *i*PhyClassifier, for quick identification and classification of diverse phytoplasmas. The website has attracted attention from researchers around the world, and served as a primary portal for getting classification and taxonomic assignment information of known and newly discovered phytoplasmas. In the new version, the *i*PhyClassifier II, additional functions including multi-locus RFLP analysis for finer differentiation of closely-related phytoplasmas, and composite RFLP pattern display for identification of strains with two sequence-heterogeneous rRNA operons will be added to the system. The *i*PhyClassfier II can be operated from any computer with an Internet connection. No special software other than a web browser is required. The *i*PhyClassfier II can also be operated from mobile devices such as iPad, iPhone, and Google phone. The URL of the web server is http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi.

***Brachypodium distachyon-Cochliobolus sativus* pathosystem—A new model for studying diseases in cereal crops**

S. ZHONG (1), S. Ali (1), R. Wang (1), Y. Leng (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.143

Cochliobolus sativus is a fungal pathogen that causes common root rot, spot blotch and kernel blight in barley and wheat. However, the molecular interaction between *C. sativus* and the cereal crops is poorly understood, partly due to the large size and complication of cereal genomes. Recently, *Brachypodium distachyon* has emerged as a new model system for studying temperate cereal crops and grass species due to its small genome size, short life cycle and many other features. To investigate the host status of *B. distachyon* to *C. sativus*, five *B. distachyon* lines (Bd1-1, Bd21, Bd2-3, Bd3-1 and Bd18-1) were inoculated with five *C. sativus* strains (ND93-1, ND90Pr, ND85F, ND4008 and Cs07-47-1) that exhibited differential virulence on barley and wheat differential lines. The results indicated that all five lines were infected by the *C. sativus* strains although their susceptibility varied depending on the *C. sativus* strains used. The strain Cs-47-1 was most virulent among the five strains tested and chosen to inoculate on a large collection of *B. distachyon* accessions of diverse background. A range of responses from a hypersensitive response (HR)-mediated resistance to full susceptibility were observed among these accessions, indicating that *B. distachyon* is a natural

host of *C. sativus*. The availability of whole genome sequences of both host and pathogen makes the *B. distachyon-C. sativus* pathosystem an attractive model for studying diseases in cereal crops.

Evolving diversity of '*Candidatus* Liberibacter asiaticus' mediated by frequent recombination and reassortment of its prophages

L. ZHOU (1), C. A. Powell (2), W. Li (3), Y. Duan (4)
(1) Indian River Research & Education Center, University of Florida/USDA-ARS, Fort Pierce, FL, U.S.A.; (2) Indian River Research & Education Center, University of Florida/IFAS, Fort Pierce, FL, U.S.A.; (3) National Plant Germplasm and Biotechnology Laboratory, USDA-APHIS-PPQ-CPHST, Beltsville, MD, U.S.A.; (4) USDA-ARS USHRL, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.144

Prophage is a highly dynamic component in the bacterial genome and plays an important role in intraspecies variations. There are at least two prophages that integrate in the chromosomes of '*Candidatus* Liberibacter asiaticus' (Las) Florida isolates. In this study, 8 different types (designated as A to H) of sequences derived from two hyper variable regions that share highly conserved sequences were identified in the two prophages, FP1 (CP001677.5) and FP2 (JF773396.1). Sequence analysis of these 8 types of sequences revealed the variations in this region may result from the frequent recombination and reassortment. Typing results using primers specific to the different types of sequences indicated that Type A and B were two major groups that located in FP1 and FP2, respectively. Las-infected periwinkle and dodder contained all types of Las populations; while psyllids only contained type A, B, C and E, and very low titer to none of D, F, G, and H. Las-infected citrus plants contained various types of Las populations, and only the type D population was associated with huanglongbing (HLB) symptoms: high titer of D with typical blotchy mottle, extreme low to none of D with vein yellowing or other atypical HLB symptoms. Similar results were obtained from global samples, indicating the dramatic variations of these prophage regions among the isolates. These intraspecies variations derived from the prophage activities may be important for the bacterial adaptation to their host plants and insects.

WITHDRAWN

Use of brassica biofumigation cover crop and plant growth-promoting rhizobacteria to manage sheath blight of rice

X. ZHOU (1), G. Liu (1), J. W. Kloepper (2), M. S. Reddy (2)
(1) Texas A&M University System, AgriLife Research, Beaumont, TX, U.S.A.; (2) Auburn University, Department of Entomology and Plant Pathology, Auburn, AL, U.S.A.
Phytopathology 102:S4.144

Sheath blight, caused by *Rhizoctonia solani*, is the most important disease of rice in the southern U. S. No leading rice cultivars have acceptable levels of resistance. Producers heavily depend on fungicides for control of this disease. Field trials were conducted at Beaumont, TX to evaluate the effects of brassica biofumigation cover crop and plant growth promoting rhizobacteria (PGPR) on severity of sheath blight. The trials were conducted as a split plot design with two main plot treatments (brassica 'Caliente 199' cover crop and fallow) and three subplot treatments (*Bacillus subtilis* PGPR strain MBI-600, azoxystrobin (Quadris) and unsprayed control). Plots were inoculated with *R. solani* prior to planting of brassica. Brassica crop was planted early spring and incorporated into soil late spring. Seed of the susceptible cultivar Cocodrie was treated with strain MBI-600 prior to seeding. At the boot stage, plots were sprayed with strain MBI-600 or azoxystrobin. Sheath blight severity was lower in plots seeded to brassica cover crop than in plots left fallow the spring. Strain MBI-600 significantly reduced disease severity in plots either incorporated with brassica or left fallow when compared to the unsprayed control. Azoxystrobin completely controlled sheath blight in plots with brassica.

WITHDRAWN

Epidemiology of *Soybean vein necrosis associated virus*

J. ZHOU (1), I. Tzanetakis (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 102:S4.144

A novel tospovirus was first identified in soybean in 2008 and was named *Soybean vein necrosis associated virus* (SVNaV) because of the distinct symptoms associated with infection; leaf necrosis along the main veins. SVNaV is of special interest to the soybean industry as it has been reported in several of the major soybean producing areas; causing severe disease in some genotypes. This communication focuses on the epidemiology of the virus including population structure, alternative hosts and vectors. The presence of SVNaV was confirmed by qRT-PCR, a more sensitive detection protocol than any method previously reported. Analysis of SVNaV populations from different geographic regions, using the virus nucleoprotein gene, revealed a high degree of similarity on both nucleotide (98%-100%) and amino acid (98%-100%) levels. Samples collected from different states did not show higher diversity compared to those collected from a single state or field, indicating that the virus has stable, homogeneous populations. Alternative host screening identified several susceptible species including vegetables, ornamentals as well as weed species commonly found in soybean fields which may function as virus reservoirs in the field. Several thrips species were tested in transmission studies, and one has proven as an efficient vector of the virus.

Development of smart spray systems to enhance delivery of pesticides in field nursery production

H. Zhu (1), E. Ozkan (2), R. D. Derksen (1), M. E. Reding (1), C. M. Ranger (1), L. Canas (3), C. R. Krause (1), J. C. LOCKE (4), S. C. Ernst (5), R. H. Zondag (6), A. Fulcher (7), R. Rosetta (8), H. Jeon (1), Y. Chen (9), J. Gu (10), H. Liu (9), Y. Shen (9), A. A. Rios (3)
(1) USDA-ARS ATRU, Wooster, OH, U.S.A.; (2) Fabe, The Ohio State University, Columbus, OH, U.S.A.; (3) Entomology, The Ohio State University, Wooster, OH, U.S.A.; (4) USDA-ARS ATRU, Toledo, OH, U.S.A.; (5) Agricultural, Environmental, and Development Economics, The Ohio State University, Columbus, OH, U.S.A.; (6) Horticulture & Crop Science, The Ohio State University, Columbus, OH, U.S.A.; (7) University of Tennessee, Knoxville, TN, U.S.A.; (8) North Willamette Research and Extension Center, Oregon State University, Aurora, OR, U.S.A.; (9) Fabe, The Ohio State University, Wooster, OH, U.S.A.; (10) College of Engineering, Nanjing Agricultural University, Nanjing, Peoples Republic of China
Phytopathology 102:S4.144

Two smart sprayer prototypes have been developed and are being evaluated with a goal of increasing pesticide application efficiency and minimizing

environmental impact in field nursery production sites. The first prototype, a modified hydraulic vertical boom system, utilizes ultrasonic sensors to detect the size and volume of liner-sized plants, and the second prototype is an air-assisted system utilizing a laser scanning sensor to measure plant structure and foliage density. Automatic controllers consisting of: a computer program, signal generation/amplification unit, pulse width modulated solenoid valves with different algorithms/circuit designs, manipulate nozzles to produce variable-rate outputs based on target characteristics and occurrence in real time. Field tests comparing standard industry sprayers, using multiple target species, were conducted using spray deposition and coverage as criteria. Field tests evaluated insect and disease control (powdery mildew on Norway maple and aphids on red oak) and determined no difference between the smart and conventional sprayers. Laboratory and field tests demonstrated that both of the smart sprayer designs had the capability to control spray outputs, matching canopy characteristics in real time, with the potential to drastically decrease pesticide usage thus reducing environmental impact and enhancing applicator safety.

Characterization and identification of *Pythium* from soybean roots in North Dakota

K. ZITNICK-ANDERSON (1), B. Nelson (2)
(1) Department of Plant Pathology, North Dakota State Unversity, Fargo, ND, U.S.A.; (2) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.145

The oomycete *Pythium* comprises one of the most important groups of seedling pathogens affecting soybean, causing pre- and post-emergence damping-off. Approximately 18 to 20 species of *Pythium* from soybean have been identified and found to be pathogenic. However, in the Northern Great Plains soybean production region, such as North Dakota and northern Minnesota, there is limited information on *Pythium* infecting soybean. The most recent information directly addressing this region dates back to 1965. The objective of this research was to identify and study the *Pythium* spp. from soybean roots in North Dakota. Soybean roots and soil samples were collected from 87 fields and isolation of *Pythium* was conducted on a water agar PARP+B and V8 P_{10}VP selective medium. Identification of *Pythium* was achieved from morphological features using the Plaats-Niterick identification key. The universal ITS primers ITS1 and ITS4 were used to amplify a portion of the 18S and 28S region of ribosomal DNA. The ITS sequences were compared with ITS sequences of known *Pythium* species available in public databases. Twenty four *Pythium* species out of 2,656 isolates were identified including well-known species such as *P. ultimum*, *P. debaryanum*, *P. sylvaticum*, and *P. perplexan* and some more obscure species such as *P. oopapillum*, and *P. kashmirense*. There were 560 isolates that could not be identified to species. Soil from each field is being analyzed and correlations between soil properties and *Pythium* species will be evaluated.

Stimulation of sexual structure production by *Pythium*

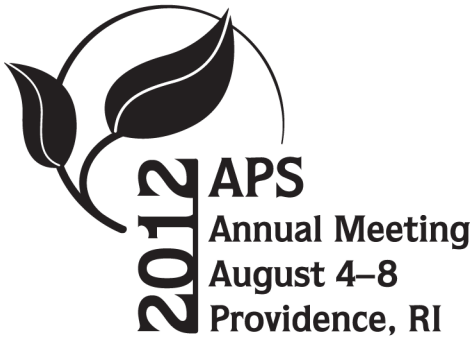
K. ZITNICK-ANDERSON (1), B. Nelson Jr. (1)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 102:S4.145

Identifying *Pythium* species based on morphological features has been a difficult challenge for mycologists. *Pythium* produces many unique distinguishing traits including both asexual and sexual structures. Proper identification cannot be achieved without the presence of both structures. Previous research indicated that turf grass clippings can stimulate the production of sexual structures. The use of grass clippings to enhance production of sexual structures was investigated using tall fescue (cultivar Grande2) and Kentucky bluegrass. The method that resulted in the greatest sexual structure production was to autoclave clippings of both grass cultivars in tap water for 20 min. Then an agar plug plus mycelium from a 5-day-old culture on potato dextrose agar was placed into a 60 mm diameter petri dish with 15 ml of the autoclaved grass clipping plus the same water used to autoclave the grass. In general, sexual structures formed within 24 to 48 hr on the edges of the grass clippings, but were not observed in the agar plugs. These structures were readily visible and abundant on the grass clippings when identifying *Pythium* species such as *P. kashmirense*, *P. ultimum*, and *P. perplexans*. When glass clippings are autoclaved but removed from the water they are autoclaved in and then placed in fresh autoclaved tap water, sexual structure production was markedly reduced. The water from the autoclaved grass clippings apparently has compounds that greatly stimulate sexual structure production.

High resolution mapping of a Ug99 resistance gene from a spring wheat landrace

J. ZURN (1), M. Newcomb (2), M. Rouse (3), Y. Jin (3), S. Chao (4), J. Sthapit (5), D. See (5), R. Wanyera (6), P. Njau (6), J. Bonman (2), R. Brueggeman (1), M. Acevedo (1)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS, Aberdeen, ID, U.S.A.; (3) USDA-ARS, St. Paul, MN, U.S.A.; (4) Cereal Crops Research Unit, Northern Crop Science Laboratory, USDA-ARS, Fargo, ND, U.S.A.; (5) Washington State University, Pullman, WA, U.S.A.; (6) Kenya Agricultural Research Institute, Njoro, Kenya
Phytopathology 102:S4.145

Disease management in wheat, *Triticum aestivum*, is critical for the preservation of global food security. The emergence of new races of *Puccinia graminis* f. sp. *tritici* are a major threat, specifically those identified in eastern Africa since 1998 (Ug99). To mitigate the global impact of the disease, researchers are currently searching for new and underutilized sources of resistance effective against Ug99 for incorporation into new wheat cultivars. A potential source for novel resistance genes may exist in landraces and wild relatives of wheat. A wheat stem rust resistance gene from a hexaploid spring wheat landrace (*SrWLR*) effective against Ug99 was previously identified from the USDA National Small Grains Collection. *SrWLR* provides resistance to TTKSK in both adult and seedling stages. Furthermore, the gene was mapped to an 8.8 cM region on chromosome 2B utilizing a recombinant inbred population and microsatellite markers. The identified region, between gwm47 and wmc332, has also been documented to contain *Sr9* and *SrWeb*. In an effort to further characterize *SrWLR* and the surrounding region, 240 recombinant inbred F5 lines were screened with single nucleotide polymorphic (SNP) markers. The polymorphic SNPs were utilized to identify closer flanking markers to *SrWLR* and provide a high resolution genomic map for future wheat research.



2012 APS Annual Meeting
Abstracts of Special Session Presentations

Biology of Pathogens

International Perspective on Fusarium Head Blight

A toxic character: *Fusarium graminearum* and mycotoxin biosynthesis
H. C. KISTLER (1)
(1) USDA-ARS, Cereal Disease Laboratory, St. Paul, MN, U.S.A.
Phytopathology 102:S4.146

Trichothecene mycotoxins produced during infection of wheat and barley by *Fusarium graminearum*, can contaminate the grains and thus render the crop unusable. Trichothecenes also may act as virulence factors that intensify the disease damage (*viz*. necrosis and blighting) done to the grain during infection. In the past five years, much has been learned about the chemical and structural triggers of trichothecene biosynthesis during plant infection. The genome sequence of the fungus also has yielded much information on the genetic adaptations and developmental events responsible for toxin synthesis. Structural changes to fungal cells that produce trichothecenes on plants and under toxin-inducing conditions *in vitro* point to morphological requirements of toxigenic hyphae. Cellular mechanisms for toxin export in the fungus are still largely unknown. The current status of trichothecene biosynthesis, regulation and transport will be summarized.

Mycotoxin production during infection of cereals
W. SCHÄFER (1), J. Boennighausen (1), J. Bormann (1)
(1) University of Hamburg, Hamburg, Germany
Phytopathology 102:S4.146

The fungal pathogen *Fusarium graminearum* is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot disease of maize. During infection, the fungus produces mycotoxins, like the trichothecenes deoxynivalenol (DON) and nivalenol (NIV). The key enzyme in their biosynthesis is the trichodiene synthase (Tri5). Disruption of the Tri5 gene restricts the infection to the inoculated wheat spikelet, independent of the chemotype (NIV or DON) and the trichothecene amount originally produced by the wild type strain. Surprisingly, trichothecenes are not essential for barley or maize colonization in greenhouse experiments. We recently cloned a reporter strain with the Tri5 promoter fused to eGFP. Monitoring mycotoxin induction during the infection process we showed a time dependent induction during infection structure development and a tissue specific production during wheat infection. Especially the developing kernel and the rachis node of wheat induce DON production by *F. graminearum*. In vitro, several different DON inducing conditions are described, among them oxidative stress, low pH and different nitrogen sources like ammonium rich amino acids and polyamines like putrescine. We will provide a model of the DON inducing conditions during wheat infection combining the above described inducers.

Identification of candidate genes for head blight and deoxynivalenol resistance
F. M. DOOHAN (1)
(1) University College-Dublin, Dublin, Ireland
Phytopathology 102:S4.146

We are investigating the mechanisms underpinning host resistance to deoxynivalenol (DON) and Fusarium diseases of wheat and barley. Using functional genomics, we identified genes associated with DON responsiveness and DON resistance. Several such genes, including a multidrug ABC transporter, directly contribute to DON resistance; attenuating this gene through virus-induced gene silencing increased the susceptibility to DON. *Pseudomonas flourescens* strain MKB158 was identified as a bacterium that reduces FHB levels, associated toxin accumulation and yield losses. Based on transcriptomic, biochemical and phenotypic studies, we deduced and confirmed that auxin is involved in bacterial priming of host resistance. Studies on another plant hormone, brassinosteroid, identified the receptor Bri1 as a key regulator of FHB resistance and toxin build-up in grain. On the basis of microarray analysis, we identified genes and pathways differentially regulated in a Bri1 mutant as compared to wild type barley. On the basis of all our studies, we can conclude that there is a biochemically diverse array of genes that can be targeted to control FHB, thus offering great scope for breeding genotypes with effective long-term resistance to FHB. However, in the context of a practical breeding programme, the effect of some such genes on other biotic and abiotic interactions must now be investigated.

The role of trichothecenes in the Triticeae-*Fusarium graminearum* interactions
G. J. MUEHLBAUER (1), S. Shin (1), J. Boddu (1), S. Gardiner (1), H. Jia (1), S. Cho (1), S. McCormick (2), W. Schweiger (3), M. Lemmens (3), F. Berthiller (3), C. Hametner (4), P. Kovalsky Paris (3), J. Torres-Acosta (3), G. Adam (3)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) USDA-ARS, Peoria, IL, U.S.A.; (3) University of Natural Resources and Applied Life Sciences, Tulln, Austria; (4) Vienna University of Technology, Vienna, Austria
Phytopathology 102:S4.146

Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, is a major disease problem for the small grain crops wheat and barley. During infection, *F. graminearum* produces trichothecene mycotoxins such as deoxynivalenol (DON) that increase the aggressiveness of the fungus and reduces grain quality. Thus, we are interested in identifying genes that protect wheat and

http://dx.doi.org/10.1094/PHYTO-102-7-S4.146

barley from the toxic effects of trichothecenes. Previous work had identified an *Arabidopsis* DOGT1 gene, encoding an UDP-glucosyltransferase (UGT), as involved in trichothecene resistance via conjugation of DON to DON-3-glucoside (D3G). We examined transcript profiles in wheat and barley during *Fusarium graminearum* infection and inoculation with the trichothecene deoxynivalenol (DON). From these experiments, we identified a set of 10 barley UDP-glucosyltransferases (UGT) that were upregulated during *F. graminearum* infection or DON treatment. We screened this set of UGTs in yeast and identified a barley UGT (HvUGT13248) gene that conferred resistance to DON via conjugation to D3G. Transgenic *Arabidopsis* overexpressing HvUGT13248 exhibited the ability to grow on media containing DON. DON feeding studies on the transgenic *Arabidopsis* showed that DON was conjugated to D3G. In contrast to prior work in *Arabidopsis* overexpression DOGT1 exhibited a dwarf phenotype due to brassinosteroid conjugation, transgenic *Arabidopsis* overexpressing HvUGT13248 did not exhibit dramatic changes in morphology and did not conjugate the brassinosteroid castasterone. More recently, we developed transgenic wheat overexpressing HvUGT13248 and showed that these lines exhibited high type II FHB resistance.

Host colonization leading to sporulation in *Fusarium graminearum*
F. TRAIL (1), D. Afton (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.147

Fusarium graminearum is unique among the major *Fusarium* pathogens of grains, in that it is sexually prolific. There is strong evidence that for *F. graminearum*, sexual spores are important dispersal agents. Infection and colonization processes for some grain crops predetermines the relative placement and quantity of sexual fruiting bodies that appear in infected plants, provided that environmental conditions are conducive. We will present our findings on the host-pathogen interactions that lead to perithecium production in infected host tissue, and discuss differences in the process as they are affected by host anatomy and maturity.

