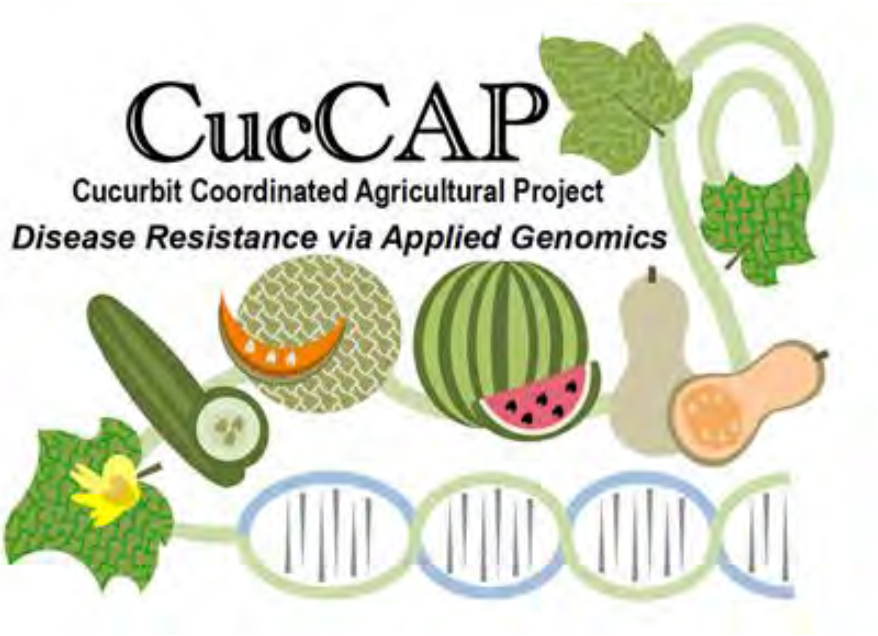
CucCAP:

Leveraging applied genomics to improve disease resistance in cucurbit crops



Annual CucCAP Team Meeting

June 4-5, 2020

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AGENDA

CucCAP team meeting – June 4-5, 2020

(All times listed are in U.S. Eastern, Daylight Savings Time)

Thursday, June 4

1:00-1:20 Arrival, welcome, introductions of participants
1:20-1:30 Overview of project progress, plans for meeting

Session I – Genomic Tools

Objective I: Develop genomic approaches and tools for cucurbit species
- Develop genomic and bioinformatic platforms for: genotyping by sequencing (GBS); sequence data processing and analysis; and genotype, phenotype and QTL databases
- Perform GBS analysis of PI collections and core populations of the four species to provide a community resource for genome wide association studies (GWAS) for current and future traits of interest
- [E/O] Provide access to cucurbit genomics tools and databases via the International Cucurbit Genome Initiative (ICuGI) website and genomics and bioinformatics workshops

1:30-1:45 Overview of progress: bioinformatics platforms and website, GBS data and analysis, publications (Fei, Reddy)
1:45-2:00 Status of core panels (seed stocks; resequencing; phenotyping)
watermelon (Levi)
melon (McCreight)
cucumber (Weng)
squash (Mazourek)

2:00-2:10 Questions/discussion

2:10-2:20 Break

Session II – Breeding for disease resistance

Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.
- Utilize genomic approaches to map resistance loci for key cucurbit diseases
- Develop and verify molecular markers for efficient trait selection and gene pyramiding
- Introgress resistances into advanced breeding lines
- [E/O] Provide web-based and face to face information via field trials, extension venues, and scientific meetings regarding breeding materials, markers, and breeding progress

2:20-3:10 **Watermelon**: Status for each disease
– where were we at the outset? Where are we now?
breeding lines, mapping/QTL, markers, introgression
Diseases: Fusarium, gummy stem blight, Phytophthora, powdery mildew, GCMMV, PRSV-W
(Levi, Kousik, Ling, McGregor, Wechter, Wehner)

3:10-3:20 Questions/discussion

3:20-3:30 Break

3:30-4:10 **Melon**: Status for each disease
– where were we at the outset? Where are we now?
breeding lines, mapping/QTL, markers, introgression
Diseases: powdery mildew, CMV, CYSDV, Fusarium
(McCreight, Kousik, Wechter, Wintermantel)

4:10-4:20 Questions/discussion

Friday, June 5

1:00-1:25 **Cucumber**: Status for each disease
– where were we at the outset? Where are we now?
breeding lines, mapping/QTL, markers, introgression
Diseases: downy mildew, Phytophthora
(Weng, Grumet, Wehner)

1:25-1:35 Questions/discussion

1:35-2:05 **Squash**: Status for each disease
– where were we at the outset? Where are we now?
breeding lines, mapping/QTL, markers, introgression
Diseases: PRSV-W, ZYMV, Phytophthora, powdery mildew
(Mazourek, Wessel-Beaver, Smart, Hausbeck)

2:05-2:15 Feedback/priorities from industry (include teleconference if needed with commodity reps)

2:15-2:25 Break

Session III – Disease control information and Economic impact

Obj. 3 Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.

- Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets
- Use a risk-based simulation model to analyze economic potential of the disease resistant cucurbit cultivars
- [E/O] Develop a centralized cucurbit disease website, including content in English and Spanish, providing information about diagnostics and links to disease clinics; disease control recommendations; disease alerts and links to forecasting tools

2:25-2:40 Socioeconomics team: Accomplishments
(Palma, Rivera)

2:40-3:15 Extension team: Accomplishments
(Schulthies, Hausbeck, Linares, Quesada, Smart, Lorscheider)

3:15-3:25 Questions/Discussion

3:25 Wrap up, final thoughts, thank yous!

CucCAP Team

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Stakeholder Advisory Board		
Organization	**Representative**	**Position**
Commodity Groups - Growers, Shippers, Processors, Marketing		
National Watermelon Promotion Board	Mark Arney	Executive Director, National Watermelon Promotion Board
National Watermelon Association	Robert Morrissey	Executive Director, National Watermelon Association
California Melon Research Board	Milas Russell	Chair Elect, California Melon Research Board President, Sandstone Melon Company
California Melon Research Board	Steve Smith	Chair, California Melon Research Board Co-Owner Turlock Fruit Company
Pickle Packers International	Brian Bursiek	Executive Vice President, Pickle Packers International
Swanson Pickles and Pickle Packers International	John Swanson	President Swanson Pickle Company; Research Board, Pickle Packers International
Martin Farms (squash grower, shipper)	Mitch Beyler	Partner, John B. Martin and Sons Farms, Inc.
Stony Brook Wholehearted Foods (squash processor)	Greg Woodworth	Founder, Stony Brook Wholehearted Foods
Seed Industry		
BASF	Jovan Djordjevic/ Suren Baliji/ Peter Kraan	Global R&D Lead, Melons and Watermelons,
HM Clause	Kishor Bhattarai Eric HOeft	Phytopathology Project Manager, HM Clause, Vegetable Seeds Division, Limagrain
Hollar Seed Company	Bruce Carle	Plant Breeder, Hollar Seed Company
Johnny's Selected Seeds	Rob Johnston/ Lindsay Wyatt	Chairman, Johnny's Selected Seeds Squash and Pumpkin Breeder
Bayer	Nischit Shetty	NAM Cucurbit Breeding Lead
Sakata Seeds	Jeff Zischke/ Nihat Guner	Director of Research, Vegetables, Sakata Seed
Syngenta Seeds Inc.	Matt Kinkade/ Sandhu Ajay	Team Lead, Watermelon Breeding Trait Project Lead, Fruity Crops

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CucCAP Project Objectives

Each objective includes integrated research and extension/outreach [E/O] approaches:

Obj. 1. Develop genomic approaches and tools for cucurbit species.

- Develop genomic and bioinformatic platforms for: genotyping by sequencing (GBS); sequence data processing and analysis; and genotype, phenotype and QTL databases
- Perform GBS analysis of PI collections and core populations of the four species to provide a community resource for genome wide association studies (GWAS) for current and future traits of interest
- [E/O] Provide access to cucurbit genomics tools and databases via the International Cucurbit Genome Initiative (ICuGI) website, and by genomics and bioinformatics workshops open to all members of the cucurbit scientific and breeding communities

Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.

- Utilize genomic approaches to map resistance loci for key cucurbit diseases
- Develop and verify molecular markers for efficient trait selection and gene pyramiding
- Introgress resistances into advanced breeding lines
- [E/O] Provide web-based and face to face information via field trials, extension venues, and scientific meetings regarding breeding materials, markers, and breeding progress

Obj. 3 Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.

- Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets
- Use a risk-based simulation model to analyze economic potential of the disease resistant cucurbit cultivars
- [E/O] Develop a centralized cucurbit disease website, including content in English and Spanish, providing information about diagnostics and links to disease clinics; disease control recommendations; disease alerts and links to forecasting tools

Disease priorities identified by the cucurbit industries:

Table 1. Major disease threats to cucurbit crop production as identified by cucurbit industry stakeholders.