New Insights into the Virulence Mechanism of Plant-Pathogenic Bacteria

The role of the type III secretion system in necrotic pathogens
A. O. CHARKOWSKI (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 102:S4.147

Necrotic pathogens prosper when plant cells die and therefore may induce programmed plant cell death during pathogenesis rather than inhibiting it by interfering with apoptotic pathways, as biotrophic or hemibiotrophic pathogens do. The necrotic soft rot pathogen *Pectobacterium* causes disease on diverse plant species and examination of its type III secretion system (T3SS) supports a model where necrotic pathogens prosper by inducing plant cell death. *Pectobacterium* and the related genus *Dickeya* appear to encode, at most, only a single type III effector. Some strains in these genera lack T3SS entirely. Deletion of the single effector, DspE, eliminates the ability of *Pectobacterium* to elicit a hypersensitive response on solanaceous plants, but does not greatly impair pathogenesis on plant stems or tubers. Unlike the hemibiotrophic pathogens *Pseudomonas syringae*, which encodes numerous type III effectors, the *Pectobacterium* T3SS appears to have little impact on plant gene regulation early in pathogenesis. DspE homologs encoded by soft rot Enterobacteriaceae pathogens are smaller than homologs encoded by hemibiotrophs. However, WXXXD/E motifs required for DspE activity in hemibiotrophs are conserved in necrotroph DspE homologs and these motifs are required for activity of the *Pectobacterium* DspE homolog.

Insights into the virulence mechanism of *Xanthomonas citri* subsp. *citri*
N. WANG (1)
(1) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 102:S4.147

The genus *Xanthomonas* is an important group of Gram-negative plant pathogenic bacteria, which infects approximately 124 monocotyledonous and 268 dicotyledonous plants. The genus, *Xanthomonas*, has become an important model organism for studying plant-microbe interactions and for understanding bacterial pathogenicity and virulence mechanisms. Among the diseases caused by members of the genus *Xanthomonas*, citrus canker is one of the most serious diseases of most commercial citrus cultivars resulting in significant losses worldwide. This devastating disease is caused by *Xanthomonas citri* subsp. *citri*. *X. citri* subsp. *citri* is spread primarily by rain splash; it enters its hosts through wounds and natural openings such as stomata and forms distinctive raised, necrotic lesions surrounded by oily, watersoaked margins and yellow chlorotic rings on leaves, stems, and fruit. Eventually, the epidermis ruptures, and massive numbers of bacterial cells emerge to the plant surface, where the bacteria are readily available for rain splash and repeated infection cycles. To understand the virulence mechanisms of *X. citri* subsp. *citri* underneath the infection cycle, we conducted genomewide microarray and RNA-Seq analyses to characterize the critical regulators including HrpX, HrpG, RpfG, and RpfC, which are critical for the pathogenicity of *X. citri* subsp. *citri*. How *X. citri* subsp. *citri* coordinates its diverse virulence factors will be presented. Genome wide mutagenesis was used to further characterize the genetic determinants of the virulence mechanism of *X. citri* subsp. *citri*. Several novel virulence factors of *X. citri* subsp. *citri* will be presented.

Ooze and rots: How enteric plant pathogens utilize cyclic di-GMP, small RNAs, and quorum sensing to regulate major virulence genes
G. W. SUNDIN (1), Q. Zeng (1), C. Yang (2)
(1) Michigan State University, East Lansing, MI, U.S.A.; (2) Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, U.S.A.
Phytopathology 102:S4.147

We have been studying the regulation of critical virulence determinants in the enteric plant pathogens *Dickeya dadantii* and *Erwinia amylovora*. Overexpression of either of two *E. amylovora* GGDEF proteins resulted in increased intracellular cyclic di-GMP levels, amylovoran synthesis, and biofilm formation. The Hfq small RNA chaperone was shown to be an important virulence factor and positively controlled type three effector translocation. A total of 10 putative small RNAs were identified in the *E. amylovora* genome, and two of these (RyhA and RprA) were found to positively regulate the expression of the type three effector DspE. In *D. dadantii*, we demonstrated that aribonuclease PNPase and regulatory small RNA RsmB function in the post-transcriptional regulation of the type III secretion system. A reduced degradation of *hrpL* mRNA was observed in D*pnp* compared to the wild type. D*pnp* also showed an RsmB transcript pattern different from the wild type, with additional shorter RsmB transcripts truncated at the 3' end present along with the full length RsmB as observed in the wild type. Functional analysis later showed that the RsmB transcripts with different nucleotides truncated at 3' end still retain some biological functions in the post-transcriptional control of *hrpL* mRNA. Our genetic and mechanistic studies contribute to our goal of an ultimate dissection of the roles of second messenger molecules and post-transcriptional regulation in enteric bacterial pathogenesis.

The role of the cell surface lipopolysaccharide molecule in *Xylella fastidiosa* biofilm formation and virulence in the grapevine host
M. ROPER (1), J. Rapicavoli (1), J. Clifford (2)
(1) University of California-Riverside, Riverside, CA, U.S.A.; (2) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 102:S4.147

Xylella fastidiosa (*Xf*) is a Gram-negative, xylem-limited bacterium that causes disease on several hosts, such as grapevine, almond, and oleander. Lipopolysaccharide (LPS) comprises approximately 70% of the outer membrane of Gram-negative bacteria, and because it is largely displayed on the cell surface, it mediates interactions between the bacterial cell and its surrounding environment. LPS is comprised of a conserved lipid A-core oligosaccharide component and a variable O-antigen portion. We are investigating the contribution of the O-antigen to *Xf* surface attachment and biofilm maturation: two critical steps for successful infection of the host xylem. Moreover, LPS has been implicated as a major virulence factor for several bacterial pathogens and we are exploring the role of LPS as a virulence determinant for *Xf*. By targeting key genes involved in O-antigen biosynthesis, we have demonstrated that a fully formed O-antigen moiety is a critical virulence factor for Pierce's disease development in grape and that depletion of the O-antigen compromises the ability of *Xf* to colonize the xylem. The O-antigen has also been implicated in host specificity for mammalian pathogens. The wide host range, yet stringent host specificity of different *Xf* subspecies affords an opportunity to study the molecular mechanisms underlying host specificity in a phytobacterial pathosystem.

The global regulatory network for the virulence of *Burkholderia glumae*, the major causal agent of bacterial panicle blight of rice
J. HAM (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 102:S4.148

Burkholderia glumae causes bacterial panicle blight of rice, which is an emerging rice disease problem in many rice-growing regions around the world including the southern United States. Production of the phytotoxin, toxoflavin, and lipase and biogenesis of flagella are crucial for the virulence of *B. glumae*. These virulence-related phenotypes are globally regulated by the *N*-acyl homoserine lactone (AHL) quorum-sensing system of *B. glumae*, which is conferred by *tofI* and *tofR* genes encoding an AHL synthase and an AHL receptor, respectively. According to our recent study, PidS/PidR two-component system controls a functional type III secretion system and toxoflavin production in *B. glumae*. In this study, additional new regulatory factors of *B. glumae* were identified through various mutational approaches and characterized in terms of their functions in virulence-related phenotypes. Among the new regulatory genes, *ntpR* encoding a LysR-family protein and *tepR* encoding an RpoN-dependent response regulator acted as negative regulators for toxoflavin biosynthesis and virulence. *tofM* encoding an RsaM homolog and located between *tofI* and *tofR* was also characterized as a new regulatory gene for toxoflavin production in this study. Further functional and genetic studies on these newly found regulatory components in conjunction with other known regulatory systems, such as the TofI/TofR AHL quorum-sensing system, would provide a better view on the global regulatory network of this pathogen for pathogenesis.

How high-throughput sequencing technology helps our understanding of plant-pathogenic bacteria (overview)
R. Bart (1), M. Sharlach (1), A. Kassen (1), N. Potnis (2), G. V. Minsavage (2), B. J. Staskawicz (1), J. B. JONES (2)
(1) Department of Plant and Microbial Biology, University of California-Berkeley, Berkeley, CA, U.S.A.; (2) Department of Plant Pathology, University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.148

Draft genome sequencing has become cost-effective and time saving with the advent of next generation sequencing methods. The increasing scale of genomics provides a rapid means for identifying virulence factors and for generating new hypotheses to explain the complexities of host-pathogen interactions. Comparative genomics has raised a number of questions as to how diverse species evolve to extend host range and as to the role of type III effectors and molecular mechanisms in driving the evolution of the pathogen. In Florida, the bacterial spot of tomato pathogen *Xanthomonas euvesicatoria* (*Xe*) was the only species associated with tomato. In 1991, *Xanthomonas perforans* (*Xp*) was identified and as of 2006 has become the predominant species in tomato fields. Until recently, only *Xe* has been pathogenic on pepper. AvrXv3 present in *Xp* strains restricted host range on pepper. We recently isolated and sequenced an *Xp* strain that is able to infect pepper. We also sequenced *Xp* strains representative of the four tomato growing regions in Florida. *In silico* analysis of the genes present in the new pepper *Xp* strain, but absent from the previously sequenced *Xp*91-118, revealed candidate virulence factors on pepper. Differences in effector suites have evolved in these closely related field strains. Phylogenetic analysis based on MLST and SNPs revealed diversity among these field strains and, when related to the geographical distribution, possible clues to their evolution.

Potato virus Y—An Old Virus and a New Problem in Potato

PVY as an emerging potato problem in North America
S. M. GRAY (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.148

In the past decade, *Potato virus Y* (PVY) has re-emerged as a significant disease affecting seed potato production and is threatening to become a tuber quality issue. The resurgence of PVY is due, in part, to widespread acceptance of potato varieties that express mild or no foliar PVY symptoms, to displacement of the ordinary PVY strain by recombinant strains that often induce mild foliar symptoms and tuber necrosis, and to increased late season spread of the virus due to changes in aphid populations. These late season infections often are not manifested in foliar symptoms, but result in infected tubers. Each of these changes hampers the detection and removal of PVY-infected plants during field inspections and rouging operations, and leads to more virus being present in seed stocks. A national PVY project, outlined in this talk, has partnered with seed certification and regulatory agencies, and grower organizations across the U.S. to address the needs of the potato industry. Our immediate goal is to work with seed certification agencies to reduce the level of virus in seed stocks below economic thresholds and to eliminate seed lots harboring the tuber necrotic strain. The longer term goals are to improve on-farm Best Management Plans to reduce the impacts of PVY on farm profitability and product quality. Project outcomes are translated to stakeholders via www.potatovirus.com that includes an open discussion forum on any aspect of PVY or other potato virus diseases.

Breeding potato for PVY resistance
S. H. JANSKY (1), X. Cai (2)
(1) USDA-ARS and University of Wisconsin-Madison, Madison, WI, U.S.A.; (2) Huazhong Agricultural University, College of Horticulture and Forestry, Wuhan, Peoples Republic of China
Phytopathology 102:S4.148

In recent years, *Potato virus Y* (PVY) has emerged as a serious disease problem in potato production areas throughout North America. One explanation for this increase in incidence is that some widely planted cultivars express mild or no symptoms, preventing effective roguing of infected plants. Furthermore, recent investigations have revealed a significant increase in the proportion of PVY recombinants ($PVY^{N:O}$) in major production areas. Effective strategies to address this emerging problem are lacking. The development of cultivars with improved levels of PVY resistance would provide one such strategy. We have identified a new source of extreme resistance to PVY^{O} and $PVY^{N:O}$ in the diploid wild potato relative *Solanum chacoense*. All plants in accession 275138 were resistant to PVY, while variability for resistance was found in accession 320285. Segregation ratios in the F1 and backcross populations indicate that PVY resistance is conferred by a single dominant gene. The 8,303 SNP array developed by the SolCAP initiative is being used to map the gene in a diploid hybrid population created by crossing a heterozygous resistant clone to a susceptible wild species clone (*S. tarijense*, PI558129). In addition, a diploid adapted fertile clone has been selected from a cross between a homozygous resistant *S. chacoense* clone and a susceptible cultivated *S. tuberosum* clone. It expresses genes for the production of numerically unreduced pollen and eggs, so when crossed to tetraploid cultivars, it produces tetraploid offspring carrying the resistance gene. In addition, it carries the dominant allele of a gene that overcomes the gametophytic self-incompatibility system, so it can be self-pollinated to create clones homozygous for the PVY resistance gene.

Modeling aphid vector flights and improved control of *Potato virus Y*
R. GROVES (1), K. Frost (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 102:S4.148

The US seed potato industry has identified PVY as its most serious disease problem and is looking for ways to reduce overall disease incidence below acceptable levels. In Wisconsin, seed potato crops are threatened by infection of PVY, vectored by several species of potato colonizing and non-colonizing species. The dispersal and movement of PVY vectors are influenced by processes operating across different temporal and spatial scales (e.g. climate, weather, or host plant condition). We describe a modeling framework that uses a generalized linear mixed modeling approach to partition the variance associated with aphid capture data. These approaches were applied to long term, multi-site and multi-year, aphid suction trap data sets from the North Central Region, Aphid Suction Trap Network. Variance estimates of aphid abundance among years significantly exceeded similar estimates among locations (regions). This suggests that factors such as season better explain variation in aphid abundance than factors driving differences among locations (e.g. surrounding habitat characteristics). These findings are consistent with the hypothesis that selected aphid species are migrating into fields from moderate to long distances. Taken together, this approach improves our understanding of the patterns of variation in aphid abundance, thereby increasing our knowledge of when the aphid vectors move into susceptible seed potato fields and when they spread the virus to highly susceptible varieties. A more accurate assessment of the phenology of the principle aphid vector flights provides an opportunity to deploy behavioral modifying crop protectants at critical periods to limit the transmission of PVY to potato.

PVY and Canadian experience

M. SINGH (1), X. Nie (2), Y. Pelletier (2), M. Fageria (3)
(1) Agricultural Certification Services/Potatoes NB, Fredericton, NB, Canada; (2) Agriculture and Agri-Food, Potato Research Centre, Fredericton, NB, Canada; (3) Agricultural Certification Services, Fredericton, NB, Canada
Phytopathology 102:S4.149

Potato virus Y (PVY) is an evolving problem throughout the world and Canada is no exception. PVY made headlines in Canada in 1990s when PVY^N was discovered in Eastern Canada, which resulted in the loss of export markets. Since then significant progress has been made in PVY research. PVY is managed in seed potatoes by visual inspection during growing season followed by post-harvest test. A national survey conducted in 2004-06 revealed that all strains of PVY were present but PVY^O was the predominant strain, which was further confirmed by a survey conducted in New Brunswick in 2009. The responses of 14 potato cultivars to five isolates belonging to four PVY strains varied significantly, ranging from local lesions to systemic necrosis and tuber symptoms depending on potato cultivar and virus strains. Field experiments on the current season PVY spread and the transmission risk by aphid species unveiled the presence of few potato colonizing aphids but many non-colonizing species, and among them, several were found to be PVY-positive by RT-PCR. Current season spread of PVY in the fields increased as the crop season progressed, and reached up to 40% at the time of harvest in some fields. PVY incidence determined from developing tubers exhibited a significant positive correlation with that from tubers after harvest using real-time RT-PCR. Application of mineral oil spray along with insecticide was found to be a very effective tool for the management of PVY in a growing crop.

Classification of PVY strains and new recombinants

A. V. KARASEV (1), S. M. Gray (2)
(1) University of Idaho, Moscow, ID, U.S.A.; (2) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.149

Currently, strains or strain groups of *Potato virus Y* (PVY) are classified based on two distinct approaches. The genetic classification groups PVY strains according to their ability to induce hypersensitive resistance (HR) responses in a set of potato indicators carrying three different *N* genes, hence five strains of PVY are defined based on this system, PVY^O, PVY^C, PVY^Z, PVY^N, and PVY^E. The molecular classification groups PVY strains according to the sequence of their genomes which are often recombinant, built of segments of parental genomes, most often of PVY^O and PVY^N parents. The two most prominent recombinant strain groups are $PVY^{N\text{-}Wi}$ and PVY^{NTN}, the latter associated with the potato tuber necrotic ringspot disease (PTNRD). Up until recently, the two classifications were not merged, due to unavailability of the PVY^Z and PVY^E sequences and lack of genetic typing of recombinant strain groups on potato indicators. In the past few years, both PVY^Z and PVY^E recombinant genomes were sequenced, and preliminary genetic typing of PVY^{NTN} was completed. This allowed development of a modified classification of PVY strains which combines genetic and molecular characteristics of recombinant and non-recombinant strain groups, and simplifies typing of new PVY isolates. Application of this modified classification to typing field isolates of PVY and search of the PTNRD genetic determinant will be discussed.

Potato seed certification and PVY

P. NOLTE (1)
(1) University of Idaho, Moscow, ID, U.S.A.
Phytopathology 102:S4.149

New strains of *Potato virus Y* (PVY) are adding to the challenges faced by seed potato certification officials across North America. The use of the traditional visual inspection to detect these new PVY strains has not been effective. This lack of effectiveness occurs because the new strains, which often cause milder symptoms in common North American varieties than the original PVY strains, are simply more difficult or even impossible to see. Poor visual detection has resulted in the certification of seed lots with high levels of PVY and has been contributing to a general increase in PVY throughout the continent. The history of PVY in the Idaho Certification system will be discussed as well as some of the reasons the virus has been on the increase over the last two decades. In response to steadily increasing levels of PVY in Idaho seed, the industry recently abandoned the increasingly ineffective visual inspections for PVY in favor of the laboratory-base ELISA test for determination of PVY during the winter seed grow out. This change has been responsible for a dramatic decrease in PVY in the Idaho Seed System and could prove to be a template for the entire North American seed potato industry.

Unifying Concepts in Plant and Animal Vector Biology

A virus at the helm: Even plant-infecting viruses modify vector behavior!

C. A. STAFFORD (1)
(1) Univeristy of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.149

Pathogenic affects have been reported for vectors of both plant and animal pathogens. Modification of vector behavior that results from these pathogenic affects is of broad adaptive significance, because parasite fitness relies on passage to a new host which predominantly occurs during feeding. We have recently shown that vector infection with a plant virus results in altered feeding behavior. Infection of the vector *Frankliniella occidentalis* (Pergande) by the plant virus it transmits [*Tomato spotted wilt virus* (TSWV), Tospovirus: Bunyaviridae] results in dramatically altered feeding behavior that may increase vector competence. We found that infected males fed more than uninfected males, with frequency of all measured feeding behaviors increasing by up to 3 fold. Notably, infected males made almost 3 times the number of non-ingestion probes, the biting behavior that is most likely to leave plant cells intact and available for initiation of virus infection. Our results demonstrate that vector infection with plant viruses, as with animal-infecting viruses, can cause increased biting rates that may serve to enhance transmission. We hypothesize that this increase in probing behavior is a conserved trait among plant and animal-infecting members of the Bunyaviridae that has evolved as a mechanism to enhance virus transmission. Understanding the similarities and differences that exist between plant and animal virus vectors and building an awareness of the advances made in both of these fields will greatly benefit researchers.

***LaCrosse virus* modifies the behavior of its mosquito vector**

B. J. BEATY (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.149

LaCrosse virus (LACV) infection modifies the mating behavior of *Aedes triseriatus* mosquitoes and conditions transovarial transmission efficiency of the virus in nature. Mosquitoes infected orally with LACV are more efficiently mated than non-infected females. Infected females exhibited significantly greater insemination rates than non-infected females. Similarly, both field collected and laboratory-colonized transovarially-infected females mated more efficiently than uninfected females, conferring a fitness advantage for LACV-infected progeny. LACV is maintained in nature by stabilized infection in superinfected (SI+) mosquitoes, which efficiently transovarially transmit (TOT) LACV. Efficient TOT is conditioned by LACV perturbation of the *Aedes triseriatus* innate immune response in ovarian follicles, resulting in increased TOT-infected mosquitoes that mate more efficiently to maintain the SI+ phenotype in nature. The LACV - *Aedes triseriatus* relationship is truly remarkable.

What makes a vector a vector: The ecological and molecular basis of vector competence in planthoppers and thrips

A. WHITFIELD (1), D. Rotenberg (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 102:S4.149

The interaction between arthropods and the viruses they transmit are complex, dynamic, and specific. Multiple biological, genetic, and environmental factors influence the competency and capacity of a vector to transmit a persistent-propagative virus. The first and most simple requisite is coexistence in time and space. The arthropod and virus must share the same plant host for some period of time for virus acquisition and transmission to occur. Once acquired, the virus must traverse barriers in the midguts and salivary glands and replicate to sufficient levels to assure transmission to a new plant. Within the vector, the virus must also survive without significantly compromising vector life-span and fecundity. Multitrophic interactions also influence vector capacity, and a virus-induced increase in plant host suitability may be one mechanism to overcome potential negative effects of virus infection on the vector. Specific examples and comparisons of vector transmission characteristics will be illustrated using a generalist virus-vector system (*Frankliniella occidentalis* and *Tomato spotted wilt virus*) and a specialist system (*Peregrinus maidis* and *Maize mosaic virus*). The application of molecular biology and -omics tools to these non-model systems has enabled

advances towards identification of viral and vector determinants of transmission, and these interactions will be discussed at the molecular and ecological scale.

The molecular basis of vector competence in mosquito-arbovirus interactions
L. C. BARTHOLOMAY (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.150

Although there are clear lessons to be learned from similarities in the molecular interactions between vectors and the viruses transmitted thereby, the potential research synergies in virus infections in insect-borne viruses of plants and animals are largely untapped. A distinct difference between vector-borne viruses is that the arthropod-borne viruses (arboviruses) of animals almost invariably depend on amplification in the vector for transmission, which is not the case for most vector-borne plant viruses. Even so, both plant and animal vector-borne viruses interact with, traverse, or infect and replicate in vector cells. It is increasingly clear that these interactions are not simple, benign events that go unnoticed by the vector. During infection with arbo-viruses including West Nile and Dengue (DENV), there is evidence for an active molecular arms race between virus replication and dissemination and the antiviral response in the mosquito; for example, DENV infection suppresses the Toll and Immunodeficiency (IMD) pathways, but the mosquito suppresses DENV, WNV and Sindbis virus infection with RNA interference (RNAi) and programmed cell death responses, particularly apoptosis. These innate immune responses are primary drivers for the compatibility of vector-arbovirus interactions and, therefore, of vector competence in blood-sucking arthropods.

Manipulation of host-derived olfactory cues by vector-borne pathogens of plants and insects
M. C. MESCHER (1)
(1) Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 102:S4.150

An increasing number of studies indicate that vector borne pathogens can alter the phenotypes of their primary hosts in ways that influence the frequency and nature of interactions between hosts and vectors. We have been exploring the effects of pathogens on host volatile emissions in a number of plant and animal disease systems. This work suggests that pathogens frequently alter host odors (as well as other aspects of host chemistry) in ways that influence the behavior of insect vectors and of non-vector insects. Moreover, pathogen transmission mechanisms appear to be an important factor shaping the evolution of effects on host-vector interactions. I will present recent results from work on viral and bacterial plant pathogens and from vertebrate disease systems, and discuss implications for disease transmission, ecology, and diagnosis.

Strategies employed by animal parasites to enhance vector transmission
H. HURD (1)
(1) Keele University, Keele, Staffordshire, United Kingdom
Phytopathology 102:S4.150

Parasitic protozoans and helminths that are transmitted from host to host by haematophagous insects have complex life cycles that often require a period of development (the pre-patent period), and sometimes asexual multiplication, in the host or vector before transmission can occur. For transmission to be successful the parasite must be in a suitable location and at a mature stage when the vector makes contact with the host. Biting insects are attracted to their hosts by the carbon dioxide, volatile chemicals and heat they emit and by their appearance. However, blood feeding is a risky foraging strategy and feeding decisions must be made to avoid host defence reactions. Using examples of parasites that infect humans causing tropical diseases such as malaria, leishmaniasis and filariasis, experimental evidence will be discussed that suggests that the attractiveness of infected hosts to the parasite's vector changes specifically when transmission stages are present. Likewise, evidence will be presented that shows that vectors infected with patent infections alter their feeding persistence and probing behaviour in ways that may enhance parasite transmission. Several parasites are also known to cause a reduction in vector fecundity and may alter the trade-off between reproduction and longevity, thus enhancing the prospects of transmission. The adaptive nature of these infection induced behavioural and physiological changes in host and vector will be discussed.

Disease Control and Pest Management

12th I. E. Melhus Graduate Student Symposium: Host Plant Resistance and Disease Management: Current Status and Future Outlook

Infection of blueberries by *Colletotrichum acutatum*: Host defenses, inheritance of resistance, and environmental effects
T. D. MILES (1), A. C. Schilder (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 102:S4.150

A disease forecasting model for anthracnose fruit rot in blueberries was developed based on temperature, wetness duration, relative humidity, and wetness interruptions. The resistance response in the blueberry cultivar 'Elliott' was also investigated using suppression subtraction hybridization and several defense-related genes as well as abiotic stress-related genes were found to be upregulated in 'Elliott'. Some genes were related to oxidative stress and higher levels of hydrogen peroxide were found. Chemical fruit extractions were performed in 'Elliott' (resistant) and 'Jersey' (susceptible), and the methanolic extract was the most biologically active. Anthocyanins and flavonols were then quantified and identified in both cultivars using HPLC-MS. 'Elliott' fruit contained more anthocyanins than 'Jersey' fruit but the same compounds were found in both cultivars. However, two unique flavonols were present in 'Elliott'. A total of 26 blueberry cultivars were screened for anthracnose resistance using several different techniques. A cut-fruit technique was promising as a rapid screening method. Resistance was positively correlated with fruit sugar content but not fruit pH. The inheritance of resistance was also investigated by inoculating fruit from F-1 populations of specific crosses between resistant and susceptible cultivars; resistance ratings based on disease incidence were highly correlated with resistance ratings predicted from previous studies.

Proteomics-based study of host-fungus interaction between soybean and *Phakopsora pachyrhizi* using recombinant inbred line (RIL)-derived sister lines
M. GANIGER (1), D. R. Walker (2), Z. Chen (1)
(1) Louisiana State University, Baton Rouge, LA, U.S.A.; (2) USDA-ARS Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, Urbana, IL, U.S.A.
Phytopathology 102:S4.150

Phakopsora pachyrhizi, the causal agent of Asian soybean rust, has the potential to cause severe yield losses as all U.S. commercial soybean varieties are susceptible. In this study, ten soybean recombinant inbred line (RIL) derived sister lines of two populations (RN06-32-2 and RN06-16-1) were evaluated for differences in response to infection by *P. pachyrhizi* (Louisiana isolates). These lines, which had previously shown differential responses to Florida soybean rust isolates, were evaluated using Louisiana rust isolates under both detached leaf assay and greenhouse *in planta* inoculation conditions. Significant differences were observed among sister lines in their responses to *P. pachyrhizi* infection under both conditions. Lines 8-a and 8-b showed immune response; lines 15-b, 16-c, 94-b and 94-c showed resistant response; lines 15-c and 16-b showed moderately resistant response; lines 94-a and 8-c showed susceptible response. To understand the compatible and incompatible host-pathogen interactions at the molecular level, we conducted a time-course study of *P. pachyrhizi* infection and compared protein profiles of 8-a (immune) and 8-c (susceptible) lines using DIGE proteomics. Based on the gel analysis we identified approximately 70 differentially expressed spots between 8-a and 8-c lines with and without *P. pachyrhizi* inoculation and currently these protein spots are being sequenced using mass spectrometry.

Characterization of resistance of *Arachis hypogaea* to *Puccinia arachidis*
I. L. POWER (1), A. K. Culbreath (1), B. L. Tillman (2)
(1) University of Georgia, Department of Plant Pathology, Tifton, GA, U.S.A.; (2) University of Florida, Department of Agronomy, Marianna, FL, U.S.A.
Phytopathology 102:S4.151

In low-input peanut (*Arachis hypogaea*) producing countries, host resistance is the best option to manage peanut rust, caused by *Puccinia arachidis* Speg. We conducted field experiments to evaluate the resistance of CRSP breeding lines to rust, in 2010 and 2011 near Gainesville, FL and in 2011 in Tifton, GA. Disease severity was assessed using the 1-9 ICRISAT scale. In 2010, 18 of the 25 genotypes demonstrated resistance to rust, including genotypes highly resistance to rust late leaf spot. In 2011 the disease pressure in Gainesville was low and the disease epidemic started late in Tifton, leading to few differences in rust severity among genotypes. Peanut rust resistance is polygenic, thus we conducted detached leaf assays in 2011 to study the components of resistance to *P. arachidis*. Detached leaflets on moist sterile paper in Petri dishes, were inoculated, and incubated at 25 degrees. There seemed to be no correlation between the numbers of pustules developed and the pustule size, as genotypes developed both few and many pustules, both large and small. RAPD and SSR markers linked to rust resistance, will be used to screen selected peanut genotypes, to identify rust resistance genes in these genotypes. To study the molecular variability of *P. arachidis*, several isolates have been collected over different peanut growing seasons in different countries. Multi-locus Sequence Typing and phylogenetic analyses will be performed to define the population structure, in relation to the geographical regions.

Transcriptomic and genetic approaches to define tomato resistance to the bacterial pathogen *Ralstonia solanacearum*
J. M. JACOBS (1), R. M. Mitra (2), B. Remenant (1), A. Milling (1), C. Allen (1)
(1) University of Wisconsin, Madison, WI, U.S.A.; (2) Carleton College, Northfield, MN, U.S.A.
Phytopathology 102:S4.151

Bacterial wilt disease, caused by *Ralstonia solanacearum* (*Rs*), is considered the most destructive bacterial plant disease due to the pathogen's broad host range, aggressiveness and worldwide distribution. Tomato growers suffer complete crop losses during severe epidemics. Control of this disease relies primarily on breeding for resistance, which comes from the quantitatively resistant tomato line H7996. H7996 is resistant to most tropical *Rs* but not the emerging race 3 biovar 2 (R3bv2) subgroup of *Rs*. To define the pathways that tomato plants use to resist *Rs* infection, we sequenced the transcriptomes of tomato roots from susceptible and resistant lines (Bonny Best and H7996, respectively) after infection by tropical and R3bv2 strains of *Rs*. These transcriptomic experiments also explored how an *Rs* virulence effector PopS targets specific defense pathways. These transcriptomes broadly describe resistant and susceptible plant behavior during early infection to define the plant defense pathways triggered by an important and widely-distributed pathogen and an effector, PopS. Our investigations revealed physiological factors with differential importance for pathogen virulence on susceptible and resistant tomato lines. Specifically R3bv2 requires plant-derived sucrose to overcome H7996 resistance and for competitive fitness during H7996 xylem growth. Overall, these findings provide significant advances in our limited understanding of tomato resistance to bacterial wilt.

Emerging Tools and Regulations Impacting the Enhancement of Disease Resistance Using Biotechnology

Using TAL effector nucleases for targeted genetic modification
B. YANG (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.151

TAL (transcription activator-like) effectors of *Xanthomonas oryzae* pv. *oryzae* (Xoo) contribute to pathogen virulence by transcriptionally activating specific rice disease susceptibility (S) genes. TAL effector nucleases (TALENs) are fusion proteins of TAL effectors and the DNA cleavage domain of FokI nuclease. TALENs are capable of inducing chromosomal DNA double-strand breaks (DBSs) at specific sites. DBS repair can lead to efficient introduction of sequence modifications or insertions through homology recombination or mutagenic insertions/deletions through nonhomologous end-joining at the site of the break, making TALEN technology a powerful genetic tool. We exploit TALEN technology to edit the specific S genes in rice to thwart the virulence strategy of *Xanthomonas oryzae* and, thereby, engineer heritable genome modifications for resistance to bacterial blight. Multiple designer TALENs are custom-engineered to precisely edit the effector binding elements within the promoters of two S genes targeted by three TAL effectors of essential virulence of Xoo. The resulting promoter modifications in transgenic plants result in loss of inducibility of S gene by the cognate TAL effectors and concomitantly loss of disease susceptibility (or gain of resistance) to pathogenic Xoo. The TALEN gene constructs can be genetically segregated out in some modified plants. The results demonstrate the feasibility of using TALENs for targeted editing of important genes for crop improvement and also raise the prospect of producing genetically modified plants without a trace of "foreign" DNA left in the genome.

Reintroduction of genetically engineered potatoes into the U.S. market
C. Rommens (1), A. STIVISON (1)
(1) J. R. Simplot Company, Boise, ID, U.S.A.
Phytopathology 102:S4.151

The spread in the production of potatoes from small regions in the Andean highlands to almost 50 million acres worldwide coincided with, and enabled, in part, the unprecedented population growth and urbanization of the 18th and 19th century. However, issues in traditional plant breeding have hampered efforts to meet evolving needs for agricultural sustainability, and currently available varieties lack many of the agronomically important traits needed to control farm input costs. Shifting consumer preferences, from high calorie diets to foods that are rich in phytonutrients, represent additional issues and have lead to a decline in the consumption of potato in the United States. The easiest and fastest way of adapting existing varieties to the needs of the 21st century is offered by genetic engineering. Currently available input traits provide resistance against some of the most important pathogens, pests, and herbicides, as well as black spot bruise. Additional quality traits enhance the antioxidant power of potato while lowering their toxin-forming potential. In many cases, it is possible to improve varieties by transforming them with native DNA only. Surveys indicate that consumers prefer such intragenic approaches over conventional transgenic methods that introduce foreign DNA into the food supply. Several genetically modified potato varieties are currently being evaluated for commercial production. With improvements in biotechnology quality management and identity preservation systems, reintroduction of the first genetically engineered varieties is expected to occur in the next few years.

Virus-meditated protection of maize from *Ustilago maydis*
T. J. SMITH (1)
(1) Donald Danforth Plant Science Center, Saint Louis, MO, U.S.A.
Phytopathology 102:S4.151

The corn smut fungus, *Ustilago maydis*, is a global pathogen responsible for extensive agricultural losses. Control of this disease using traditional breeding has met with limited success because natural resistance to *U. maydis* is organ-specific and is quantitatively inherited. Here we present a transgenic approach using *Totivirus* antifungal protein KP4 expressed constitutively in transgenic maize. Transgenic maize plants expressed high levels of extracellular KP4 with no apparent negative impact on plant development and displayed robust resistance to *U. maydis* challenges to both the stem and ear tissues in the greenhouse. More broadly, these results suggest that ahigh level of organ independent broad-spectrum corn smut resistance might be afforded by transgenic coexpression of KP4 and other *Totivirus* antifungal proteins KP1 and KP6.

Historical perspective of regulation and deregulation of biotech crops
S. A. TOLIN (1)
(1) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 102:S4.151

Over 25 years have passed since recombinant DNA techniques were successfully used to introduce foreign genes into plants. Biosafety Level 3 was required to prevent release to the environment, which was prohibited by the early Guidelines for Research with Recombinant DNA Molecules promulgated by NIH under direction of the highest levels of the U. S.

government. All Federal-funded research agencies were asked to comply with the NIH guidelines. This position resulted from intense scientific discourse ending in the compromise to assess and manage potential and conjectural risks by containment, but enabling research to continue. The USDA agreed, but pointed out that prohibiting release would preclude the power of biotechnology to agriculture. Useful discoveries in biotechnology in many areas were soon recognized as marketable products, resulting in an analysis of extant Federal Agency authorities for regulation of pharmaceutical, industrial and agricultural products. The 1986 Coordinated Framework for Regulation of Biotechnology clarified regulation of crop plants across three agencies, with approval of all needed prior to commercialization. Small scale field tests of plants was regulated by USDA-APHIS Plant Pest Quarantine and at first required permits from APHIS as well as EPA. Risk assessment and risk management discussions have continued, both nationally and internationally. Perspectives and results enabling deregulation will be presented.

Current processes involved in biotech crop deregulation

P. SPAINE (1), J. M. Cordts (1)
(1) USDA/APHIS/Biotechnology Regulatory Services, Riverdale, MD, U.S.A.
Phytopathology 102:S4.152

USDA/APHIS has been one of three primary federal agencies involved with the regulation of genetically engineered organisms since 1987. Its primary roles relate to permitting to allow field trials of genetically engineered (GE) organisms and making determinations of non-regulated status, which can lead to eventual commercialization of products. The process of obtaining non-regulated status typically requires conducting one or more years of field research under APHIS' oversight (a permit or notification) followed by submission of a Petition to the Agency documenting why the GE organism does not pose a plant pest risk. Typical data submissions include molecular and environmental characterizations that describe both the genotype (i.e., information about the inserted gene(s)) and phenotype of the organism. The phenotypic characterization typically includes a comparison with a closely related organism lacking the new gene(s) or with a representative range of related organisms as controls. Comparisons encompass a number of characteristics (e.g., growth habit, vigor, fecundity, outcrossing, reproduction, biotic and abiotic stress adaptations, cultivation practices, potential impacts on non-target organisms, etc.). Following a positive finding by the Agency, the organism may be moved and used in the environment without further APHIS oversight. Other U.S. government agencies involved in regulation of GE organisms include the FDA (assesses food safety) and the US EPA (deals with safe use of pesticides and pesticide labels).

Fungicides to Promote Plant Physiological Benefits in Crops

Influence of ethylene inhibitors on plant physiology, biomass, and yield

F. E. BELOW (1), J. W. Haegele (1), A. S. Henninger (1), F. Cantao (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.152

Doubling average crop yields in the next 40 years to feed a growing world population will require simultaneous improvements in genetics and crop management, as well as alleviation of biological and environmental stresses. Because many of the physiological responses to stresses are modulated by the plant hormone ethylene, we are investigating technologies that alter the level of, or the sensitivity to, ethylene as means of protecting the corn crop from stress. We have examined the competitive ethylene inhibitor 1-MCP, which decreases plant sensitivity to ethylene, aminoethoxyvinylglycine (AVG), which decreases ethylene biosynthesis, and strobilurin-based fungicides which in addition to disease control cause a late-season leaf greening thought to be associated with ethylene biosynthesis. We have applied these compounds at different growth stages, under different environmental conditions, and with varied crop management in order to evaluate when, and how, the control of ethylene can be used to improve the productivity of corn. Collectively, our data suggests that altering ethylene level (or sensitivity) alone cannot guarantee higher yields, but rather ethylene control in combination with multiple management factors that impact productivity has the greatest opportunity to increase biomass production and grain yield.

Limitations of small plots for crop enhancement effects in corn

E. C. TEDFORD (1)
(1) Syngenta, Greensboro, NC, U.S.A.
Phytopathology 102:S4.152

In the previous symposium on this topic in 2009, the main focus of my presentation was on the effects of strobilurin fungicides on various physiological processes in treated plants. Today's presentation will concentrate on elucidation of yield benefits from strobilurin fungicides as influenced by plot size. Data over three years, from independent and Syngenta small-plot trials, have indicated a 2-3 Bu/A increase in yield in strobilurin-treated plots over untreated controls. These results were similar to those reported by University reseaerchers as non-beneficial to growers. By contrast, data from commercial plots (≥ 20 acres) over the same period have indicated yield benefits of 10-15 Bu/A. To explore these discrepancies in the magnitude of yield benefits, Syngenta conducted trials in 26 locations in 2011 across the corn-belt with 2 hybrids per location. To eliminate border effects, these were large plot trials (5 acres per hybrid). The yield results from these 52 hybrid-by-location replications were consistent with earlier data from commercial-size un-replicated trials. Quadris at V5, Quilt Xcel at R1, or the combination of the two provided average yield benefits of 5.2, 11.2, and 14.2 Bu/A, respectively, over the untreated control. These data from 2011 suggest that the earlier results from commercial-size plots were a good indicator of yield benefits from the application of Quadris and/or Quilt Xcel at the recommended timings. By inference, yield data from small plot trials may be less accurate indicators of the benefits of strobilurin fungicides on commercial corn production in the relative absence of disease.

Disease management vs. insurance applications: What have we learned about fungicide use in corn?

K. WISE (1)
(1) Purdue University, Lafayette, IN, U.S.A.
Phytopathology 102:S4.152

The increased use of foliar fungicides in United States corn production is the result of several coinciding factors. These factors include the increased demand and value for corn, a shift in crop production practices that favor disease development, and the labeling and promotion of the quinone-outside inhibitor (QoI) fungicide group for use in field crops. QoI fungicides, commonly referred to as strobilurin fungicides, are marketed for management of biotic and abiotic stresses, and are promoted to increase yield even in the absence of disease. An analysis of 10 years of corn fungicide data indicates that when final foliar disease severity is greater than 5%, the average yield response from a fungicide application is 9.6 bu/A. In contrast, fungicide applications made in low disease pressure environments resulted in an average yield response of only 1.5 bu/A. This analysis reinforces recommendations to use fungicides in response to disease pressure or a disease threat for optimum efficacy and profitability.

Review of large-scale field trials in potatoes

T. A. ZITTER (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.152

Vydate C-LV (oxamyl, a carbamate) is widely recognized for the control of nematodes and insects. In potato evidence exists for nematode control with a concomitant increase in tuber yield. A special Local Need 24(c) was granted to upstate potato growers for the in-furrow use of the product in 2008. Vydate has unique systemic characteristics, such that when applied to the soil it is absorbed and translocated upward, and when applied to above ground tissue it moves downward into the root system for plant protection. Little attention has been directed to any side benefits that may accrue when Vydate is applied at-planting for its effects on soilborne pathogens. Field studies conducted in 2008 and 2009 using Chieftain (widely adapted) and Andover (vulnerable to stress) addressed the effects that in-furrow (1X) and subsequent lower canopy spray of Vydate C-LV (2X) would have on overall tuber appearance and the occurrence of black dot (BD), black scurf (BS), silver scurf (SS), and common scab (CS). Enhanced yields were noted with the Vydate treatments for both varieties in 2008 and for Andover in 2009. Significant improvement in tuber appearance was noted in both varieties in both seasons with either 1X or 2X applications. BD occurrence on both tubers and stems was markedly reduced for both varieties in both seasons, and especially so for Andover. Similar trends were noted for reduced occurrence of tuberborne BS and SS, and less so for CS.

Grafting as an Alternative to Soil Fumigation for Disease Management in Vegetable Production

The pros and cons of cucurbit grafting in the United States

R. L. HASSELL (1)
(1) Clemson University CREC, Charleston, SC, U.S.A.
Phytopathology 102:S4.153

From its research beginnings in the 1920's, cucurbit grafting in Asia has now become the predominantly practiced growing method; currently 95% of watermelon and oriental melon are grafted in Japan, Korea and Taiwan. As a result of the phase-out or reduction of soil fumigants, especially methyl bromide, grafting has only recently been considered as a practice for cucurbit growing in the United States. Grafting presents a promising option for soil-borne pathogen control with such diseases as Fusarium wilt, Monosporascus vine decline, Phytophthora blight and root knot nematode. However, grafting enhances tolerance to abiotic stress, increases water and nutrient use efficiency, extends harvest periods, and improves fruit yield and quality in certain cucurbits. These appealing advantages, as well as the grafting successes in Asia and Europe, make cucurbit grafting an important topic of study in the United States. The problems associated with grafting in the United States centers around economics, namely, the cost of producing the grafted transplant. These costs include added facility and supply costs and increased labor demands. Added facility and supply costs stem from the need for space to grow twice as many seedlings per grafted plant as well as provide a controlled environment for graft healing. Finally, grafting requires an increase in labor, not only in the skill required to produce grafted transplants, but the increased costs under our standards and the availability of such labor. In order for grafting to be successful in the United States these costs need to be taken seriously and research conducted to reduce these costs. Current research involves mechanization, as well as new and more efficient methods. Cucurbit grafting is a promising process; requiring study to create both understanding and innovation that will allow the United States to use this tool to enhance the opportunities afforded the grower.