Disease	Identified as commodity funding priority[a]	Also affects:
Downy mildew	cucumber	melon, watermelon, squash/pumpkin
Fusarium wilt	watermelon	melon, cucumber
Gummy stem blight	watermelon	melon, cucumber, squash/pumpkin
Phytophthora rot	cucumber, watermelon, squash/pumpkin	Melon
Powdery mildew	melon, watermelon, squash/pumpkin	Cucumber
Viruses (CMV[b], CYSDV[c], PRSV-W[d], CGMMV[e]	melon[b,c], watermelon[d,e]	cucumber[c,e], squash/pumpkin[b,d]

Project Structure – Team Organization

CucCAP Teams			
Team	**PD, Co-PDs and Co-PIs**		**Institution[a]**
	PD: Rebecca Grumet	(RG)	MSU
Watermelon	***Team Leader: Amnon Levi***	(AL)	ARS-SC
	Shaker Kousik	(SK)	ARS-SC
	Kai-Shu Ling	(KSL)	ARS-SC
	Cecilia McGregor	(CM)	UGA
	Lina Quesada	(LQ)	NCSU
	Pat Wechter	(PW)	ARS-SC
	Todd Wehner	(TW)	NCSU
Melon	***Team Leader: Jim McCreight***	(JM)	ARS-CA
	Shaker Kousik	(SK)	ARS-SC
	Pat Wechter	(PW)	ARS-SC
	Bill Wintermantel	(BW)	ARS-CA
Cucumber	***Co-PD, Team Leader: Yiqun Weng***	(YW)	ARS-WI
	Rebecca Grumet	(RG)	MSU
	Mary Hausbeck	(MH)	MSU
	Todd Wehner	(TW)	NCSU
Squash	***Team Leader: Michael Mazourek***	(MM)	CU
	Christine Smart	(CS)	CU
	Linda Wessel-Beaver	(LWB)	UPR
Genomics/bioinformatics	***Team Leader: Zhangjun Fei***	(ZF)	BTI
	Umesh Reddy	(UR)	WVSU
	Amnon Levi (watermelon)	(AL)	ARS-SC
	Mike Mazourek (squash)	(MM)	CU
	Pat Wechter (melon)	(PW)	ARS-SC
	Yiqun Weng (cucumber)	(YW)	ARS-WI
Socioeconomics	***Team Leader: Marco Palma***	(MP)	TAMU
	Louis Ribera	(LR)	TAMU
Extension/Outreach	***Team Leader: Jonathan Schultheis***	(JS)	NCSU
	Lina Quesada (watermelon)	(LQ)	NCSU
	Mary Hausbeck (cucumber)	(MH)	MSU
	Jim McCreight (melon)	(JM)	ARS-CA
	Angela Linares Ramírez	(ALR)	UPR
	Christine Smart (squash)	(CS)	CU
	Zhangjun Fei (bioinformatics)	(ZF)	CU

[a]Institution abbreviations: ARS-CA (Salinas), SC (Charleston), WI (Madison); BTI-Boyce Thompson Inst; CU-Cornell Univ; MSU-Michigan St Univ; NCSU-North Carolina St Univ; TAMU- Texas A&M Univ; UGA-Univ Georgia; UPR-Univ Puerto Rico; WVSU-West Virginia St Univ

SUMMARY OF CucCAP ACCOMPLISHMENTS

OBJECTIVES

The CucCAP project provided the opportunity for 21 research groups from 11 universities and USDA laboratories to collectively engage our expertise in plant breeding, genetics, genomics, bioinformatics, plant pathology, extension and economics to leverage applied genomics to improve disease resistance in cucurbit crops.

Our objectives were to:

1. Develop genomic and bioinformatics breeding tools for cucurbit crops
2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars
3. Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.

OVERVIEW of CucCAP ACCOMPLISHMENTS

- Developed state-of-art centralized databases, genomic and bioinformatic tools for cucurbit crops

-Genotyped by sequencing (GBS) USDA PI collections for watermelon, melon, cucumber, and squash, and developed genomically-informed core populations

-As appropriate to the crop-disease combination (*described below*), identified sources of disease resistance, characterized inheritance, identified QTL, developed markers for marker-assisted selection (MAS), released resistant breeding lines

-Developed a centralized, web-based resource providing information for cucurbit disease control

-Provided training opportunities for 12 post-docs and 30 graduate students (fully or partially supported by CucCAP) as well as an additional 12 post-docs, 26 graduate students, and 88 undergraduates in the areas of bioinformatics, genomics, plant breeding, plant pathology, extension, economics

-Published and presented:

104 *(125*)* refereed publications

144 extension publications, web-materials, or webinars

212 presentations at scientific conferences, universities

151 presentations in commodity-based venues

*(*125 includes additional related publications by CucCAP team, e.g., analysis of fruit quality traits facilitated by CucCAP genomic resources, reports of new cucurbit diseases or cucurbit cultural practices)*