IPM diversification: Advancing the science and practice of grafting tomatoes to manage soilborne pathogens

F. J. LOUWS (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.153

Soilborne pathogens persistently limit optimum production in fruiting vegetable production systems. Preplant fumigation has been a core management tactic but the phase out of methyl bromide and increased regulatory requirements has spurred increased interest in alternative or complimentary disease management practices. Use of grafted plants has been a core method to manage numerous soilborne pathogens in many tomato production regions of the world. The Southeast USA tomato production region offered an opportunity to evaluate the efficacy of selected rootstocks (RS) to manage a diversity of soilborne pathogens. Multiple on-farm-research and research station experiments demonstrated the efficacy of grafting to manage bacterial wilt (*Ralstonia solanacearum*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*, race 1) and root knot nematode (*Meloidogyne incognita*, race 1). Serendipitously, several RS were discovered to offer near complete control of southern stem blight (*Sclerotium rolfsii*) and multiple experiments confirmed efficacy, a first report of commercially useful resistance to this important pathogen. Verticillium wilt (*Verticillium dahliae* race 2) severity was suppressed using selected RS; mechanisms need to be elucidated. Assessment of the diversity, dynamics and pathogenicity of pathogens and increased knowledge of host:parasite interactions undergirds RS selection and advances the science of grafting as a viable IPM diversification tactic. Participatory research and outreach programs advanced the practice of grafting in high tunnel, open field, organic and conventional tomato production systems.

Grafting on hybrid squash and bottle gourd rootstocks to manage Fusarium wilt of watermelon

A. P. KEINATH (1), R. L. Hassell (1)
(1) Clemson University, Charleston, SC, U.S.A.
Phytopathology 102:S4.153

Fusarium wilt of watermelon is caused by *Fusarium oxysporum* f. sp. *niveum* (FON). FON is not pathogenic on other cucurbit species, although it can infect them. As part of our research on cucurbit rootstocks, we evaluated if FON was able to infect rootstocks, spread to the *Fusarium*-susceptible seedless watermelon scion, and cause wilt after inoculation in greenhouse experiments. Grafted seedlings were inoculated by dipping roots into a microconidia suspension of FON race 1 or 2. Two to 3 weeks later, scion stems were cut above the first true leaf, disinfested and cultured on Komada's medium. Inoculated non-grafted Tri-X 313 and Tri-X 313 grafted onto *Citrullus lanatus* var. *citroides* Ojakkyo wilted, while the other inoculated rootstocks and healthy controls were symptomless. Recovery from scions grafted onto Ojakkyo (90% for race 1 and 63% for race 2) and from non-grafted Tri-X 313 (90 and 91%) was greater in inoculated than control treatments and greater than the other five rootstocks. Mean recovery of *F. oxysporum* from scions grafted onto *Lagenaria siceraria* Emphasis, Macis and WMXP3945 and *Cucurbita moschata* x *C. maxima* Shintosa Camel and Strong Tosa did not differ between inoculation treatments or among the five rootstocks. In a field infested with races 1 and 2, <2% of Tri-X 313 grafted onto bottle gourd and hybrid squash wilted compared with 53% of non-grafted Tri-X 313. Grafted plants yielded more fruit than non-grafted and self-grafted controls (P<0.01).

Grafting as a production system component for nematode management in Florida vegetables

N. K. BURELLE (1), E. N. Rosskopf (1), M. G. Bausher (1), G. G. McCollum (1)
(1) USDA-ARS, Fort Pierce, FL, U.S.A.
Phytopathology 102:S4.153

Trials in Florida evaluated grafting for control of root-knot nematode (*Meloidogyne incognita*) in tomato, cantaloupe, and pepper. Pepper rootstocks assessed included 'Charleston Hot', 'Carolina Wonder', 'Charleston Belle', 'Mississippi Nemaheart', and 'Carolina Cayenne', which were resistant to galling. The non-grafted and self-grafted 'Aristotle' scion, and several other pepper rootstocks were susceptible. In inoculated microplot trials conducted with tomatoes, juveniles (J_2) of *M. incognita* in soil were similar among all tomato rootstocks, but the number of J_2 in roots was higher in non-grafted 'FL-47' than in any grafted rootstocks. In cantaloupe, *Cucumis metuliferus* rootstock reduced galling while 'Tetsukabuto' did not reduce galling or J_2 in soil or roots. Grafted tomatoes and melons in a double-crop field trial showed that 'FL-47' tomato on 'Multifort' rootstock had the healthiest roots, but the number of *M. incognita* J_2 recovered from roots was similar for all rootstocks. Galling was highest in non-fumigated plots with non-grafted plants. For melon, *M. incognita* J_2 in soil did not differ, but J_2 in roots were higher in 'Tetsukabuto' than in *C. metuliferus*. *C. metuliferus* had less galling following all tomato rootstocks and in all fumigant treatments compared with the non-grafted 'Athena' and 'Tetsukabuto'. Grafting can provide some control of root-knot nematodes under subtropical conditions and can be combined with other soil treatments for more effective control.

Grafting eggplant to manage soilborne diseases: An international perspective

S. A. MILLER (1), M. A. Rahman (2), M. S. Nahar (2), G. Norton (3), E. Rajotte (4)
(1) The Ohio State University, Wooster, OH, U.S.A.; (2) Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh; (3) Virginia Tech, Blacksburg, VA, U.S.A.; (4) Penn State University, University Park, PA, U.S.A.
Phytopathology 102:S4.153

Eggplant (*Solanum melongena*) is one of the most important vegetable crops in South Asia, widely produced on marginal soils in tropical climates by resource-limited smallholder farmers. Bacterial wilt, caused by *Ralstonia solanacearum*, and root knot, caused by *Meloidogyne* spp., are arguably the most important soilborne diseases of eggplant in this region. In Bangladesh, losses of 30-100% due to bacterial wilt in eggplant have been reported. Consumer preferences for eggplant fruit shape, size and color vary widely within the region, resulting in a least 100 locally or regionally preferred varieties in Bangladesh alone. Therefore disease-resistant varieties released from breeding programs are not always accepted by farmers and consumers. Several rootstocks have been identified that confer resistance to one or both of these diseases, including *S. melongena* EG203 (AVRDC), and wild *Solanum* species *S. torvum* (turkey berry) and *S. sisymbriifolium* (sticky nightshade). The local variety 'Chega' grafted onto *S. sisymbriifolium* rootstock survived 22-29 days longer, produced 250-280% more fruit and provided 3-4X higher income than non-grafted 'Chega' in bacterial wilt-infested fields in two locations in Bangladesh. The potential for, and challenges to, widespread adoption of grafting technology among smallholder farmers will be presented.

Issues and Opportunities in Regulatory Sciences at EPA

Reviewing biopesticides in the United States
G. S. TOMIMATSU (1)
(1) U.S. Environmental Protection Agency, OPP BPPD, Washington, DC, U.S.A.
Phytopathology 102:S4.154

Biopesticides offer attractive and environmentally-benign components in IPM strategies for organic growers, and include biochemical and microbial active ingredients, and plant-incorporated protectants. They are distinguished from conventional chemical pesticides by their natural occurrence, target pest specificity, low volume use, lack of persistence in the environment and in general, low toxicity to humans. Before a pesticide is registered in the U.S., risk assessments are conducted to ensure that there are no unacceptable risks to human health and the environment when the pesticide is used in accordance with label instructions. The U.S. Environmental Protection Agency (US EPA) uses a tiered testing paradigm for evaluating hazards and exposures of pesticide uses. Information and data necessary to assess hazards are established in data requirements designed to address the primary disciplines of product characterization and manufacturing, human health, and environmental effects. Data requirements are grouped in a tiered testing framework and potential risks are determined first from estimates of hazard and exposure under "worst-case" scenarios (Tier I). Subsequent testing (Tiers II, III and IV) may be required to assess adverse effects under more realistic use or environmental conditions, especially when lower-tiered studies suggest potentially unacceptable risks. The US EPA has over 30 years of experience in preparing risk assessments to inform its registration decisions for biopesticides. The presentation will provide an overview of microbial pesticides and the tiered risk assessment approach for biopesticide regulation.

Biotechnology for disease management: Regulatory considerations
C. A. WOZNIAK (1)
(1) U.S. Environmental Protection Agency, OPP BPPD, Washington, DC, U.S.A.
Phytopathology 102:S4.154

Biotechnology, or more specifically, recombinant DNA (rDNA) technology, has demonstrated real promise for managing plant diseases through genetic means. To date, however, few of these rDNA-derived products have realized commercial success. The use of viral coat protein or other viral sequences as modulators of RNA interference mechanisms have worked well in potato, papaya, yellow squash and European plum for viral resistance. Of these, only papaya and squash are currently in commercial use with plum to follow soon. Other disease resistant plants are in development, such as peanut expressing oxalate oxidase providing resistance to *Sclerotinia minor*, and inverted repeat transgenes using an RNAi-based approach to silence mycotoxin production by *Aspergillus* and *Fusarium* in maize. These types of products would be licensed under FIFRA as plant-incorporated protectants (PIPs), which are registered as pesticides by the US EPA. PIPs consist of the genetic material and their expression products as expressed *in planta* for the management of pests, including plant disease organisms. Insect resistant maize plants expressing the d-endotoxin from *Bacillus thuringiensis* have demonstrated reductions in fumonisin content of seed as a consequence of reduced insect feeding damage and subsequent fungal colonization. This presentation will focus on the regulatory aspects of the development of biotech derived products for plant disease management.

The National Clean Plant Network: Ensuring Disease-Free, Vegetatively Propagated Fruit Tree Planting Stock

The National Clean Plant Network
E. S. RUDYJ (1)
(1) USDA, Riverdale, MD, U.S.A.
Phytopathology 102:S4.154

The National Clean Plant Network (NCPN) was established by the Farm Bill of 2008. Members provide high quality plant material free of targeted pathogens that cause economic loss to agriculture. NCPN contributes to the global competitiveness of U.S. specialty crop producers by generating desirable planting stock, increasing yields of healthy, high quality produce, and establishing rigorous standards for clean plant programs. The network is coordinated by USDA's Animal and Plant Health Inspection Service (APHIS), the Agricultural Research Service (ARS), and the National Institute of Food and Agriculture (NIFA). Partners include States, universities, non-profits, and industry. To support NCPN operations, 19 clean plant centers or programs located in 15 States received around $5 million in NCPN funding in FY 2011 for pathogen diagnostics, therapy, and establishing foundation plantings. To date, five specialty crops are fully operational under the NCPN banner: fruit and nut trees; grapes; citrus; berries; and hops. Other specialty crops are also considering program entry. NCPN stakeholders build and operate network governing bodies, identify emerging clean plant issues, and strengthen linkages to allied activities such as nursery certification programs. NCPN has also organized working groups, including clean plant education and outreach to industry.

***Plum pox virus* case study: The eradication road is paved in gold**
R. A. WELLIVER (1)
(1) Pennsylvania Department of Agriculture, Harrisburg, PA, U.S.A.
Phytopathology 102:S4.154

The National Clean Plant Network (NCPN) plays a critical role in decreasing the negative impacts of viruses spreading via distribution of propagative material. In the world of temperate tree fruit, the high costs associated with virus introduction have been illustrated recently in the story of *Plum pox virus* (PPV), first detected in North America in 1999 in Pennsylvania. Although the route of introduction has never been proven, it is assumed to have entered as infected plant material. Intensive survey indicated that the virus was confined to a relatively small area, so an eradication plan was implemented. The eradication goal was achieved – but only after ten years, $53 million dollars, and the destruction of 1600 acres of stone fruit orchards. The mission of the NCPN is to produce and distribute virus-tested stock. Because industry is a vital part of the network, the system is constantly adapted to meet industry needs, making it less tempting to move untested stock that may harbor virus. Because the NCPN supports strong communication among virologists, USDA, state certification personnel, and growers, it is uniquely suited to uncover new viruses making their way into the nursery stream. The NCPN also draws together a group that could be quickly mobilized for virus surveillance activities, if needed. For each PPV introduction that is prevented by the work of the NCPN, the network is paid for ten times over.

New threats on the horizon for the fruit tree industry
M. FUCHS (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 102:S4.154

Disease management options based on the use of pathogen-tested, clean material are essential to limit the dissemination of viruses, phytoplasmas and viroids, and to mitigate their impact on fruit trees. USDA recently created the National Clean Plant Network (NCPN) to help protect specialty crops from the spread of economically harmful pathogens. Through NCPN, Clean Centers for fruit trees (NCPN-FT Centers) ensure the availability and maintenance of disease-tested, clean foundation stocks and propagation material through extensive testing and pathogen elimination therapy. Coordination of diagnostic guidelines and establishment of national standards are also important NCPN-FT activities. Amid the numerous successes and accomplishments of NCPN-FT, pathogens that are known or not in the U.S continue to threaten the sustainability of the fruit tree industry, stressing the need for continued efforts to (i) explore novel approaches to accelerate the safe introduction and distribution of pathogen-tested, clean propagation material, (ii) better harmonize certification efforts across regional and national boundaries, (iii) set realistic certification standards that include current pathogen detection technologies, (iv) coordinate the implementation of science-based eradication programs, and (v) communicate more effectively on the benefits of pathogen-indexed, clean material.

Diagnosing and cleaning up viruses in imported fruit tree nursery stock
K. C. EASTWELL (1)
(1) Washington State University, I.A.R.E.C., Prosser, WA, U.S.A.
Phytopathology 102:S4.154

New technology is rapidly expanding new opportunities to assist clean stock programs in the detection and elimination of viruses from vegetatively propagated perennial plants. Viruses cripple the economic sustainability of all segments of the temperate tree fruit industry, reducing or eliminating profit margins from the production and sales of trees and fruit. Additionally, domestic and international movement of nursery stock becomes complicated or restricted by efforts to minimize further dissemination of disease agents. Since peak profitability occurs soon after new cultivars are released, clean stock programs must balance the speed demanded by industry and the exceptional hygiene required of the final product. Critical translational research is needed so that benefits of new technologies can be fully captured by clean plant programs. To assist in the adaptation of new detection strategies, the etiology of severe disease can now be established with the assistance of high throughput sequencing methods. Advances in the understanding of the innate plant virus defense systems are informing the design of effective virus elimination strategies to produce pathogen-free trees. Outputs of these activities will support agencies charged with verifying the absence of exotic pathogens in transported material, and will benefit innovative and progressive fruit growers trying to compete in the global marketplace.

Quantifying the economic benefits of the National Clean Plant Network for the tree fruit industry in the Pacific Northwest

C. F. SEAVERT (1), J. Julian (1)
(1) Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 102:S4.155

This project is an attempt to quantify the economic value of plant certification programs, specifically the NCPN, to the tree fruit growers in the Pacific Northwest through a Net Present Value assessment of the industry. Production budget models were developed for apple, cherry, pear and peach production systems based upon published Extension budgets, tree fruit growers, USDA market data, and Washington State tree fruit industry reports. Net Present Value estimations were calculated using AgProfitTM software. The NPV estimate took into account production system cultivars and orchard architecture. Five scenarios were developed: 1) The current situation, 2) 10 percent reduction in yield and 7 percent reduction in harvest costs, 3) 20 percent reduction in yield and 15 percent reduction in harvest costs, 4) 30 percent reduction in yield and 20 percent reduction in harvest costs, and 5) a shift in productions systems and cultivars back to 2001 conditions. These scenarios were selected based upon feedback from industry stakeholder that suggested certified plant material increased orchard health thus without the NCPN, orchard yields would be lower. Additionally, it was suggested certified plant material fostered investment in new cultivars and orchard systems due to less risk from orchard diseases related losses. The results estimate the current NPV of grower returns in the PNW to be $2.6 billion (assuming an 8% discount rate over twenty years). Under the assumptions in this study, without plant certification programs, reductions in grower returns range from $828 million under scenario five to $4.7 billion under scenario four.

The industry's perspective on the National Clean Plant Network

W. L. HEUSER GALE (1)
(1) International Plant Management, Inc., Lawrence, MI, U.S.A.
Phytopathology 102:S4.155

The US tree fruit industry is a very specialized area of farming that competes in a global market. Fruit varieties are licensed, marketed and sold to consumers in every part of the world. The ability to have clean foundation material available across international borders is essential to maintain our competitive edge. Additionally, fruit growers produce in very high density, intensely productive systems. Our plant material must be clean in order to maintain these systems. We will explore the past, present and future benefits of the National Clean Plant System for the US tree fruit industry.

Diseases of Plants

Advances in Detection Technologies: Application in Plant Pathogen and Disease Detection

Next-generation diagnostics: Eliminating the excessive sequence processing associated with next-generation sequencing using EDNA

W. L. SCHNEIDER (1), A. H. Stobbe (2), J. Daniels (2), A. S. Espindola (2), R. Verma (2), T. Blagden (2), J. Fletcher (2), F. Ochoa-Corona (2), C. Garzon (2), P. R. Hoyt (2), U. Melcher (2)
(1) USDA-ARS FDWSRU, Fort Detrick, MD, U.S.A.; (2) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.155

Deep sequencing (or next-generation sequencing) has altered the molecular biology landscape in many ways, including the development of the field of metagenomics. Not surprisingly, deep sequencing using metagenomics principles have been applied to diagnostics. The massive volumes of data generated by individual deep sequencing runs increases the likelihood of finding pathogens present at low levels. However, sorting through the volumes of data, particularly if diagnosis is the primary concern, can be cumbersome. Significant portions of any metagenome are irrelevant for pathogen detection, and the assembly of complete genomes is not actually necessary for pathogen diagnostics. The initial objective of this work was to develop bioinformatic tools for streamlining sequence data analysis for diagnostic purposes. The bioinformatic process, termed E-probe Diagnostic Nucleic acids Analysis (EDNA) avoids assembly and GenBank BLAST steps while successfully finding nucleic acid signatures of microbes of interest. *In silico* simulations indicated that EDNA was both sensitive and specific in the detection of viruses, prokaryotic and eukaryotic organisms. Initial experiments using infected plant samples suggest that EDNA can detect the same range of organisms in real metagenomes, provided the pathogen titers are sufficient. EDNA also features a high degree of flexibility, allowing adjustment of both specificity and sensitivity levels to suit the needs of the user.

All plant virus chip: Shifting from proof to use

B. BAGEWADI (1), D. C. Henderson (2), K. Fischer (3), R. L. Jordan (4), D. Wang (5), K. L. Perry (6), U. Melcher (7), J. Hammond (8), C. M. Fauquet (1)
(1) Danforth Plant Science Center, Saint Louis, MO, U.S.A.; (2) USDA-ARS, Beltsville, MD, U.S.A.; (3) University of Utah, School of Medicine, Salt Lake City, UT, U.S.A.; (4) USDA-ARS-BA, Molecular Plant Pathology Lab, Beltsville, MD, U.S.A.; (5) Washington University, School of Medicine, Saint Louis, MO, U.S.A.; (6) Cornell University, Ithaca, NY, U.S.A.; (7) Oklahoma State University, Stillwater, OK, U.S.A.; (8) USDA-ARS Floral and Nursery Plants Research Unit, Beltsville, MD, U.S.A.
Phytopathology 102:S4.155

The need to screen increasing numbers of plant viruses in a given sample has resulted in the development of highly parallel methods of detection and identification such as DNA microarray and next generation sequencing. Based on our results with a prototype universal plant virus microarray (UPVM) here we present the development and validation of a full UPVM to detect and identify all known plant viruses, viroids and satellites. A total of 9600 60-mer oligonucleotide probes were selected from 302,230 candidates representing every taxon. After confirming the capacity of selected probes to identify and discriminate viral species by *in silico* analysis, probes were synthesised without any linker or modifications and spotted on poly-l-lysine coated glass slides. The UPVM was validated with non-amplified amino-allyl labelled cDNA from nine healthy plants and more than 30 plants infected with characterized viruses. Validation is in progress with more samples from ATCC and 'unknown' samples. High-titre viruses could easily be detected and identified. For low-titre virus samples several strategies including increasing total RNA for labelling, depleting the host rRNAs and random-amplification are being tested. Comparison of hybridization profiles of healthy and infected plant samples identified a few non-specific reactions. Microarray data was also analyzed by hierarchical clustering and a predictive algorithm, T-Predict, to identify one or more viruses in a mixed infection.

The results of QBOL deposited in the Q-bank database to support plant health diagnostics

P. BONANTS (1), M. Edema (2)
(1) Plant Research International, Wageningen, Netherlands; (2) NVWA, National Plant Protection Organization, Wageningen, Netherlands
Phytopathology 102:S4.155

The rate of introduction and establishment of damaging plant pests and diseases has increased steadily over the last century as a result of expanding globalisation of trade in plant material, climate change, EU expansion, and by a recognised decline in the resources supporting plant health activities.