ACTIVITIES, RESULTS, OUTPUTS

Activities, Results, Outputs by Objective

Objective 1: Genomic and bioinformatics breeding tools for cucurbit crops

A. Development of the Cucurbit Genomics Database (http://cucurbitgenomics.org). The CucCAP bioinformatics team developed the Tripal toolkit-based Cucurbit Genomics Database (CuGenDB) (http://cucurbitgenomics.org) (Zheng et al., 2019). This comprehensive, multifunctional genomic resource, which is continually being updated, contains all available genome and expressed sequence tag (EST) sequences, genetic maps, sequence annotation and transcriptome profiles for cucurbit species, and provides a central portal to store, mine, analyze, integrate and disseminate rapidly accumulating genomic and genetic data in cucurbits. The Cucurbit Genomics Database provides a critical resource for cucurbit genomics and breeding widely used by academic and industry researchers throughout the world.

In addition to key functions such as BLAST and genome browser capabilities, novel analytic capacities were developed, including 'RNA-Seq' modules for differential gene expression analyses facilitating comparisons among experiments, and a 'Synteny Viewer' to identify and visualize conserved syntenic regions within and across species. The synteny viewer will benefit our efforts to inquire whether QTL identified for resistance to a specific disease in one cucurbit crop can provide guidance for potentially relevant genomic regions in other crops. Several other genome databases have since incorporated 'Synteny Viewer', indicating the broad value of this tool.

B. ***Genetic characterization of the U.S. National Plant Germplasm System (NPGS) collections for cucumber, melon, watermelon, and squash.*** Germplasm collections are a critical resource for plant breeders. They are frequently the first place to turn for new sources of valuable traits, including disease resistance. Molecular genetic characterization allows us to evaluate the extent and nature of variation that exists within our collections to help manage and preserve diversity and better facilitate breeding efforts. For each of the cucurbit crops there are approximately 1000-2000 PIs in the U.S. collections

The CucCAP project performed genotyping by sequencing (GBS) to genetically characterize the full USDA NPGS PI collections for cucumber, melon, watermelon (Citrullus sp.), and squash (Cucurbita pepo). For each species, 24,000-48,000 filtered SNPs were identified with an average density of 1 SNP per 5-15 kb, providing approximately 3000 markers per chromosome. The GBS data and identified SNPs are freely available.

The SNP data allow for numerous analyses providing information about the genetic structure of the PI collections. Principal component analysis (PCA), K-means, and phylogenetic analysis provide

insight into evolutionary and domestication trajectories and relationships among the accessions (Wang et al., 2018; Wu et al., 2019). Understanding of the genetic relationships among accessions also informs breeders about relationships among sources of disease resistance.

C. ***Development of core populations as a community resource for genetic mapping.*** The genetic data from the PI collections for the four crops was used to identify a subset of ~300 accessions that capture >95% of the allelic diversity present within the collections. The selected accessions were supplemented with historical cultivars providing key horticultural and disease resistance traits. These functional panels provide a set of diverse lines, associated sequence data, SNP datasets and genetic maps for future genotypic and genome-wide association (GWAS) analysis of any traits of interest. The functional panels will be re-sequenced for high resolution mapping. Self-pollinations and seed multiplication are currently underway for each of the four crops. Re-sequencing has been initiated for cucumber.

While the resequencing data will ultimately provide higher resolution mapping, the GBS data allowed for preliminary GWAS results. In cucumber, regions associated with disease resistance were identified for anthracnose, downy mildew, gummy stem blight, and root knot nematodes (*Meloidogyne incognita* race 3) (Wang et al., 2018). In watermelon, QTL were identified for resistances to bacterial fruit blotch, powdery mildew race 2W, and the watermelon strain of *Papaya ringspot virus* (Wu et al., 2019).

Objective 2: Genomic-assisted breeding for disease resistance in cucurbit crops

CucCAP breeding efforts focused on the priority diseases identified by each industry through consultation with growers, shippers and processors as represented by commodity organizations and with seed companies with significant cucurbit breeding programs. The importance of these problems was underscored by research investments cucurbit stakeholder groups have made to combat these diseases.

A. Identify QTL for resistance, develop markers to facilitate marker assisted selection (MAS). For each crop and multiple diseases, progress has been made to identify QTL and develop markers. Molecular markers were developed for 12 crop-disease combinations; QTL for an 13 additional crop-disease combinations were mapped (Table 1). Several are already being used by the seed industry. Specific examples are provided by crop below in the following sections.

B. Introgress disease resistance into cucurbit crops. In concert with QTL identification and marker development, the four CucCAP crop teams (watermelon, melon, cucumber, squash) are developing and releasing breeding lines with resistances to the priority diseases. Outputs include - identification of new sources of resistance; release of breeding materials; analysis of inheritance of resistance; and incorporation of resistance into commercial breeding material.

Table 1. QTL identified and markers developed for cucurbit crop diseases					
Disease	**Crops**	**QTL**	**Markers/ Gene**	**Teams**	**Publications**
Alternaria	Melon	chr 10, 12		Wechter, Levi	Daley et al. 2017
Angular leaf spot	Cucumber	chr 5	STAYGREEN	Weng	Wang et al. 2018
Anthracnose	Cucumber	chr 5, 7	STAYGREEN	Weng, Wehner, Fei	Pan et al. 2018 Wang et al. 2018; Wang et al., 2019
Bacterial fruit blotch	Watermelon	multiple		Levi, Wechter, Wehner, Fei	Branham et al., 2019b; Wu et al 2019
CYSDV	Melon	chr 3,5	STS	Wintermantel, McCreight	
Downy mildew	Cucumber Watermelon	chr 1,4,5 In progress	SSR, STAYGREEN	Weng, Wehner Wechter	Wang et al. 2018, 2019
Fusarium wilt	Watermelon Melon	R1. chr 9 (C. amarus) R2. chr 1, 9 (C. amarus) R1. chr1 (C. lanatus) R1. chr 2,7,11	KASP KASP KASP KASP	Levi, Wechter, McGregor Wechter, Levi	Meru and McGregor 2016; Branham et al. 2019a Branham et al. 2017, 2020 Fall et al. 2018; Branham et al. 2018b Branham et al. 2018a
Gummy stem blight	Watermelon Cucumber	multiple chr 2,5,7	In progress	McGregor Wehner, Fei	Wang et al.,2018
Phytophthora fruit rot	Cucumber Watermelon Squash	ARR chr3 Young fruit chr5 In progress In progress		Grumet Kousik Smart	
Powdery mildew	Squash Cucumber Melon Watermelon	chr. 10 chr 2,5,6 chr 4,5,10,12 chr 2	CAPS SSR ClaPMR2	Mazourek, Weng, Wehner Wechter, Kousik, McCreight Kousik, Wechter, Levi	Holdsworth et al. 2016 Wang et al. 2018 Wu et al.,2019
PRSV	Squash Watermelon	chr 16 chr 3	KASP	Mazourek, Wessel-Beaver Ling, Levi, Wechter	Branham et al., 2019b
Target leaf spot	Cucumber	chr 6	SNP/SSR	Weng	
ZYMV	Watermelon Squash	chr 3 In progress		Levi, Ling Mazourek, Wessel-Beaver	Branham et al., 2019b

Examples of breeding accomplishments:

Watermelon. Multiple sources of resistance, QTL and markers have been developed for Fusarium wilt races 1 and 2 (Branham et al., 2017, 2018b, 2019a,b; Fall et al., 2018; Meru and McGregor, 2016). QTL and molecular markers also have been developed for *Papaya ringspot virus*, *Zucchini yellow mosaic virus* (Branham et al., 2019b) and gummy stem blight, and QTL have been identified for bacterial fruit blotch (Branham et al., 2019a) and powdery mildew. Breeding lines with foliar resistance to multiple powdery mildew isolates were released (Kousik et al., 2018a); lines with resistance to powdery mildew and Phytophthora fruit rot are being used to develop inbreds with red flesh and increased brix. Breeding is in progress to develop gummy stem blight resistant lines with good fruit quality. A source of resistance to *Cucumber green mottle mosaic virus* was identified and a resistant breeding line (Ling and Levi, 2019) has been approved for release.

Melon. A major QTL and marker has been developed for *Fusarium* race 2 in melon (Branham et al., 2018a). Additional QTL have been identified for *Alternaria* (Daley et al., 2017) and powdery mildew. Breeding is underway to transfer resistance to powdery mildew and Fusarium wilt into commercial melon types. Inheritance studies have been performed for resistance to *Cucurbit yellow stunting disorder virus* (McCreight et al., 2017); QTL identification is in progress. Six lines have been identified with high resistance to *Cucumber mosaic virus*. Virus resistances are being transferred into western shipper type melons. Multiplex PCR-based systems were developed for identification and quantification of four common viruses infecting melon.

Cucumber. A major QTL and underlying gene conferring resistance to downy mildew (DM) and several other diseases (angular leaf spot, anthracnose) was identified (Pan et al., 2018; Wang et al., 2018, 2019). Sources of resistance to the new DM strains were tested in multiple years and locations (VandenLangenberg et al., 2016) and QTL identified (Wang et al., 2016). Marker-assisted backcrossing to introgress resistance into pickling cucumber is underway. Advanced inbreds of pickling and slicing types selected for yield, earliness, quality and downy mildew resistance are being prepared for release. A breeding line with young fruit resistance to Phytophthora (*P. capsici*) fruit rot was released (Grumet and Colle, 2017) and QTL have been identified for young fruit and age-related resistance. QTL and markers for resistance to powdery mildew (Wang et al., 2018b) and target leaf spot have been identified.