Furthermore there is a constant decline in the number of taxonomic specialists in the different disciplines (mycology, bacteriology, etc.) capable of identifying plant pathogens, and funds to support this type of work are difficult to obtain. Also the number of other specialists in phytopathology and other fields, which are vital for sustaining sound public policy on phytosanitary issues, are diminishing. These problems affect all members of the EU and other nations. In this context QBOL (www.qbol.org), an EU project on DNA barcoding, started in 2009 to generate DNA barcoding data of quarantine organisms and their taxonomically relatives to support plant health diagnostics. The data are included in a database, called Q-bank (www.Q-bank.eu). Q-bank now consists of a dynamic open-access database of quarantine plant pests & diseases and look-alikes, linked to curated and publicly accessible reference collections. It contains sequence and morphological data including photographs, nomenclatural and diagnostic data of specimens available in reference collections. Within Q-bank curators from many countries with expertise on taxonomy, phytosanitary and collection issues for the different groups have been appointed and links with other databases have been made; this in order to provide Q-bank an international role in supporting plant health agencies. The results of the QBOL project will be presented as well as the Q-bank database.

CANARY: Serological detection sees a new dawn
Z. LIU (1), H. Bowman (1), K. Rappaport (1), L. Levy (2)
(1) USDA APHIS PPQ CPHST, Beltsville, MD, U.S.A.; (2) USDA APHIS PPQ CPHST, Riverdale, MD, U.S.A.
Phytopathology 102:S4.156

CANARY (Cellular Analysis and Notification of Antigen Risk and Yield) is a new serological method invented by scientists at the Massachusetts Institute of Technology. The technology uses engineered mouse B-cells that express membrane-bound, pathogen-specific antibodies and a calcium sensitive bioluminescent protein. Cross-linking of the antibodies by even minute amounts of the specific pathogen leads to elevation of intracellular calcium and the emission of light. The amplified light output is detected using an easy-to-use luminometer. The method is fast, specific (equivalent to other immunoassays) and sensitive (similar to PCR), and has been used for medical diagnostics and monitoring of bio-warfare agents. USDA APHIS and MIT developed plant-pathogen-specific-cell lines for detection of *Ralstonia solanacearum*, *Xylella fastidiosa* CVC strain and to the genus-level for *Potyvirus* and *Phytophthora*, and are developing cell lines for Citrus greening and *Citrus leprosis virus*. We employed simple procedures for testing bacterial pathogens with less than a minute detection and demonstrated analytical sensitivity of 3 CFU/test for *R. solanacearum*. For detection of *Phytophthora* and viruses, capture protocols are used in the sample preparation to enhance the CANARY performance. CANARY has the potential to improve the detection of plant pathogens in many economically and environmentally important plant species.

Pathogen signatures—Beyond nucleic acids & proteins
L. LEVY (1)
(1) USDA APHIS PPQ CPHST, Riverdale, MD, U.S.A.
Phytopathology 102:S4.156

Plant pathogen detection and diagnosis has characteristically involved morphology, isolation and the use of polymerase chain reaction (PCR) or a form of serological detection on strips or in microtiter plates. While these are the standard tools of our trade and widely proven, new technologies used in other disciplines are worth looking into for advancement or supplementation of the tools used to perform these activities. New technologies such as isothermal amplification and CANARY B-cell technology are beginning to show promise for diagnosticians and also require fewer reagents, have smaller footprints and do not require advanced-trained personnel. Additional technologies can also be applied to the detection of plant pathogens based on target properties such as volatiles or other chemical signatures. The potential use of detector dogs combined with rapid traditional diagnostics may be a realistic solution for plant inspection stations or ports of entry in the future. The integration of technologies used for bio-warfare agent detection or point of care diagnostics with more traditional technologies will be discussed. In addition, the integration of several different processes used in traditional diagnostics into single simplified systems used for detection or isolation will be briefly presented.

Isothermal amplification: So many names, are there differences?
M. R. SUDARSHANA (1)
(1) USDA-ARS, Davis, CA, U.S.A.
Phytopathology 102:S4.156

Polymerase chain reaction (PCR) has been the method of choice for the detection of microbial pathogens and in many pharmacogenomics applications. The assay is dependent on a thermal cycler to facilitate repeated cycles of denaturation, primer annealing and DNA synthesis to generate diagnostic DNA products using thermostable DNA polymerases. Unlike conventional-PCR, isothermal amplification (IA) does not need a thermal cycler to amplify nucleic acid targets and allows simultaneous strand separation, primer annealing and DNA synthesis at a constant temperature. Loop-mediated isothermal amplification, helicase-dependent DNA amplification, rolling circle amplification, nucleic acid sequence-based amplification, nicking enzyme amplification reaction and recombinase polymerase amplification represent IA methods currently in use. However, they differ in the way amplification is achieved, use of gene specific primers and choice of DNA polymerases. Some protocols include other nucleic acid modifying enzymes and ssDNA binding proteins to work in concert with DNA polymerases. Amplified products of IA may look similar to those of conventional PCR, recognized by dsDNA binding dyes and fluorescent probes, or represent massive amounts of DNA with loopy structures detectable without any additional reagents. Despite some problems in adopting the IA assays for routine detection, they have the potential to provide high throughput screening without expensive instrumentation.

Bioenergy Crops and Disease

Bioenergy crops and disease agents: Research and industry status
R. S. NELSON (1)
(1) Samuel Roberts Noble Foundation, Inc., Ardmore, OK, U.S.A.
Phytopathology 102:S4.156

Considerable resources are being directed toward developing plants with high and/or easily convertible levels of lignocellulose to serve the bioenergy industry. With this allocation of resources comes a responsibility to fully assess factors that can seriously limit this potential. Disease is one of these factors and we currently have the ability to analyze the influence of disease on these plants proactively (i.e. prior to full commercialization) rather than reactively. This talk will introduce the plant species under consideration as lignocellulosic biofuel crops. Their potential for yield improvement and reduced recalcitrance to biofuel production will be presented. Since the following talks in this session will detail the microbes that infect these species and their negative influence on yield or recalcitrance these topics will be only briefly summarized here. As an interesting contrast, a short overview of the use of microbes to enhance bioenergy crop yield and study recalcitrance will be presented. Supported, in part, by The BioEnergy Science Center, which is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science, and the Samuel Roberts Noble Foundation, Inc.

The impact of disease on biofuel production
D. HAEFELE (1), S. Barr (2)
(1) Pioneer Hi-Bred, A DuPont Business, Johnston, IA, U.S.A.; (2) DuPont, Wilmington, DE, U.S.A.
Phytopathology 102:S4.156

Plant disease can reduce both biomass productivity per unit land area (e.g. grain or lignocellulosic biomass yield per hectare) and the potential biofuel yield of that biomass substrate per unit dry matter (ethanol yield potential per bushel of corn). Plant disease may also contaminate biomass substrate with microbial metabolites (e.g. mycotoxins) that reduce the value of biofuel co-products from a higher value feed ingredient to a product valued only for its thermal energy content. Environmental impact (LCA) and production economics of biofuels are strongly influences by these three factors that are mediated, at least in part, by plant disease. Biofuels are in the midst of an intense period of technology development, scientific investigation, and socio-political debate of costs and benefits. The definition of biofuel is expanding rapidly beyond yeast catalyzed ethanolic fermentation of sugars derived from the enzymatic hydrolysis of starch biomass. Both new substrates (e.g. lignocellulosic biomass), new processes (e.g. lignocellulosic pretreatment technologies), and new products (e.g. isobutanol) are expected to be in commercial use within a matter of months. The environmental merits of biofuels are the subject of an intense popular debate and numerous scientific and engineering studies. The use of life cycle analysis (LCA) to evaluate

environmental impacts is now common place in both drafting legislation and rulemaking by enforcement agencies (e.g. USEPA and CARB). In this context of rapid change we will use the 1st generation biofuel ethanol as an example of the potential for plant disease to impact economic and environmental analyses of biofuels.

Viruses of bioenergy crops

B. O. AGINDOTAN (1), M. E. Gray (2), C. A. Bradley (2)
(1) Energy Biosciences Institute, University of Illinois, Urbana, IL, U.S.A.; (2) Energy Biosciences Institute & Department of Crop Sciences, University of Illinois, Urbana, IL, U.S.A.
Phytopathology 102:S4.157

Present and future energy challenges including crude oil volatility, energy dependence, sustainability, and environmental concerns are the motivators for renewed interest in non-fossil fuel sources of energy. An attractive approach is to produce alcohol from abundantly available biomass, using their hemi-cellulose and cellulose as substrates. Biomass crops are usually not consumed by humans and livestock and those having huge biomass yield are attractive feed-stocks for cellulosic ethanol production. The most prominent bioenergy crops are *Miscanthus* species (*Miscanthus x giganteus*), switchgrass (*Panicum virgatum*), elephant grass (*Pennistum purpureum*), energycane (*Saccharum* hybrid), and energy sorghum (sweet *Sorghum* hybrid); while the most prominent biomass trees are *Poplar* species and willow (*Salix alba* 'Vitellina-Tristis'). *Miscanthus* is not native to America and different varieties of this crop are being imported from Asia. Some of these crops are being improved upon to increase their biomass yield and reduce their lignin content to increase the efficiency of the downstream ethanol production processes. Viruses post great challenges to biomass production because of their potential to reduce biomass yield if they cause dwarfism or induce intensive mosaic or necrotic symptoms. Introduction of these bioenergy crops, especially the improved ones, into new environments might make them more vulnerable to new viral infection. There are also the concerns that these crops may be reservoirs of potent viruses of cultivated food crops. Identification of potential viruses of these crops is vital for the control and management of future potential viral threats as the crops are cultivated in commercial scale, and in multiple locations and environments. In this presentation, I will report research findings on potential viral threats of some of these bioenergy crops.

The potential of increased virus susceptibility in grasses modified for biofuel production

C. M. MALMSTROM (1), A. C. Schrotenboer (2), P. Trebicki (1)
(1) Michigan State University, East Lansing, MI, U.S.A.; (2) Trinity Christian College, Palos Heights, IL, U.S.A.
Phytopathology 102:S4.157

Human experience with crop domestication and agricultural improvement suggests that development of new crop species can shift virus dynamics in a region. Will development of modified native perennial grasses as bioenergy feedstocks thus shift virus dynamics in the US Midwest? Wild populations of prairie grasses have inhabited central North America for millennia. But selection for biofuel-favored traits such as reduced lignin content and fast growth will change these species' phenotypes and potentially their disease susceptibility. Here we consider the influence of selection for biofuel-valued traits in *Panicum virgatum* (switchgrass) on its interactions with one widely-distributed group of aphid-vectored viruses (Luteoviridae: Barley and cereal yellow dwarf viruses). A common prairie element, switchgrass is now a favored candidate for bioenergy feedstock. We examined relationships between switchgrass growth rates and tissue digestibility in field-grown populations that ranged from near-wildtype to highly selected cultivars and compared these traits with measures of switchgrass attractiveness to aphid vectors and susceptibility to virus infection. We found that vector preference and population-level virus infection rates were not strongly influenced by foliar digestibility but increased with biomass accumulation rates. To reduce risk of pathogen movement from fuel to food crops, we recommend screening of new bioenergy feedstocks for virus susceptibility.

Hunt for sources of rust resistance in the bioenergy crop, switchgrass (*Panicum virgatum* L.)

S. R. Uppalapati (1), Y. Ishiga (1), D. Serba (1), L. J. Szabo (2), M. C. Saha (1), K. S. MYSORE (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.; (2) Cereal Disease Laboratory, USDA-ARS, St. Paul, MN, U.S.A.
Phytopathology 102:S4.157

Several fungal pathogens have been identified on ornamental and native strands of switchgrasss (*Panicum virgatum* L.). Diseases of switchgrass have been largely neglected and pathogens could become the major limiting factors to biomass quality, and yield of switchgrass; especially when planted in monocultures. Leaf and stem rust caused by *Puccinia emaculata* is a major emerging disease in switchgrass research fields of Oklahoma. To identify genetically diverse source(s) of rust resistance, we evaluated half-sibling families from upland (Summer and Cave-in-Rock) and lowland (Alamo and Kanlow) switchgrass populations in both field growth chamber assays. Our results revealed a high degree of genetic variation within and among switchgrass populations. Alamo in general showed moderate resistance to *P. emaculata*, while Summer was highly susceptible. These results suggested a potential for improvement of rust resistance via the selection of the resistant individuals within the population. In addition, we have also initiated programs to identify and incorporate traits that confer nonhost rust resistance. Using forward-genetics screen in *Medicago truncatula*, we identified an *irg1* (*inhibitor of rust germ tube differentation1*) mutant that failed to promote pre-infection structure differentiation of *P. emaculata* due to complete loss of the abaxial epicuticular wax crystals and reduced surface hydrophobicity. An update on integrated approaches undertaken to mitigate rust disease and will be discussed.

Response of sorghum modified for bioenergy to grain and stalk fungal pathogens

D. L. FUNNELL-HARRIS (1), S. E. Sattler (1), L. K. Prom (2), P. F. Dowd (3), J. F. Pedersen (1)
(1) USDA-ARS, Grain, Forage and Bioenergy Research, Lincoln, NE, U.S.A.; (2) USDA-ARS, Crop Germplasm Research, College Station, TX, U.S.A.; (3) USDA-ARS, Crop Bioprotection Research, Peoria, IL, U.S.A.
Phytopathology 102:S4.157

Sorghum is a versatile feedstock crop for cellulosic biofuels production. Reducing lignin content results in increased ethanol conversion efficiency. Two mutations in lignin biosynthesis were used to decrease lignin content and alter its composition. Near isogenic lines were developed with *brown midrib (bmr)-6* and *bmr12*, which are deficient in cinnamyl alcohol dehydrogenase and caffeic acid *0*-methyltransferase, respectively. Assessing *Fusarium* spp. infection of field-grown grain showed a significant reduction in *Gibberella fujikuroi* isolates in *bmr* grain, as compared with wild-type (WT); in particular, *Fusarium proliferatum* colonization was limited in *bmr12* grain. *Fusarium* sp. cf. *bullatum* colonization was absent in *bmr12*, while this genotype was common in *bmr6* and WT grain. Peduncles inoculated with *Fusarium thapsinum, Fusarium verticillioides* or *Alternaria alternata* had significantly smaller mean lesion lengths on *bmr6* or *bmr12* plants, relative to WT. Basal stalk inoculations with *Fusarium* stalk rot pathogens showed that *bmr* lines were as resistant as wild-type lines. Responses of *bmr* plants to foliar pests, including *Colletotrichum sublineolum* and chewing insects, provided further evidence that reduced lignin lines were not more susceptible, and in some cases were more resistant, to common sorghum pathogens and other pests as compared to WT lines.

Schroth Faces of the Future—New Frontiers in Plant Bacteriology

Minerals influence interactions between the bacterium *Xylella fastidiosa* and its host plants

L. DE LA FUENTE (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 102:S4.157

Xylella fastidiosa (Xf) is a pathogen restricted to live inside the xylem of the plant host or mouth parts of insect vectors. Once inside the xylem the bacterium forms biofilms believed to be responsible for disrupting water flow to the aerial parts of the plant. Evidence from different laboratories indicates that water deficit is not the only cause of disease development. We hypothesize that infection with Xf causes changes in the mineral status of the host plant that are used by the bacterium to accentuate its virulence. Infection of *Nicotiana tabacum* with Xf caused significant changes in the mineral and trace elements (viz. the 'ionome') of the plant, in particular increasing Ca and Mg, and reducing K and P. Changes in the ionome observed under greenhouse conditions in tobacco were confirmed in field samples from various plant hosts naturally infected with Xf, including grapes, blueberries and pecan. *In*

vitro studies showed that specific minerals (notably Ca) added to the media increased the ability of the bacterium to attach to surfaces, form biofilm and move. These traits were studied using microfluidic chambers, where also the process of biofilm formation is being mathematically modeled. Responses to metals are being investigated in a collection of Xf isolates from diverse host plant and geographic origins. Our studies are characterizing how minerals, main components of xylem fluid, shape diseases caused by Xf in diverse hosts.

Draft genomic sequence of rice pathogens and nonpathogens: Insights in biology, diversity, and diagnosis
L. R. TRIPLETT (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.158

The declining cost of sequencing has made bacterial draft genomes a valuable tool for functional, comparative, and diagnostic information. We recently obtained draft genome sequence from a collection of poorly understood strains of rice-associated bacteria: multiple diverse strains of the pathogen *Xanthomonas oryzae*, including variants from Africa and the United States, strains of the rice bacterial pathogens *Burkholderia glumae* and *Pseudomonas fuscovaginae*, and two previously uncharacterized species of nonpathogenic *Xanthomonas* commonly associated with rice seeds and leaves. The weakly virulent US strains of *X. oryzae* were highly divergent from other studied *X. oryzae* strains, suggesting a long existence in the US. These US strains lack full or partial transcriptional like effectors (TALEs) common to other *Xanthomonas* pathovars. The lack of TALEs made them a useful tool for studying individual TALE roles in a panel of rice cultivars, a study which yielded several novel insights into TALE functional variability among hosts and pointed to possible sources of resistance. Intriguing finds from the other pathogen and nonpathogen draft genomes will also be reviewed, along with implications for future research. Finally, a rapid automated pipeline was developed to design sets of specific diagnostic primers by comparing draft and/or completed genomes; performance analysis and testing of the primers demonstrated the program to be a highly efficient means of diagnostic primer design. These studies demonstrate how draft genomes of non-model international isolates, isolates of low virulence, and plant-associated nonpathogens can improve diagnostic test development and reveal secrets of pathogen biology and diversity.

Effector proteins as probes to understand molecular mechanisms underlying plant-bacterial interactions and as markers for detecting bacterial diseases
W. MA (1)
(1) University of California-Riverside, Riverside, CA, U.S.A.
Phytopathology 102:S4.158

Bacterial pathogens have evolved a multitude of effectors that exert essential virulence functions in plant hosts during infection. Since effectors directly target components of host immune system, research on how effectors promote infection provides critical information not only on strategies taken by pathogens to infect plants, but also on plant defense mechanisms. Using both model and natural pathosystems with high impacts in agriculture, research in my laboratory aims to functionally characterize effectors secreted through the type III secretion system. In particular, our research has been focusing on the *Pseudomonas syringae* effector HopZ1, which belongs to the widely distributed YopJ family of type III effectors. We identified the direct targets of HopZ1 in the natural host soybean. Our data demonstrated that HopZ1 directly suppresses the isoflavonoid biosynthetic pathway, thereby promoting bacterial infection in soybean. In addition to our long-term goal, which is to apply knowledge obtained from basic research on effector functions to the development of sustainable and novel disease intervention strategies, we are also committed to develop shorter-term applications that can immediately benefit growers. One such application is to use effectors to develop novel serological detection methods for endemic bacterial diseases. I will discuss our recent progress on marker development for citrus bacterial diseases including citrus stubborn disease and Huanglongbing.

***Streptomyces* secondary metabolism and scab disease development: To what extent are these two processes linked?**
D. R. BIGNELL (1)
(1) Memorial University, St. John's, NF, Canada
Phytopathology 102:S4.158

Potato common scab is an important crop disease caused by the *Streptomyces*, which are Gram-positive, filamentous soil bacteria. The best characterized scab-causing streptomycete, *S. scabies*, has a world-wide distribution and is the main causative agent of scab disease in North America. A key virulence determinant produced by *S. scabies* is the phytotoxic secondary metabolite thaxtomin A, which targets cellulose biosynthesis in the plant host. The production of thaxtomin A is controlled by the pathway-specific regulator, TxtR, as well as five additional regulators that are conserved in other *Streptomyces* species. In addition, the recently available genome sequence of *S. scabies* 87-22 has revealed the presence of additional secondary metabolite biosynthetic gene clusters (SMGCs) that may contribute to the pathogenicity of this organism. One such SMGC is predicted to synthesize molecules similar to the coronatine (COR) phytotoxin produced by *Pseudomonas syringae*, and recent functional analyses have confirmed a role for this SMGC in *Streptomyces*-plant interactions. Current research is focused on elucidating the mode of action of these virulence-associated COR-like metabolites and how production of these metabolites is regulated in *S. scabies*. In addition, the role(s) of other *S. scabies* SMGCs in plant pathogenicity is being addressed in order to provide a more complete picture of the virulence strategies used by this pathogen during host-pathogen interactions.

Thousand Cankers Disease: A Threat to Eastern Black Walnut Throughout Its Native Range and Beyond

The distribution and impact of thousand cankers disease in walnut species in the western United States
N. TISSERAT (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.158

Thousand cankers disease (TCD) was likely present in black walnut along the Wasatch Front Range in Utah and areas of the Columbia Gorge and Willamette Valley in Oregon as early as the late 1980's. TCD in black walnut is now distributed in the West as far south as New Mexico, as far north as Idaho and Washington, and as far east as Colorado. Disease severity has intensified in communities where it has been found and in many locations mature black walnuts have been eliminated, or nearly so, by TCD. The walnut twig beetle (WTB) and cankers caused by *Geosmithia morbida* also have been associated with a decline of southern and northern California walnuts in their native range. TCD has not yet significantly impacted the English walnut industry in California, although individual trees have been killed. The WTB and *G. morbida* have not been found in the native range of little walnut, although this species is susceptible to the fungus. Both the WTB and *G. morbida* are widespread in Arizona walnut throughout its range in the southwestern US, but only in association with damaged or senescing branches. The population structure of *G. morbida* in the West is complex. Haplotype diversity in isolates collected thus far from Arizona walnut, the putative native host of the beetle and fungus, is different from isolates collected from black walnut and other walnut species in the West.