Squash and Pumpkin. A QTL was identified and marker developed for powdery mildew resistance in squash (Holdsworth et al., 2016). New sources of resistance to Phytophthora crown rot in *C. maxima* and *C. moschata* (Mantooth et al., 2017; Kousik et al., 2018c) have been identified. A mapping population

has been developed for resistance to Phytophthora in zucchini (*C. pepo*) and QTL identification is in progress. A QTL has been found for resistance to PRSV-W in squash and QTL identification is in underway for ZYMV. Genetic analysis of resistance to PRSV-W and ZYMV from 'Nigerian Local' and 'Menina' indicate potentially complementary sources (Seda-Martinez et al., 2019); resistance is being introgressed into advanced breeding lines of tropical pumpkin (*C. moschata*).

Objective 3. Analysis of cost of production and readily accessible information for cucurbit disease diagnosis and control.

A. Economic analysis. Macro and micro economic variables (interest rates, input costs, production windows, existing crop budgets) were collected and 13 representative farms developed for different crops and production regions: watermelon - 3 CA, 3 FL, 1 TX; melon - 3 CA, 1 TX; cucumber - 1 PA; pickles - 1 NC). Production costs and risks analyses were developed using a risk-based simulation model indicating probabilities of profit or loss depending on crop, region, and farm size.

B. Cucurbit disease diagnostic and control information. The CucCAP extension team developed the CucCAP website (https://cuccap.org/) to provide a centralized source of information 'one stop shopping' for cucurbit disease diagnosis and control. Cuccap.org, which has had more than >63,000 page views, provides diagnostic resources and disease control recommendations with links to plant disease clinics, production guides with disease control recommendations, disease alerts, links to forecasting tools, and links to state, regional, and national watermelon, melon, cucumber and squash commodity websites. A set of 16 cucurbit disease fact sheets with pathogen biology, diagnostic guides, and symptom and pathogen pictures at the macro and micro level, were developed for the website addressing detection and control of anthracnose, downy and powdery mildew, Fusarium wilt, *Phytophthora capsici*, gummy stem blight, *Papaya ringspot virus* and *Zucchini yellow mosaic virus*, among other diseases in cucurbits. Thirteen of the fact sheets were translated into Spanish and are accessible through https://cuccap.org/espanol/. The CucCAP website is continually updated with disease outbreaks, Cooperative Extension news posts, current factsheets, events calendar, and production manuals. The 'CucCAP Chronicle' newsletter is distributed monthly. The extension team also provides consulting, conference calls, diagnostics, disease management recommendations, field days, demonstration plots, workshops, agent in-service training, and publishes extension articles, bulletins, disease management reports, and cultivar evaluations.

IMPACTS

The CucCAP proposal described how information would be translated into products and delivered.

How information will be translated into products and delivered to end-users

(from the original CucCAP proposal)

(a) Sources of disease resistance will be made available to the cucurbit breeding community through publications and germplasm releases.

(b) Information regarding pathogen populations, distribution and spread, virulence, control measures and germplasm under development, will made available through web postings, extension venues and publications.

(c) Genomics databases, tools, and bioinformatics platforms to facilitate genomics-assisted breeding will be made available through workshops, publications, and cucurbitgenomics.org.

(d) Markers and sequence information for disease resistance traits will be made available to the breeding community through publications and web-based databases.

(e) Improved germplasm and breeding lines for use in cultivar development will be made available to seed companies through publications and germplasm releases.

(f) Economic analyses of farm-level impacts will be distributed via extension venues and publications.

As described above, each of these outcomes has been accomplished.

CucCAP provided the opportunity for extensive collaboration among breeders, geneticists, genomicists, bioinformaticians, plant pathologists, extension specialists, and economists to develop genomic and breeding tools to incorporate disease resistance in cucurbit crops and provide relevant disease control information. Collectively the CucCAP team:

- developed a centralized website providing genomic tools and databases; provided GBS and SNP data for full USDA PI collections of watermelon, melon, cucumber and squash (*Cucurbita pepo*) and developed genomically-informed core populations for each crop;
- identified sources of disease resistance, characterized inheritance, identified QTL, developed markers for screening, released resistant breeding lines, and have worked to introgress resistance into commercially valuable materials;
- developed informational materials and provided centralized access to diagnostic resources, disease control recommendations, disease alerts and forecasting tools for cucurbit crops (https://cuccap.org/; http://cucurbitgenomics.org/).

TEAM REPORTS

Genomics and Bioinformatics Team

Team members:

Zhangjun Fei (Boyce Thompson Institute)
Umesh Reddy (West Virginia St. Univ.)
Amnon Levi (USDA, ARS)
Yiqun Weng (USDA, ARS)
Michael Mazourek (Cornell University)
Pat Wechter (USDA, ARS)
Rebecca Grumet (Michigan State University)

(a) Obj. 1. Develop common genomic approaches and tools for cucurbits	Personnel/Institution	Yr 1	Yr 2	Yr 3	Yr 4
1.1. Develop genomic and bioinformatics platforms					
1.1.1. Genotyping by sequencing	ZF (BTI)	X	X	X	
1.1.2. Sequence data processing/analysis	ZF (BTI)	X	X	X	X
1.1.3. ICuGI database development	ZF (BTI)	X	X	X	X
1.1.4 Community standardized nomenclature	YW (ARS-WI), AL (ARS-SC) JM (ARS-CA). MM (CU)		X	X	
1.1.5. Genomic, bioinformatics workshops	ZF (BTI), UR (WVSU), members of crop teams		X	X	X
1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)					
1.2.1. GBS of cucurbit species, establish molecular-informed core populations	ZF (BTI), RG (MSU)	X	X		
- watermelon	AL (ARS-SC)	X	X		
- melon	JM (ARS-CA)	X	X		
- cucumber	YW (ARS-WI),	X	X		
- squash	MM (CU)	X	X		
1.2.2. Population genetics and GWAS analyses	UR (WVSU), ZF (BTI)		X	X	X
- watermelon	AL (ARS-SC)		X	X	X
- melon	JM (ARS-CA)		X	X	X
- cucumber	YW (ARS-WI), RG (MSU)		X	X	X
- squash	MM (CU)		X	X	X

Work completed

1.1. Develop genomic and bioinformatic platforms for cucurbit crops

1.1.1. Genotyping by sequencing

In closely working with Cornell Genomic Diversity Facilty, we have set up the genotyping-by-sequencing (GBS) platform for the cucurbit species.

1.1.2. Sequence data processing/analysis

We have established a GBS data analysis pipeline based on TASSEL-GBS (http://www.maizegenetics.net/tassel).

1.1.3. ICuGI database development

We have re-implemented the ICuGI database (now named Cucurbit Genomics Database (CuGenDB), and the new URL: http://cucurbitgenomics.org/) using the GMOD Tripal system (http://gmod.org/wiki/Tripal) and the Chado database schema (http://gmod.org/wiki/Chado). The newly designed and developed database was released in May 2017. Currently the database contains genome sequences of melon, watermelon (97103 and Charleston Gray), cucumber (Chinese Long and Gy14), wild cucumber (*Cucumis sativus* var. *hardwickii* PI 183967), four *Cucurbita* species (*C. pepo*, *C. maxima*, *C. moschata and C. argyrosperma*), bottle gourd and wax gourd. Genome

syntenies between any two of the sequenced cucurbits have been identified and a synteny viewer have been implemented in the database. An "expression" module has been developed in the database using RNA-Seq datasets publicly available for cucurbit species, mainly collected from NCBI Sequence Read Archive (SRA). A set of tools to mine and analyze the RNA-Seq datasets, such as heatmap view of expression profiles and differential gene expression analysis, were implemented. The synteny viewer and the expression module have been packed as Tripal extension modules which can be implemented in other genomic databases developed using the Tripal system. Development of tools and interfaces to analyze and integrate genotype and phenotype data is ongoing. A manuscript describing the database has been published (Zheng et al., 2019, Nucleic Acids Research, 47:D1128).

1.1.4 Community standardized nomenclature.
The cucumber community has developed a set of vocabularies for QTL mapping (Wang et al. 2020, Horticulture Research, 7:3). Vocabularies for fruit size, weight and shape in QTL mapping across cucurbit crops were also recommended (Pan et al. 2020, Theor Appl Genet 133:1).

1.1.5. Genomic, bioinformatics workshops
A workshop on the Cucurbit Genomics Database was held at the Solcuc2017 meeting in Sept. 2017 at Valencia, Spain. A talk on the database was presented at the CUCURBITACEAE 2018 in November 2018 at Davis, California.