From discovery to regulation: A pathologist's perspective of thousand cankers disease in eastern United States
M. WINDHAM (1)
(1) Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 102:S4.158

Thousand Cankers Disease (TCD), is an insect (*Pityophthorous juglandis*)/ fungal (*Geosmithia morbida*) complex that threatens black walnut (*Juglans nigra*). In early August, 2010, TCD was confirmed in Knox County in east Tennessee and this was the first report of TCD within the native range of black walnut. Since then, TCD has been found in five additional counties in east Tennessee. In 2011, the disease was reported in Virginia and in Pennsylvania. TCD may be hard to detect because the initial symptoms mimic those associated with drought stress. Since much of the eastern U.S. had suffered from drought in the last seven years, many hardwood species have displayed symptoms consistent with drought and this may have allowed TCD to stay hidden for several years in affected areas. To date, all reports of TCD in eastern outbreaks have been in urban areas or in trees along roadways. No trees in a forest environment have had a case of TCD confirmed. Since *P. junglandis* is considered a native insect by APHIS, the disease has not been regulated on a national basis. Regulation has been left to individual states and regulation has not been consistent. For example, in Tennessee, counties

surrounding counties with known outbreaks of TCD are regulated. However, when a county with TCD is next to a county in another state, the adjacent county is not regulated since it is outside of Tennessee's jurisdiction. This may make quarantines ineffective.

Evolution, diversity, and ecology of the genus *Geosmithia* with emphasis on *G. morbida*
M. KOLARIK (1)
(1) Institute of Microbiology of the Academy of Sciences of the Czech Republic, Prague, Czech Republic
Phytopathology 102:S4.159

Bark beetles are associated with diverse fungal symbionts. One of these fungi, with poorly known biology belong to the genus *Geosmithia* (Ascomycota: Hypocreales). The current knowledge is based on samples from western Palearctic, USA and several other regions. 50 species of the genus (22 published, 11 formally described) were found until now. *Geosmithia* spp. are typically associated with phloeophagous bark beetles, bostrichids and weevils, and over of 90% from the screened insects species were found to be obligate or facultative vectors. A derived ecological strategy, found in three *Geosmithia* spp., represents association with ambrosia beetles, where they act as primary ambrosia fungi. Several species occur on alternative substrates or can live as endophytes of healthy trees. One species, *G. morbida*, was proven to be a pathogenic fungus causing tissue necroses on walnuts. *Geosmithia* community pattern and presence of primary ambrosia fungi suggest the long-term and stable association between *Geosmithia* and bark beetles. *G. morbida* belongs to clade of hardwood-associated species. Major enzymatic activities, utilization of C, N, P, S sources (BIOLOG microarrays), secondary metabolites and antimicrobial activities of *G. morbida* are presented. Physiological fingerprinting places *G. morbida* among strict specialists confirming its specificity to a single host and long term co-evolution.

Life history of the walnut twig beetle, *Pityophthorus juglandis*, and its association with *Geosmithia morbida*, causal agent of thousand cankers disease in the United States
S. J. SEYBOLD (1)
(1) USDA Forest Service, Pacific Southwest Research Station, Davis, CA, U.S.A.
Phytopathology 102:S4.159

The walnut twig beetle (WTB) is a native phloeophagous (phloem-feeding) insect that has recently been associated with the newly described fungus, *Geosmithia morbida*. This insect-fungal complex is known as thousand cankers disease. In northern California male WTB colonize *Juglans* branches and stem material by tunneling into the phloem where they produce an aggregation pheromone that attracts females and more males. The pioneer males are joined by two or more females and together they construct a largely transverse gallery system in the phloem. Both sexes carry the conidia for *G. morbida* and the pathogen appears to be introduced into host *Juglans* phloem during gallery construction. The pheromone has been used to demonstrate that WTB flies nearly year round with trap catches absent in December and very low in January. Peak flights occur in May/June and August/September; another major flight occurs in March/April when overwintering adults emerge. Overwintering larvae develop in spring and likely emerge in May. In the summer, most of the beetles fly between 1800 and 2000 h (crepuscular flight), but this diurnal pattern appears to shift as temperatures cool in the fall. The bulk of the WTB responding to pheromone-baited traps are females (0.59 to 0.83 relative to total number of beetles captured). An analysis of larval head capsule widths showed that WTB has three larval instars. The efficacy of the pheromone for detecting WTB was demonstrated over a range of population densities in eastern and western states.

Thousand cankers disease: A recently emerging disease of eastern black walnut in the eastern United States
G. J. GRIFFIN (1)
(1) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 102:S4.159

Eastern black walnut (EBW) is an important forest and landscape tree in the eastern U.S. Since the discovery of thousand cankers disease (TCD) in TN in 2010 and subsequently in VA and PA in 2011, the threat of this disease to the EBW resource has been of increasing concern. The rate of spread of TCD in the East is yet to be defined. Many EBW trees have been killed by this disease in the West, often within a few years. As environmental conditions in the West are quite different than in the East, it is not clear if the progression of this disease in the East will be the same as in the West. EBW is a mesic tree species, native to moist coves and alluvial soils. In contrast, many EBW trees are planted in the East on sites that often experience seasonal drought or other stresses that may affect TCD development. In 2011 in TN, I observed little or no new development of TCD external symptoms (wilt and branch dieback) on EBW trees in research plots, and increase in live crown ratings (recovery) on some trees was observed early in 2011. The rainfall for the growing season was 53% greater in 2011 than in 2010, when severe TCD symptoms occurred. It is possible that TCD on individual EBW trees in the East will not be continuously progressive, as was for chestnut blight, but be intermittently progressive, depending on the occurrence of environmental conditions favorable to disease development. Movement of infested EBW products is of special concern for TCD spread to new areas.

Ecology and Epidemiology

Exploring the Micropolis: Sampling, Identifying, and Analyzing the Diversity of Microbial Communities

Metagenomics for complex microbial communities
S. TRINGE (1)
(1) DOE Joint Genome Institute, Walnut Creek, CA, U.S.A.
Phytopathology 102:S4.159

Molecular techniques have revolutionized microbial ecology and constant advances in next-generation sequencing have dramatically improved our ability to explore both dominant and rare members of microbial communities at ever-greater depths. Until recently, shotgun metagenomics of complex communities was largely restricted to gene-centric analyses that highlighted important functional distinctions between communities, but not between individual populations within those communities. Current gigabase and even terabase scale projects are overcoming the obstacles to genome assembly from complex metagenome data and enabling new levels of functional exploration. I will review the sequencing technologies in use for metagenomics and describe their application to complex communities including soils and sediments.

From metagenomics to metabolomics: Communication in the rhizosphere
J. HANDELSMAN (1)
(1) Yale University, New Haven, CT, U.S.A.
Phytopathology 102:S4.159

Small molecules rule the rhizosphere. This has been clear since the first definition of the rhizosphere in 1904, but it emerged much later that microorganisms are central to the dynamic conversation that creates the metabolic profile of the rhizosphere. Although plants initiate the conversation by pumping photosynthate from their roots, microorganisms rapidly respond by modifying plant chemistry and contributing metabolites of their own. Roles of certain microorganisms and metabolites have been elucidated, but a comprehensive portrait of the microbial membership, chemical composition, and dynamic responses of the rhizosphere has eluded us. Recent technical advances provide a new opportunity to tackle the complexity of the rhizosphere. Analysis of 16S rRNA gene sequences provides a census of both culturable and as-yet-uncultured bacteria in the rhizosphere community. Metagenomic sequence analysis offers insight into the genetic potential of the community, and functional metagenomics provides an activity-based approach to discovering new genes from uncultured organisms through functional expression in surrogate hosts. New methodology for mass spectrometry now promises a complete metabolomic portrait of the rhizosphere in real time. These methods used in concert have the potential to reveal new dimensions of the rhizosphere, particularly its variation over space and time and responses to microbial perturbation.

Metagenomics in fungal community ecology—Combining new and old technologies to maximize our understanding of complex fungal communities
M. E. SMITH (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.159

Emerging DNA sequencing technologies are making it quicker, cheaper, and easier to generate massive sequence data from environmental samples. This

deluge of information can quickly overwhelm our capacity to interpret results, understand how fungal communities are structured, and identify keystone species. In this talk I will provide a perspective based on my research in natural ecosystems and based on studies of fungi that are not well represented in sequence databases. I will discuss how the new, enhanced sequencing capacity ironically increases our reliance on reference databases and therefore necessitates more molecular systematic studies of fungi as well as widespread sequencing directly from fungal herbaria and culture collections. I will also discuss the importance of phylogenetically-focused sampling and how combinations of molecular and non-molecular approaches could be used to enhance our understanding ofe nvironmental sequences. I will argue that fungal systematics and ecology are inextricably linked and that both are necessary to adequately comprehend the ever-expanding flood of new sequences.

New frameworks in disease ecology that address the micropolis
K. A. GARRETT (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 102:S4.160

The revolution in sequencing technologies offers an exciting new perspective on full microbial communities - and on both microbial effects on plant health and microbial responses to climate. Components of plant health and plant productivity can be considered part of the extended phenotype or phenome of environmental microbial communities. Progress in plant genomics supports new levels of characterization of plant responses to microbial communities, which have potential for construction of a more nuanced understanding of the extended phenome in the context of epidemics. Two types of network models may prove useful for conceptualizing the roles of microbial communities in disease ecology. In one type, nodes of the network represent individual taxa. Approaches for modeling the interactions of these communities may be analogous to genetic networks and food webs. In another type, nodes of the network represent physical locations. This case includes epidemic network models of the movement of pathogens, and may be extended to include the movement of communities of microbes. When such models treat the host as habitat for within-host modeling, they may also include the movement of forms of information associated with microbes, such as microbial and plant signaling molecules.

Welcome to the micropolis: How metagenomics can enhance plant pathology research
K. D. BRODERS (1)
(1) University of New Hampshire, Durham, NH, U.S.A.
Phytopathology 102:S4.160

Although plant pathologists and microbiologists have been investigating the impact of soil microbial communities for several decades, we still have an incomplete picture of how microbial diversity affects crop yield, disease severity, and ecosystem function. As new sequencing technologies have become cheaper and more accessible it provides an opportunity to investigate the interactions between soil microbial communities, plant hosts, and the soil environment at greater spatial and temporal scales. While the power and depth of data generated from soil ecosystems is unprecedented, using these new sequencing technologies in the absence of generally acknowledged standards may prove counterproductive. In this talk I will address these issues within the larger context of soilborne and root diseases in agricultural and forest ecosystems, and discuss some of the key aspects of meta '-omics' analyses and how they can be used to provide a more comprehensive picture of the complex interactions of soilborne pathogens with their biotic and abiotic environments. Finally, I will present examples of how these new sequencing technologies can be integrated into traditional plant pathology research to investigate many of the unanswered questions associated with how soil microbial communities effect plant production and how we can harness the diversity of these communities for improved crop production and ecological function.

It's a Mixed Up World: Hybridization and Horizontal Gene Transfer in Plant Pathogens and Endophytes

***Verticillium longisporum*—A hybrid pathogen with an expanded host range**
P. INDERBITZIN (1), R. M. Davis (1), R. M. Bostock (1), K. V. Subbarao (1)
(1) University of California-Davis, Department of Plant Pathology, Davis, CA, U.S.A.
Phytopathology 102:S4.160

Hybridization plays a central role in plant evolution, but its overall importance in fungi is unknown. It has been suggested that new plant pathogens could arise by hybridization between formerly separated fungal species. Evolution of hybrid plant pathogens from non-pathogenic ancestors in the fungal-like protist *Phytophthora* has been demonstrated, but in fungi, the most important group of plant pathogens, there are few well-characterized examples of hybrids. We focused our attention on the diploid hybrid and plant pathogen *Verticillium longisporum*, the causal agent of the Verticillium wilt disease in crucifer crops. To address questions related to the evolutionary origin of *V. longisporum*, we used phylogenetic analyses of seven nuclear loci and a dataset of 203 isolates of *V. longisporum*, *V. dahliae* and related species. We confirmed that *V. longisporum* was diploid, and originated three different times, involving four different lineages and three different parental species. All hybrids shared a common parent, Species A1 that hybridized respectively with Species D1, *V. dahliae* lineage D2 and *V. dahliae* lineage D3, to give rise to three different lineages of *V. longisporum*. Species A1 and Species D1 are as yet unknown taxa. *Verticillium longisporum* likely originated recently, as each *V. longisporum* lineage was genetically homogenous, and comprised Species A1 alleles that were identical across lineages. The three different hybrid lineages may differ in virulence.

Emergence of *Phytophthora* pathogens by hybridization
E. GOSS (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 102:S4.160

Interspecific hybridization is known to be a diversifying force in plants and is increasingly being recognized as an important evolutionary process in the emergence of pathogens. There are multiple instances of interspecific hybridization in the genus *Phytophthora* and they suggest that hybridization between *Phytophthora* species can lead to the emergence of pathogens with novel host ranges. Interspecific hybridization has been hypothesized to be a consequence of the global movement of *Phytophthora* species. Has interspecific hybridization contributed to the evolution and diversification of *Phytophthora* pathogens? These questions will be discussed in the context of known hybrids, with a focus on *P. andina*.

The role of horizontal gene transfer in bacterial crop pathogen emergence
B. A. VINATZER (1), R. Cai (1), C. L. Monteil (2), C. E. Morris (2)
(1) Virginia Tech, Blacksburg, VA, U.S.A.; (2) INRA PACA, Avignon, France
Phytopathology 102:S4.160

Traditionally, *Pseudomonas syringae* has been viewed as a crop pathogen and consequently research has focused on strains isolated from crops. However, sampling precipitation, snow pack, leaf litter, and surface water has revealed an immense genetic diversity of *P. syringae* in these non-agricultural substrates. Interestingly, very close relatives of the tomato pathogen *P. syringae* pv. *tomato* (Pto) have been found among the *P. syringae* in these non-agricultural substrates. Some of these strains are as aggressive on tomato as Pto under laboratory conditions. Multilocus sequence typing and analysis of selected virulence genes show that these environmental Pto-like strains share identical alleles with Pto at some loci and that recombination between Pto-like strains and Pto has occurred during evolution of Pto. The implications of these findings for crop pathogen emergence and evolution will be discussed.

Hybrids hybrids everywhere: The role of hybridization in the evolution of *Neotyphodium* grass endophytes
K. D. CRAVEN (1), S. R. Ghimire (2), C. Young (1), N. D. Charlton (1), G. Swoboda (1), B. Hall (1), M. Afkhami (3)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.; (2) RTI International, Research Triangle Park, NC, U.S.A.; (3) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 102:S4.160

Interspecific somatic hybridization has played a prominent role in the evolution of seed-transmissible, mutualistic fungal endophytes collectively known as the epichloae. Current theory suggests that hybridization events may provide increased herbivore protection for the host plant through pyramiding of bioactive alkaloid genes. Additionally, these endophytes typically lack a

sexual cycle and thus, hybridization may alleviate the destabilizing effects of deleterious mutations that accumulate in the fungal genome. Here, we analyzed the genetic diversity of epichloid endophytes infecting populations of a single host grass species endemic to California, *Bromus laevipes*. In total, 58 isolates representing samples from 12 populations were analyzed and grouped based on intron sequences of translation-elongation factor 1-alpha (*tefA*) and beta-tubulin (*tubB*). We also grouped the isolates based on both morphological characters and alkaloid chemotypes to evaluate congruency between classification schemes. Preliminary data suggest that some of these hybrids were formed from different progenitor species while others share the same progenitor species, but may have involved distinct genotypes with differing alkaloid profiles. These findings suggest that the diversity of hybrid endophytes within single host species can reflect that observed more broadly within the grass subfamily Pooideae.

Genomic characterization of the conditionally dispensable chromosome in *Alternaria arborescens* provides evidence for horizontal gene transfer
T. MITCHELL (1)
(1) The Ohio State University, Department of Plant Pathology, Columbus, OH, U.S.A.
Phytopathology 102:S4.161

Fungal plant pathogens cause serious agricultural losses worldwide. *Alternaria arborescens* is a major pathogen of tomato, with its virulence determined by the presence of a conditionally dispensable chromosome (CDC) carrying host-specific toxin genes. Genes encoding these toxins are well-studied, however the genomic content, organization, origination of the CDC is not known. To gain a richer understanding of the molecular determinants of virulence and the evolution of pathogenicity, we performed whole genome sequencing of *A. arborescens*. Presented will be the *de-novo* assembly of the CDC and its predicted gene content as well as hybridization data validating the CDC assembly. Predicted genes were functionally annotated through BLAST. Gene ontology terms were assigned, and conserved domains were identified. Differences in nucleotide usage were found between CDC genes and those on the essential chromosomes (EC), including GC3-content, codon usage bias, and repeat region load. Genes carrying PKS and NRPS domains were identified in clusters on the CDC and evidence supporting the origin of the CDC through horizontal transfer from an unrelated fungus was found. We provide evidence supporting the hypothesis that the CDC in *A. arborescens* was likely acquired through horizontal transfer. We also identified several predicted CDC genes under positive selection that may serve as candidate virulence factors.

"Left of Boom!" Information: Form, Content, and Use in Epidemic Prediction

Information in multiscale epidemiological models
C. C. MUNDT (1), P. Skelsey (2), P. S. Ojiambo (3), K. A. Garrett (2)
(1) Oregon State University, Corvallis, OR, U.S.A.; (2) Kansas State University, Manhattan, KS, U.S.A.; (3) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 102:S4.161

Food, fiber, and energy demands over the next 50 years call for substantial increases in agricultural productivity while addressing crucial issues of environmental impact and resource sustainability. One approach to this challenge is to design agricultural landscapes that are resistant to the establishment and spread of plant disease epidemics. pidemic processes occur over different spatial scales, ranging from infection of an individual plant cell to the dispersal of propagules within and between continents. In contrast, practical limitations usually restrict epidemiological studies to a much narrower spatial scale. Thus, there is a great need to better understand how epidemic processes translate across disparate spatial scales. We will discuss modeling approaches that make predictions across spatial scales that are relevant to the influence of landscape heterogeneity on disease spread. The focus will be on the degree to which processes may be scale-dependent versus scale-neutral, how the issue of scale influences the impact of landscape heterogeneity on prediction of epidemic spread, and the degree to which predictions of different models are congruent. One clear conclusion of the modeling efforts is that there is a crucial lack of biological data to adequately parameterize and validate currently available models.

Transportation grids as early indicators and warning—The use of census and travel data for prediction of disease incursions
T. R. GOTTWALD (1), T. D. Riley (2), M. S. Irey (3), S. R. Parnell (4)
(1) USDA-ARS, Fort Pierce, FL, U.S.A.; (2) USDA APHIS PPQ, Orlando, FL, U.S.A.; (3) United States Sugar Corp., Clewiston, FL, U.S.A.; (4) Rothamsted Research, Harpenden, United Kingdom
Phytopathology 102:S4.161

Detection of initial introductions of exotic pathogens and pests is challenging because they occur in very low incidence. The earlier the detection, the more likely the pathogen can be eliminated or the epidemic slowed, lessening impact over multiple years. Finding point introductions across a broad geographic landscape of mixed agricultural and residential areas requires substantial manpower and fiscal resources, and often goes undetected for prolonged periods until incidence exceeds the lower threshold of sampling sensitivity. The Census/Travel model utilizes probable pathways, parses regions into smaller areas (ex. zip code), and predicts the most likely locations for introduction. This geospatial method uses US census and international travel data combined with a pathosystem's epidemiological characteristics, i.e., latency, reproductive rate, etc. The model generates a risk index map that is linked to a survey optimizer that calculates the number of samples to be taken in each area based on risk, estimates manpower and fiscal requirements and ranks foreign countries by their relative risk. The model is independent of pathosystem and can be used to predict introductions of vectored and non-vectored plant, human, and animal diseases/pests. Risk maps have been generated for several exotic plant and human/animal diseases: ex. malaria, dengue fever, yellow fever, chagas, and Chikungunya, and has validated known points of introduction for prior disease introductions.

Emergence of unified concepts of disease in textual surveillance data
C. S. THOMAS (1), N. P. Nelson (2)
(1) University of California-Davis, Department of Plant Pathology, Davis, CA, U.S.A.; (2) Georgetown University Medical Center, Washington, DC, U.S.A.
Phytopathology 102:S4.161

Technology has increased the amount of text based information available for use in biosurviellance. Large text-based repositories are evolving to include relational databases of test results, citizen observations, social networks, expert opinion and news media. Additionally, database interconnectivity is increasing rapidly. Human health surveillance has pioneered syndromic analysis of diagnostic test results, pharmaceutical purchase, absenteeism in schools and the workplace, and other case count-based analysis to inform situational awareness. With the development of the National Plant Diagnostic Network Repository, national and regional syndromic clinic analysis is now possible. Initially, local repositories consisted of open form text that was unstandardized. Analysis showed certain words are used more frequently than others. As technology and data streams improve, and media becomes near-real time, it is possible to recognize indicators and warning of potential or emerging outbreaks earlier. Additionally, public media analysis indicated the progress of an epidemic can be discerned based on what terms the communicators choose. Such an understanding of a communicator's choice of terms can be exploited to identify epidemic stages and severity. Biosurveillance programs have developed "taxonomies" of word choices to identify escalation of an event and tipping points as very early indicators and warning of an outbreak. Syndromic and media case examples are presented.

Advantages and challenges of using Internet media for disease detection and tracking
N. P. NELSON (1)
(1) Georgetown University Medical Center, Washington, DC, U.S.A.
Phytopathology 102:S4.161

The use of Internet media data for the early detection and situational awareness of infectious disease events has evolved into a globally recognized field. Internet media has the advantage of being timely -- available in any language from local to international sources. Published success stories include tracking events associated with mass gatherings, pandemic influenza, the aftermath of natural disasters, food shortages and plant pest and disease outbreaks. However, sifting through the vast array of information on the Internet, ranging from traditional text media to social media is challenging, e.g., detecting a significant signal among the vast noise of misinformation and non-specific reports. In addition, identifying anomalous activity without an established baseline of media activity for a given disease in a particular region is difficult. Recognizing false positive and false negative events is also problematic due to the lack of official reports or delays in diagnostic testing. As such, applying traditional epidemiological analytical approaches to Internet data is often not possible. Despite these obstacles, many recent

advances in the field, such as mapping of Internet data using visual analytic tools, forecast modeling and social networking analysis have demonstrated the great potential of media as a real-time complimentary approach to traditional public health methods of disease surveillance.

Putting information to use: Decisions at different scales
S. SAVARY (1), A. H. Sparks (2), A. Nelson (2), N. McRoberts (3), P. D. Esker (4)
(1) INRA, Castanet-Tolosan, France; (2) IRRI, Manila, Philippines; (3) University of California-Davis, Davis, CA, U.S.A.; (4) Universidad de Costa Rica, San Jose, Costa Rica
Phytopathology 102:S4.162

Information pertaining to crop health may be gathered at the field, district, eco-regional, or global scales. These scales have been addressed in the case of rice in tropical Asia. This allowed cross-scale comparisons of crop health information and management. At the field scale, cropping practices (e.g., crop rotation, crop management), i.e., medium to short term decisions have a strong bearing on crop health. At the district scale, results further highlight the links between production situations and crop health, and the emergence of new crop health problems linked with technology shifts (e.g., new plant material). At the eco-regional to global scales, simulation and GIS analyses suggest that eco-regions can be grouped according to epidemic risks (ER) and their attached uncertainty for five very different rice diseases. This latter scale brings about risk categories depending on diseases, i.e. (1) world areas of disease endemicity (high ER mean and low ER variance), (2) areas with low ER mean and low ER variance, and (3) areas where ER is variable, and ER variance is high. Durable control measures are required in the first category, but not in the second. The third category implies the highest uncertainty level, requiring better prediction to deploy tactical and short-term decisions timely. Combining different geographical scales enables different systems perspectives for disease management, and information use along the decision time-line.

Resolving the Species-Population Interface in Asexual Fungi: New Tools to Address an Old Problem

Asexuality across the kingdom Fungi and the taxonomic challenges of species delineation
P. W. CROUS (1), J. Z. Groenewald (1)
(1) CBS Fungal Biodiversity Centre, Utrecht, Netherlands
Phytopathology 102:S4.162

Recent changes to the International Code of Nomenclature for algae, fungi and plants have significant implications for practical plant pathology. Other than the abolishment of Latin and the implementation of name registration, asexual genera will be integrated with sexual genera of fungi, and a single name will be used to communicate about species. By employing DNA-based techniques, genera and species can be linked in the absence of all stages of their life cycle. To complicate matters, however, many genera are either poly- or paraphyletic, and many pathogens are in fact species complexes. Even though it is clear that the phenotype conveys limited information about true genealogical relationships, close to 80 % of all novelties described per year still lack DNA sequence data, which represents one of the biggest challenges facing our community. Although the internal transcribed spacer region has been selected as the universal fungal barcode, it only successfully identifies 73% of the taxa screened across kingdom Fungi to species level, suggesting that secondary barcodes will be needed to provide accurate identifications. This is especially true for species in many important plant pathogenic fungal genera such as *Botryosphaeria*, *Calonectria*, *Cercospora*, *Colletotrichum*, *Ilyonectria*, *Mycosphaerella*, *Phoma* and *Pseudocercospora*. Based on these findings, it is clear that even though many diseases are associated with species complexes, some of these species are in fact also complex in that they have a complicated ecology and life cycle.

Using comparative genomics for species resolution in *Alternaria*
B. M. PRYOR (1), B. Wang (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.162

Systematic study in the genus *Alternaria* provides a unique opportunity to examine speciation processes across different fungal lifestyles and microbial ecology. The genus encompasses nearly 200 species, but most fall into two very distinct sister clades: Section Alternata, which represents wide-spread facultative pathogens, and Section Porri, which represents host-specific facultative sabrobes. Twelve species from each Section have been sequenced using Illumina Next Generation Sequencing technology and functionally annotated based on gene prediction in one reference genome for each Section: *A. arborescens* for Section Alternata and *A. solani* for Section Porri. Whole genome comparisons revealed orthologs in high synteny within groups rather than between, supporting Section designation. Homology was much lower among host-specific species within Section Porri than among nonhost-specific species within Section Alternata, suggesting ancestral barriers to genetic exchange by host despite common sympatric associations. Gene ontology analysis also revealed differential enrichment in certain functional categories, suggesting that each Section encompasses some distinct metabolic pathways. Genomic polymorphisms revealed most species are clearly delimited by host within Section Porri, but indicated genetic exchange among clusters of species within Section Alternata, which may support their collapse into fewer taxonomic units.

Challenges and opportunities for species recognition in *Fusarium* provided by genomics
D. M. GEISER (1), B. Park (1), S. Kang (1), K. O'Donnell (2)
(1) Penn State University, University Park, PA, U.S.A.; (2) NCAUR USDA-ARS, Peoria, IL, U.S.A.
Phytopathology 102:S4.162

In the last twenty years, there has been a complete overhaul of *Fusarium* systematics at the species level, as molecular phylogenetic approaches have facilitated the recognition of species boundaries using evolutionary criteria. While genealogical concordance principles have provided excellent guidance for recognizing species boundaries, in cases such as the *F. oxysporum* complex and *F. solani* species complex, non-concordance of gene genealogies due to possible unorthodox evolutionary patterns have been confounding factors. In addition, complete genome sequences of members of these groups and associated experimental results have led to hypotheses that large portions of their genomes have origins outside *Fusarium*, and support the potential for horizontal gene transfer. Can genealogical concordance principles be utilized to recognize species boundaries in a high background of interspecific gene exchange? Can signatures of speciation events be identified from complete genome sequences in such a background? In this talk, I will develop a framework for addressing these basic questions, and propose some approaches that may allow species recognition in groups that show dynamic genome evolutionary patterns.

***Cladosporium*: Current concepts, diversity, and taxonomy**
F. DUGAN (1)
(1) USDA-ARS WRPIS, Pullman, WA, U.S.A.
Phytopathology 102:S4.162

By dominance of aerobiota and on plants and in soil, members of the genus *Cladosporium* (anamorphic *Davidiella*) are collectively amongst the most common eukaryotic life forms. Although G.A. De Vries (1952) delimited 23 taxa and M.B. Ellis (1971, 1976) 43 taxa within *Cladosporium*, the most well known guides to fungi in air, food, seed, and soil, as well as guides to fungi from veterinary or clinical settings, have usually provided descriptions for 2-4 species, most commonly *C. cladosporioides*, *C. herbarum*, and *C. sphaerospermum*. Some contemporary, licensed technology for molecular diagnosis of molds also uses only these three species names. However, these three species are best regarded as species complexes, each encompassing the taxon *sensu stricto* plus several to numerous additional named species, and sometimes with multiple distinct, but as yet un-named, clades. Internal transcribed spacer (ITS) regions are insufficient for delineation of species in *Cladosporium*, although species complexes are reasonably identified by ITS and individual species by multi-gene topologies. Well over 700 species names have been published since 1816. Determination of correct names for given taxa, and segregation of *Cladosporium*-like taxa, are on-going but sometimes impeded by absence or condition of type material.

Comparative genomics and bioinformatic tools for studying evolution and speciation in fungi
J. E. STAJICH (1), T. J. Poorten (2), T. Y. James (3), D. Rodriguez (4), D. Ilut (4), K. Zamudio (4), E. B. Rosenblum (5)
(1) University of California-Riverside, Riverside, CA, U.S.A.; (2) University of Idaho, Moscow, ID, U.S.A.; (3) University of Michigan, Ann Arbor, MI, U.S.A.; (4) Cornell University, Ithaca, NY, U.S.A.; (5) University of California-Berkeley, Berkeley, CA, U.S.A.
Phytopathology 102:S4.162

The patterns of genome-wide variation from population genomic studies can be used to identify the basis for adaptation, dispersal range, and degree of hybridization among strains. *Batrachochytrium dendrobatidis* is a diploid chytrid fungus and an amphibian pathogen. It is currently one of the most important emerging invasive disease affecting biodiversity of frogs worldwide. Using whole genome sequencing we can extract patterns of population genomic to test the hypothesis that the fungus is cryptically sexual and undergoing recombination, or that the diploid represents an asexual lineage that undergoes a parasexual or other chromosome copy number based changes to shuffle genetic content. Further comparisons among closely related species were used to polarize changes in the genome focus on likely recent adaptations that are specific to the pathogen. Using the map of polymorphisms from genome sequencing and genotyping, comparative genomics for gene order conservation comparisons, and phylogenetic approaches, we can identify loci that adhere to and violate expectations for genome evolution and indicate targets of selection or related to rearrangements that have impacted compatibility between strains or species. Tools for bioinformatics and databases and software to enable these comparisons for enabling these studies and recently developed approaches will be demonstrated.

Right of the Boom: Deciding to Act, React, or Let Go in a Fluid Data Environment

Even when data are fluid a decision must be made
P. H. BERGER (1), L. G. Brown (1)
(1) USDA-APHIS-PPQ-CPHST, Raleigh, NC, U.S.A.
Phytopathology 102:S4.163

Regulatory agencies respond to the introduction of quarantine diseases in a complex and often rapidly changing environment. Biology of pathogen-host relationships, technology adoption, disease management practices, risk perceptions, risk aversion strategies, resources, and economics all play a role when deciding on the response that occurs to the Right of Boom! In the early stage of an outbreak, information is typically fluid and frequently very limited, conditions are dynamic, and external influences (political, regulatory, scientific, and industry) can change depending on authorities, resources, and relationships with each other and to the situation in question. While much depends on the stage of an emergency in progress, Plant Protection and Quarantine has developed a generic framework for decision making to increase the likelihood that responses will be consistent and predictable. In the very early stages of an emergency, however, decisions can be influenced by perceptions or other factors rather than analytically sound principles. The goal here is to explore how information flows and the fundamental structure for a regulatory support framework that adapts deliberately and predictably to a fluid data environment.

Use of law enforcement indicators and warning to prevent and respond to a crime
M. M. KREITNER (1), L. Lee (1)
(1) FBI, Washington, DC, U.S.A.
Phytopathology 102:S4.163

As part of the FBI's top priority in investigating terrorism, the FBI investigates suspected terrorist attacks or attempted attacks on US food or agriculture. These investigations involve potential indicators that develop from a joint partnership with other federal, state, and local partners, the industry, public health, and academia. Discussion will include potential targets, groups or individuals that represent this threat, why the food and agriculture sector would be targeted, where information could be researched, threat agents of concern, and the impact this threat would have if an agroterrorism attack were to occur.

The role of epidemiology research in shaping regulatory plant pathology
J. J. MAROIS (1)
(1) University of Florida, Quincy, FL, U.S.A.
Phytopathology 102:S4.163

Regulatory actions in plant pathology are designed to minimize risk to trade, food security and economic vitality. Understanding the epidemiological aspects of a pathosystems allows regulatory agencies to respond in a scientific manner after an event occurs (Right of Bomb). Additionally, the use of science-informed decisions provide increased accountability for the policy regulatory agencies as response strategies are determined, and greater likelihood that mitigation efforts will be successful. For example, after several decades of Left of Bomb activities investigating the potential impact of soybean rust in North America, soybean rust was discovered in the U.S. in late 2004. During the 2004-2005 off season, a series of organized responses allowed for a coordinated effort to quickly register fungicides under Section 18 and Section 3 guidelines so that the soybean industry had the appropriate tools in place to combat the disease in the 2005 season. At the same time, a national *ipm*PIPE website was developed that conveyed regularly updated distribution information from sentinel plots established for the most part by the land grant universities and funded by USDA Risk Management Agency. This critical epidemiological data addressing the spread of the disease was credited by the US Government Accountability Office of saving the soybean industry up to $299,000,000 in reduced fungicide applications in 2005 alone. Other pathosystems will be discussed as well.

From boom to busted: Trade concerns and disputes under the WTO's SPS Agreement
L. M. PEARSON (1)
(1) Imperial College-London, London, United Kingdom
Phytopathology 102:S4.163

Every year disease outbreaks, of varying intensity, occur throughout the world from both unpredictable occurrences and known underlying risks. Many risks are exacerbated by globalization and the increasing volume of international trade. Emergency measures taken to control a problem and subsequent regulations established to prevent future issues must effectively deal with the risks based on the best available science under uncertainty. Despite these efforts, conflicts among trading partners still arise. This paper analyzes all Specific Trade Concerns citing the Sanitary and Phytosanitary (SPS) Agreement of the World Trade Organization (WTO) from 1995 to 2011. There are 327 concerns raised of which 81 have a primary concern regarding plant health regulations. Analysis will show the common types of dispute, countries involved, most contested rule violation, and the impact of trade patterns on dispute likelihood. A framework is presented to aid in regulatory design that considers international commitments in light of the patterns of dispute.

Making and implementing program decisions in regulatory plant pathology
T. S. SCHUBERT (1)
(1) Florida Department of Agriculture & Consumer Services, Gainesville, FL, U.S.A.
Phytopathology 102:S4.163

Regulatory plant pathology (Rpp) strives to modify human behavior as it relates to plant health. It can assume many forms besides the obvious quarantine in the quest to minimize pest damage. Major goals are primarily to prevent or delay infection, or secondarily to minimize the spread and impact of pests as much as possible. Combinations of pure Rpp with other means of plant disease management are common. Rpp problems are usually urgent due to the dispersal potential of the pest, the brief window of economic opportunity and the limited useful life of the commodity. Such conditions preclude long, thoughtful decision-making, and call for speedy responses based on information in hand. Anticipating and preparing for potential incursions is fundamental, but adaptations and adjustments on the fly are frequently required. Phytopathologists naturally tend to presume that biological scientific criteria for decision-making are the most important or even the exclusive criteria, and may be dismayed to learn that economics, ecology, logistics, politics, public relations and legal matters also have a say in the decision process. Subordination of biological components to these other criteria should be expected under certain circumstances. Advocating for the biological science aspects in the combined arena is vital. Rpp practitioners would benefit from more direct interaction, interdisciplinary training and cooperation in these other criteria to optimize regulatory decisions and implementation.

A case-based analysis of information sources, sinks, and loops in regulatory plant pathology programs
N. MCROBERTS (1), P. S. Ojiambo (2), G. Hughes (3)
(1) University of California-Davis, Davis, CA, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.; (3) Scottish Agricultural College, Edinburgh, United Kingdom
Phytopathology 102:S4.163

The collection, meaning, quality and use, of information are major themes in the presentations during the Left of Boom and Right of Boom symposia. Formal information theory has not been widely used in plant pathology, but nonetheless impressive progress has been made in understanding what needs

to be known to assess risks from new disease outbreaks and how to use knowledge to manage outbreaks that do occur. Notwithstanding this hard earned empirical advance in methodology it is instructive to apply simple elements of information theory to typical decision situations in regulatory plant pathology in search of further gains and to identify generic concepts. We review the concepts of information entropy, channel capacity and relative entropy in the context of networks of decision makers who are collectively attempting to manage uncertain situations. To illustrate possible applications for these information theoretic concepts we use a re-analysis of past decision-making problems in which several symposium participants were involved. Some of the individual information theoretic statistics we discuss are relevant to issues raised in other papers in the symposia and we refer to these where possible.

Molecular/Cellular/Plant-Microbe Interactions

Genetics, Genomics, and Proteomics Approaches to Elucidate Arthropod-Vector Specificity

The effect of temperature on '*Candidatus* Liberibacter solanacearum' gene expression

T. W. FISHER (1), R. He (2), J. Munyaneza (3), J. Crosslin (4), J. K. Brown (1)
(1) University of Arizona, Tucson, AZ, U.S.A.; (2) Washington State University, Pullman, WA, U.S.A.; (3) USDA-ARS YARL, Wapato, WA, U.S.A.; (4) USDA-ARS YARL, Prosser, WA, U.S.A.
Phytopathology 102:S4.164

Zebra chip is a devastating disease of potato. The putative causal agent, '*Candidatus* Liberibacter solanacearum'(Lso), is a fastidious bacterium transmitted by the potato psyllid *Bactericera cockerelli* Sulc. Little is known about this complicated vector-pathogen relationship, but temperature has been shown to significantly affect the progression of ZC disease development. In this study, novel RNA sequencing (RNA-Seq) technologies were used to monitor infected and uninfected potato psyllid gene expression changes reared at different temperatures. The overall pattern observed for psyllid and Lso gene expression at two temperature extremes (18 and 30C), compared to the moderate or 'baseline' temperate (24C, where potato psyllids have historically performed optimally) was analyzed with respect to the presence and absence of Lso infection. The results indicate that temperature has a profound effect on psyllid physiology, and also to some extent (as is possible to determine) on Lso gene expression owing to some bacterial transcripts inadvertently carried over during RNA isolation. Preliminary comparisons among Lso infected psyllids at the three temperatures tested indicate that some of the misexpressed genes are involved in psyllid development and in Lso pathogenesis.

Host switching in the vector-borne plant pathogen *Xylella fastidiosa*

R. ALMEIDA (1)
(1) University of California-Berkeley, Berkeley, CA, U.S.A.
Phytopathology 102:S4.164

Insect-borne plant pathogens often have complex life histories. One important question for many of these pathogens is how to regulate the expression of genes required for colonization of plant and insect hosts. Another essential question is how to prepare for the switch from one host to another. The plant and insect colonizer *Xylella fastidiosa*, a bacterium that requires insect vectors to disperse among immobile host plants, is an ideal model system to address such questions. In insects and plants *X. fastidiosa* occurs in distinct phenotypic states that are controlled by a density-dependent signal (cell-cell signaling) and environmental cues (host polysaccharides). Because *X. fastidiosa* traits that are involved in plant colonization are incompatible with insect colonization, and vice-versa, transitioning between hosts represents an abrupt and absolute environmental change. Experimental data with this system will be used to highlight broader questions in the field.

Functional transcriptomics of *Begomovirus*-whitefly transmission

J. K. BROWN (1)
(1) The University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.164

Emergent plant viruses in the genus *Begomovirus* are transmitted by the whitefly *Bemisia tabaci* sibling species group, resulting in crop damage, worldwide. To gain insights into the molecular and cellular basis of whitefly-mediated begomovirus specificity, and to identify whitefly effector molecules involved in transmission we employed two complementary approaches, proteomics and transcriptomics. The partial proteome was determined by multidimensional chromatography and tandem mass spectrometric LC-LC-MS/MS analysis of whitefly adults and extirpated guts. To aid in functional genomics analysis and the development of a search library for protein identification we determined the transcriptome for adult whiteflies and the gut of the AZ-B biotype of *B. tabaci*. Twelve MudPIT runs yielded 597 unique proteins. Approximately 500 unique proteins were identified from whitefly adults, while 400 were identified in gut samples. Proteins were identified by searching translated, annotated ESTs from well-studied insects (GenBank) and the whitefly transcriptome (19% annotatable), and proteins in the Insecta database. The greatest numbers of hits were to pea aphid, fruit fly, and then to body louse. Real-time PCR amplification of selected whitefly genes revealed differential expression in time-course AAP studies comparing viruliferous (SLCV) with nonviruliferous whiteflies suggestive of putative effector functions. [Note: The JK Brown, L Breci, J Crowe, D Gang, and C Soderlund teams contributed equally to this research].

Using proteomics and mass spectrometry to explore the dynamic virus-vector interface

M. L. CILIA (1)
(1) USDA-ARS, Ithaca, NY, U.S.A.
Phytopathology 102:S4.164

A majority of plant viruses and a large number of important animal viruses are transmitted by insect vectors. Nearly all insect-transmitted animal viruses are internalized and circulate in their insect vectors, while plant viruses are divided between those that are carried on the cuticle linings of mouthparts and foreguts and those that circulate in their vectors. Members of the Luteoviridae are economically important viruses in staple food crops and are retained in the phloem of host plants. Phloem-retention facilitates circulative transmission by aphid vectors. This presentation will highlight our efforts to develop and apply advanced proteomics technologies to enable us to explore the dynamic virus-vector interface. Several examples of proteomics data will be discussed to illustrate the power of these technologies to further our basic understanding of the molecular pathways involved in circulative transmission in plants and aphids and the excellent agreement of our data with previously published studies on the biology of circulative transmission. Finally, examples from our data will also be presented to show how proteomics technologies can enable us to develop novel strategies that disrupt virus movement within and between hosts.

Comparative functional genomics to elucidate psyllid-'*Ca.* Liberibacter asiaticus' and solanacearum interactions

M. VYAS (1), T. Fisher (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 102:S4.164

The Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama and the potato psyllid (PoP) *Bactericerca cockerelli* Sulc. are vectors of one or several '*Candidatus* Liberibacter' species that infect citrus and solanaceous crops, respectively. ACP transmits '*Ca*. L. asiaticus', the causal agent of huanglongbing, or greening disease of citrus. PoP transmits '*Ca.* L. solanacearum', the pathogen associated with zebra chip disease of potato and vein-greening disease of tomato. To elucidate genes involved in pathogen infection of the psyllid host and psyllid-mediated transmission, we have employed comparative transcriptome profiling using infected and uninfected ACP and PoP nymphal and adult instars. Comparative *in silico* expression analysis within and between both psyllid species revealed conserved as well as unique transcripts. A number of genes involved in psyllid nutritional and innate immune system pathways were differentially expressed in infected and '*Ca*. Liberibacter'-free psyllids, making them candidates for functional analyses. Also gene expression profiles varied between infected adult-nymph species pairs, suggesting different cues may operate at different developmental stages of '*Ca.* Liberibacter' spp. psyllid pathogenesis. Transcript profiling for ACP and PoP vector-'*Ca.* Liberibacter' complexes will aid in elucidating and functionally characterizing genes essential for '*Ca*. Liberibacter' infection and pathogenesis of the psyllid host, and for psyllid-mediated transmission.

Pathogen Effectors and Host Targets

Hotspots in viral siRNA accumulation in maize
V. VANCE (1), S. Mlotshwa (1), C. Johnson (2), A. Wahba Foreman (1), G. Pruss (1), V. Sundaresan (2), K. Scheets (3), L. Bowman (1)
(1) University of South Carolina, Columbia, SC, U.S.A.; (2) University of California-Davis, Davis, CA, U.S.A.; (3) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 102:S4.165

RNA silencing is a sequence-specific RNA degradation mechanism that serves as an antiviral defense pathway in plants. Most plant viruses have single stranded RNA genomes and replicate via double stranded RNA (dsRNA) replication intermediates. The viral dsRNA triggers antiviral silencing in the host. It is processed by Dicer-like ribonucleases to produce short interfering RNAs (siRNAs) that incorporate into a RNA-induced silencing complex (RISC). Within RISC, the viral siRNA acts as a guide to direct the complex to complementary target RNAs, which are then destroyed. In this way, viruses provide the molecular tools (siRNAs) that lead to their own destruction, providing a potent and specific antiviral defense. Here we report an analysis of the population of viral siRNAs that accumulate during infection of maize with three different viruses. In each case, we find that viral siRNAs comprise a large proportion of the total small RNAs in infected cells and that viral siRNAs are generated along both strands of the entire genome. However, the analysis identified a few regions of the viral genome that generated very high levels of siRNAs. The characteristics of these "hotspot" viral siRNAs will be discussed. The data raise the intriguing possibility that these abundant viral siRNAs mediate an additional level of antiviral silencing by targeting host genes that are required for efficient viral replication.

The fungal effector AvrPiz-t suppresses host innate immunity by targeting the RING finger E3 ligases APIP6 and APIP10 in rice
C. PARK (1), S. Chen (1), G. Shirsekar (1), B. Zhou (2), C. Khang (3), P. Songkumarn (1), M. Bellizzi (1), Y. Ning (4), B. Valent (3), G. Wang (1)
(1) The Ohio State University, Columbus, OH, U.S.A.; (2) Zhejiang Academy of Agricultural Sciences, Hangzhou, Peoples Republic of China; (3) The Kansas State University, Manhattan, KS, U.S.A.; (4) Chinese Academy of Agricultural Sciences, Beijing, Peoples Republic of China
Phytopathology 102:S4.165

Although the function of effector proteins of plant bacteria and oomycete pathogens has been elucidated in the recent years, the information for plant fungal effectors is still lacking. Here we showed that the avirulence effector AvrPiz-t from the rice blast fungus *Magnaporthe oryzae* preferentially accumulates in the biotrophic interfacial complex (BIC), and is translocated into rice cells. Ectopic expression of *AvrPiz-t* in transgenic rice causes suppression of the flg22- and chitin-induced reactive oxygen species (ROS) generation and enhances susceptibility to *M. oryzae*, indicating that AvrPiz-t has virulence function to suppress the PAMP triggered immunity (PTI) in rice. Interaction analyses show that AvrPiz-t suppresses the E3 ligase activity of two rice RING E3 ligases, APIP6 and APIP10, and in return, the two E3 ligases ubiquitinate AvrPiz-t *in vitro*. We also found that AvrPiz-t promotes the degradation of APIP6 and APIP10 *in vivo*. Silencing of *APIP6* and *APIP10* in the non-*Piz-t* background leads to a significant reduction of flg22-induced ROS generation, suppression of defense-related gene expression and enhanced susceptibility against *M. oryzae*. Interestingly, silencing of *APIP10* in the *Piz-t* background causes strong cell death and a significant accumulation of the Piz-t protein. Taken together, our results demonstrate that AvrPiz-t suppresses the host innate immunity through manipulating the two positive PTI regulators APIP6 and APIP10 in the non-*Piz-t* plants. In contrast, APIP10 degradation by AvrPiz-t in the *Piz-t* background leads to the accumulation of Piz-t and triggers a battery of defense responses to prevent the rice blast infection.

Effector promoters play a major role in delivery of *Magnaporthe oryzae* effectors into living rice cells
C. KHANG (1), B. Valent (2)
(1) University of Georgia, Athens, GA, U.S.A.; (2) The Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 102:S4.165

Magnaporthe oryzae secretes effectors to cause the globally important rice blast disease. Some effectors that accumulate preferentially in Biotrophic Interfacial Complexes (BICs) enter the host cytoplasm across host-derived membrane, but other effectors that are not BIC-localized remain within the membrane. To determine gene sequences controlling effector BIC-targeting and host translocation, we analyzed transgenic *M. oryzae* expressing chimeric genes between PWL2 (BIC-localized and host translocated) and BAS4 (nonBIC-localized and host non-translocated). The in planta localization patterns for the mCherry fusion proteins were determined by confocal microscopy. We have found that Pwl2p, when expressed under control of the BAS4 upstream regulatory sequence (URS), showed impaired BIC localization, host translocation, and avirulence activity. On the contrary, Bas4p, when expressed under control of the PWL2 URS, gained host translocation. We have also determined that reciprocal swapping of 5' UTR between PWL2 and BAS4 genes did not alter respective protein localization specificity. Thus, effector promoters play the critical role in determining the preferential BIC-localization and delivery of effectors into host cells. Consistent to this, PWL2 is strongly upregulated in the BIC-associated hyphal cells. New quantitative assays for effector gene expression levels and protein delivery into host cells will also be presented.

***Pseudomonas syringae* type III effectors: Evolution, distribution, and host targets of a bacterial Monkey Wrench Gang**
M. LINDEBERG (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 102:S4.165

Over 30 closed and draft *P. syringae* genome sequences are now publically available with 57 Type III effector families confirmed and likely representing the near-complete super-repertoire for the species. The type III effectors have proven to be important tools for deconvoluting the intricate network of defense responses in diverse host plants, and the accumulated data on their activities have revealed the central role they play in suppression of the 2-layered plant defense response composed of PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). A core set of effectors is widely distributed among the sequenced strains and acts by disrupting vesicular trafficking critical to effective PTI, while other less well-conserved effectors inhibit various stages in PAMP perception through interference with signaling kinases. As host plants have evolved to recognize and trigger immunity in response to PTI-suppressing effectors, additional effectors have been deployed by the pathogen to disrupt ETI, contributing to an endless evolutionary cycle of detection and evasion. Insights into coordinated functional roles of individual effectors are revealed through analysis of effector family distribution among the major *P. syringae* clades and by disassembly and reassembly of the effector repertoire of *P. syringae* pv *tomato* DC3000. Distribution of effectors among sequenced genomes and documentation of their cellular locations, molecular functions, and involvement in diverse biological processes as captured using Gene Ontology Annotation can be found at the PPI Website (http://www.pseudomonas-syringae.org/).

Functional characterization of the conserved modular domains found in the oomycete RXLR effector superfamily
S. D. KALE (1)
(1) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 102:S4.165

The Oomycota consists of a diverse grouping of predominately plant and animal pathogens, evolutionary distinct from fungi yet parallel in the mechanism of infection. A number of plant and animal pathogenic oomycetes deploy small-secreted proteins, known commonly as effectors, to modulate intrinsic host functions and defense responses. The genus *Phytophthora*, a key collection in the Oomycota, consists of several infamous plant pathogens that adversely affect natural ecosystems and domestic agriculture. Genome sequencing of several *Phytophthora* species has in part lead to the discovery of a large super-family of putative effector proteins. The hallmark of this super-family is a highly conserved N-terminal RXLR motif. The RXLR motif from several oomycete effectors have been shown to facilitate translocation into host cells in a pathogen independent manner via phosphatidylinositiol-3-phosphate (PtdIns-3-P) receptor mediated endocyotsis. Interestingly, several plant mutualistic and pathogenic fungi also deploy RXLR-like effectors that enter via a PtdIns-3-P receptor mediated endocytosis pathway. Here we present a detailed NMR study of the RXLR effector Avh5 interactions with the head group of PtdIns-3-P as well as several functional assays characterizing the respective C-terminal domains of *Phytophthora sojae* effectors Avr1b and Avh5 to further elucidate the mechanism of effector cell entry and intracellular function.

Cyst nematode effectors and their targets
T. J. BAUM (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 102:S4.165

Plant-parasitic cyst nematodes secrete effector proteins through their stylets into host root cells. These proteins are crucial for the development of specialized feeding sites (syncytia) and for mediating susceptibility through the suppression of plant defenses and manipulation of a variety of host factors. Functional characterization of effectors revealed the mechanisms by which cyst nematodes modify cell wall structure, negate host defenses, alter auxin and polyamine signaling and change plant gene transcription. Of particular interest is an altered expression of microRNA396 as a potential consequence of effector activity. microRNA396 was determined to be a key developmental regulator of syncytium formation. This microRNA post-transcriptionally regulates the expression of the Growth Regulating Factor 1 (GRF1) and GRF3 transcription factor genes in the syncytium. The miR396-GRF1/GRF3 regulatory unit is involved in the initiation of the syncytium induction/formation phase and then the transition into the maintenance phase. Genome-wide gene expression profiling revealed major roles of this microRNA396 regulatory unit in controlling host plant gene expression, explaining about 50% of the gene expression changes described in the syncytium. Ongoing functional characterizations of nematode effectors promise to provide an understanding of how plant-parasitic nematodes drive normal root cells toward novel developmental pathways required for successful parasitism.

Professionalism/Outreach

Everything a Scientist Should Know About Politics, Funding, and Public Opinion

Policy 101: A not-so-boring look at how government works
K. EVERSOLE (1)
(1) Eversole Associates, Bethesda, MD, U.S.A.
Phytopathology 102:S4.166

In an increasingly bipartisan political climate filled with repeated stalemates over the budget policy of the United States and other countries, it is reasonable to ponder whether scientists can have an impact on governmental policies or on science, education, and extension funding decisions. Whether one is "inside the beltway" or beyond the suburbs of Washington, it is hard to not become disillusioned with the political process. Despite the disenchantment, with a little knowledge and the right tools, it remains possible for ordinary citizens and scientists to affect change in policies and funding. This presentation will provide a brief refresher on how laws are made, with a focus on the steps that lead to appropriation of funds for scientific research. The inner workings of the United States federal government will be viewed from an insider's perspective. Importantly, a constructive discussion about engagement in the political process and the tools needed for engagement will be presented.

International funding cooperation
D. BECK (1)
(1) National Science Foundation, Arlington, VA, U.S.A.
Phytopathology 102:S4.166

Within an increasingly global context for research collaboration, science funding agencies in the U.S. and their international counterparts are employing a variety of different mechanisms to allocate resources for such collaboration, to facilitate the establishment of new scientific partnerships, to engage the next generation of scientists in international research, and to leverage resources for greater impact. This presentation will provide an illustrative overview of approaches being used by science funding agencies to develop such cooperative programs. And, it will describe specific initiatives of the National Science Foundation that are intended to advance opportunities for international collaboration in science, engineering, and education. International funding cooperation.

Getting engaged in public policy work is easy
M. L. LEWIS IVEY (1)
(1) The Ohio State University, Medina, OH, U.S.A.
Phytopathology 102:S4.166

Public policy engagement (PPE), within the realm of plant pathology, is a mechanism to define a framework of ideas and values that can be adopted by government to positively impact regulatory measures, funding priorities and laws that address specific issues or needs of members of The American Phytopathological Society (APS) and their stakeholders. Contrary to popular belief, PPE extends beyond lobbying and involves a diverse continuum of activities. Whether you are engaging media–social or otherwise, educating stakeholders, disseminating research, sharing resources, building networks, or supporting grassroots organizations you are working along a range of activities that can influenceo pinions and promote ideas that affect public policy. One's level of involvement in public policy is a matter of choice and can be as simple as writing a letter or sending a "tweet" to a decision maker or as involved as becoming an APS-Public Policy Board intern or board member or speaking in Washington. Public policy activities can occur on a local, state or national scale and engaging decisions makers within each division is equally important. Whether one dives right in or eases in slowly, engaging in public policy activities provides one with the opportunity to raise the profile of issues that are important to plant pathologists, stakeholders and the community as a whole.

Scientists: Almost as prestigious as firefighters
A. RECORDS (1)
(1) APS Policy Fellow, Silver Spring, MD, U.S.A.
Phytopathology 102:S4.166

How do you view the public? Surveys indicate that most scientists view the public as homogeneous, undereducated, and misled – at least when it comes to science. How does the public view scientists? The answer may come as a surprise. This presentation will explore misconceptions of both scientists and the public, while addressing the influence of these views on science policy. The relationships between science, the public, and policymakers will be reviewed from modern-day, historical, and global perspectives. The role of scientist as advocate will be discussed.

Practice and Management of Microbial and Plant Germplasm Collections

The National Plant Germplasm System (NPGS) and GRIN-Global
C. A. GARDNER (1)
(1) USDA-ARS, Ames, IA, U.S.A.
Phytopathology 102:S4.166

The National Plant Germplasm System (NPGS) is a cooperative effort by public and private organizations to preserve plant genetic diversity. Federal and State personnel at 20 sites are responsible for 547,000 unique accessions of a wide array of plant genetic resources representing 14,325 species and 2,355 genera. Included are the National Center for Genetic Resource Preservation, which conducts preservation research and provides back-up for all collections; 18 active sites responsible for seed- and clonally-propagated species; and the National Germplasm Resources Laboratory, responsible for the Germplasm Resource Information Network, a system for collection and dissemination of germplasm information. Examples of integrated plant-associated microbial research with genebank collections will be presented. Collections of plant-associated microbes are very important; no collection is prepared to accept and maintain the variety of material being isolated and characterized by researchers. The NPGS, in partnership with the Global Crop Diversity Trust and Bioversity International, developed the GRIN-Global System to provide a database flexible, multi-lingual, license-free information management system to meet global plant genebank needs. Released internationally in 2011, NPGS staff members are working to implement GRIN-Global. This system should be assessed for its potential value for managing information associated with other biological collections, including microbes.

Management of germplasm collections and associated data via informatics tools: Opportunities and challenges
S. KANG (1)
(1) Department of Plant Pathology, Penn State University, University Park, PA, U.S.A.
Phytopathology 102:S4.166

Germplasm collections often connect past, present, and future research endeavors by preserving key research specimens and making them widely available. They also serve as a critical reference resource for taxonomic

research, preserve specimens from biodiversity surveys, and supply materials for various practical applications. To fully realize the value of germplasm collections, we should go beyond mere physical preservation. These collections must be connected intimately to active research programs and their associated expert personnel and data resources so as to ensure their continuous growth and integrity. Since many different types of data are generated using germplasm collections, resulting data should be organized in a format that is readily available and should also be linked to individual specimens. Given the need for maintaining and integrating diverse and large datasets to support research communities, it is essential to establish a cohesive and user-friendly cyberinfrastructure supporting the management of collections. Using a few projects as examples, I will present several key issues in creating value-added germplasm collections via the use of informatics tools.

Plant germplasm curation—Best practices

D. ELLIS (1)
(1) USDA-ARS, National Center for Genetic Resources Preservation, Fort Collins, CO, U.S.A.
Phytopathology 102:S4.167

The curator of any collection of living organisms, be they microbe, animal, insect or plant, has the ultimate responsibility for the maintenance of the genetic true-to-type that is inherent in each individual accession in their collection. With plants, field regenerations are required for larger collections, and challenges with plant size, reproductive system, pollination and maintenance of genetic integrity are dealt with using isolation tents, individually introduced pollinators, hand pollination and hand harvesting. Reproductive system and effective population size are also important considerations. Barcoding at all stages from seedbank to field to harvesting to cleaning to shipping aids in tracking accessions. These are not museum collections, consequently access to information is critical for users to decide which accessions to order, test and utilize, and of primary concern to derive maximum benefit from the collections. Transparent, web-based databases with easy-to-find, relevant accession characterizations, both phenotypic and molecular, greatly facilitate use. Finally, one cannot over-emphasize the crucial importance of backing-up collections in a geographically-distant secure site; if the active collection suffers a catastrophic loss, a complete set of accessions is available for current use and for future generations.

Experience with best practice guidelines for microbial germplasm repositories at the Fungal Genetics Stock Center

K. MCCLUSKEY (1), A. Wiest (1), R. Schnittker (1)
(1) University of Missouri-Kansas City, School of Biological Sciences, Kansas City, MO, U.S.A.
Phytopathology 102:S4.167

The Organization for Economic Cooperation and Development published best practice guidelines for Biological Resource Centers in 2007. Similarly, the International Society for Environmental and Biological Repositories publishes best practice guidelines with regular updates. While the latter is more appropriate for collections of tissue specimens, and neither deal with herbaria or natural history collections, these together provide a virtual step-by-step guide for the management of ex situ microbial germplasm repositories. Because neither guideline gives detailed instructions on how to implement best practices it is valuable to share practices from existing collections. The Fungal Genetics Stock Center has over fifty years of experience with techniques and implements modern data and management practices. For example, the FGSC uses at least two formats to store every isolate, has an off-site back-up, works from established standard operating protocols, and has clear strain accession and archiving policies. Moreover, the FGSC has trained staff and appropriate facilities to carry out the operations of a biological resource center. These simple examples form the foundation of best practice guidelines for culture collections.

From culture collection to genetic resource centre: The Dutch approach

P. W. CROUS (1), G. J. Verkley (1)
(1) CBS Fungal Biodiversity Institute, Utrecht, Netherlands
Phytopathology 102:S4.167

The CBS Culture Collection (1904), is the largest public service collection for living fungi in the world. CBS and other established fungal collections harbour a great hidden diversity that, within a relatively small time frame, can most effectively be surveyed by a DNA barcoding approach. CBS has therefore acquired funding to generate DNA barcodes of its entire collection. Faced with a great diversity of undescribed and cryptic taxa, taxonomic types have the prominent function of anchoring existing species names. Taxonomic type sequence information is rare, because few types have to date been subjected to DNA sequencing. The barcode sequences of the more than 8000 ex-type cultures will be of tremendous value for fungal taxonomy and its user community. Furthermore, the CBS yeast collection holds almost 9000 strains including the 2240 ex-type strains of all described species. The resulting barcode data set will be an invaluable reference source for yeast research. For more than 100 years, strains entering the CBS collections have been identified based on state-of-art techniques at the time of accession. DNA barcoding the holdings will allow to update identifications and to recognize strains in need of further research, which will not only increase the value of the collection, but will aid the user community. The DNA barcoding approach and online database identification tool will also prove invaluable to confirm the identity of strains selected for whole genome analysis.

DNA barcoding and next-generation sequencing—Opportunities and challenges for reference biological collections

C. A. LEVESQUE (1)
(1) Agriculture & Agri-Food Canada, Ottawa, ON, Canada
Phytopathology 102:S4.167

With the advent of DNA barcoding and next generation sequencing, reference biological collections have never been as relevant and important. In Canada, significant investments are currently being made to process large numbers of accessions for barcoding and to sequence the genomes of high risk plant pathogens. DNA barcoding can be seen as horizontal genomics whereby one or a few short markers are sequenced by Sanger technology for a very large number of accessions. In order to have the “barcode” keyword, sequences in GenBank must be from an approved marker, must be from a properly vouchered specimen or strain, and must have electropherograms attached. The International Barcode of Life (iBOL) initiative is supporting such activity and receives a very significant portion of its funding from Genome Canada. There are currently over 250,000 genuine barcodes in GenBank and 1.5M in the database of iBOL. Detection of microorganisms can now be done by matching millions of sequences generated by new technologies to reference barcodes. The cost of whole genome sequencing is also coming down very rapidly, making it possible to sequence the genomes of all species of a genus. Maintenance and support of reference biological collections are essential in this new era of barcode and genome sequencing as they provide the foundation to a new rapidly evolving technology. Without the reference collections, the science coming from next generation sequencing is difficult to interpret or even irreproducible.