1.2. Perform GBS analysis of PI collections, establish core populations of the four species, and provide community resource for genome wide association studies (GWAS)
1.2.1. GBS of cucurbit species, establish molecular-informed core populations
We have finished GBS for all cucumber, melon, watermelon, *Cucurbita pepo*, *C. maxima* and *C. moschata* accessions collected from the USDA National Plant Germplasm System (**Table 1**). After removing accessions with insufficient reads and merging duplicated accessions, a total of 1,564 cucumber, 2,077 melon, 1,365 watermelon, 852 *C. pepo*, 463 *C. maxima* and 314 *C. moschata* accessions have been genotyped (**Table 2**). We have finished processing the GBS data and SNP calling for all species.

We obtained a total of 1.71, 1.57 and 0.88 billion GBS reads with expected barcodes for melon, cucumber and watermelon, respectively. From these reads, a total of 54,192,089, 76,860,960 and 34,621,369 unique tags were obtained, and 743,545, 593,678 and 388,298 tags with at least 10 reads were used for SNP calling for melon, cucumber and watermelon, respectively. A total of 89,377, 114,338 and 62,258 SNPs were called in melon, cucumber and watermelon, respectively, and 27,846, 23,828, and 25,930 SNPs were obtained by applying criteria of missing data rate < 0.5 and minor allele frequency (MAF) > 0.01 (**Table 3**).

Table 1 Summary of cucurbit GBS

Batch	DNA plate No.	Multi-plex Level	Crop	DNA Submission Date	Data Release Date
1	8	96	cucumber	4/13/2016	7/12/2016
2	9	96	cucumber	5/2/2016	7/12/2016
3	11,12,13,14	384	cucumber	8/24/2016	10/18/2016
4	2,5,6,16	384	cucumber	9/23/2016	11/21/2016

5	1,4,7,15	384	cucumber	10/3/2016	11/21/2016
6	31,34,35,36	384	watermelon	10/19/2016	11/21/2016
7	37,38,39,40	384	watermelon	10/31/2016	1/3/2017
8	41,42,43,44	384	watermelon	11/4/2016	2/15/2017
10	3,10,17,46	384	cucumber	1/20/2017 & 2/2/2017	5/31/2017
11	50,51,52,53	384	melon	2/14/2017	5/5/2017
12	54,55,56,57	384	melon	2/22/2017	5/5/2017
13	58,59,60,61	384	melon	3/2/2017	5/5/2017
14	62,63,64,65	384	melon	3/16/2017	5/5/2017
15	66,67,68,69	384	melon	3/23/2017	5/5/2017
16	21,32,33,70	384	melon & watermelon	3/23/2017	5/31/2017
17	71,72,73,74	384	1melon&3squash	4/19/2017	6/13/2017
18	75,76,77,78	384	squash	5/31/2017	7/11/2017
19	22,23,79,80	384	squash	8/18/2017	9/25/2017
26	81,82	192	C. maxima	3/1/2018	3/22/2018
27	83,84	192	C. maxima	3/1/2018	3/22/2018
29	27	96	C. maxima	3/20/2018	10/9/2018

Table 2 Summary of cucurbit accessions genotyped using GBS

	melon	**cucumber**	**watermelon**	***C. pepo***	***C. moschata***	***C. maxima***
Total No. of plants genotyped	2090	1604	1377	854	318	463
No. accessions with low reads	5	3	11	0	0	0
No. accessions genotyped more than once	8	36	1	1	4	0
Final No. accessions genotyped	2077	1564	1365	852	314	463

Table 3 Summary of GBS SNPs

	No. raw SNPs	**No. SNPs with missing rate <0.5 and MAF <0.01**
melon	89,377	27,846
cucumber	114,338	23,828
watermelon	62,258	25,930
C. pepo	108279	47544
C. moschata	85345	46859
C. maxima	49904	4787

A core collection selection strategy has been developed. Briefly, a total of ~400 accessions were selected for each species. Around 300 accessions which represent the majority of the genetic diversity of the germplasm, based on the core collection analysis using GenoCore (Jeong et al., 2017, PLoS ONE 12:e0181420), were selected. Another ~100 accessions with interesting traits and/or parents of mapping/breed populations were selected. In the final core collection, if a selected line is known to be derived from a PI accession that is also in the final core collection, then the corresponding PI should be replaced with the most closely related one on the phylogenetic tree. Accessions in the final core collection whose genomes have already been resequenced should also be replaced by the most closely related ones on the phylogenetic tree, unless they harbor very interesting/important traits. Based on this strategy, core collections of melon, cucumber, watermelon and *Cucurbita* spp. have been established. For example, the melon core collection contains 384 accessions and captures 98.96% of all allelic diversity in the melon germplasm we have genotyped, and the cucumber core collection contains 395 accessions, of which 354 are from the GBS collection and captures 95.9% of all allele diversity, and 41 are historical varieties with important horticultural and disease resistance traits. Principal component analysis (PCA) of the melon and cucumber core collections showed similar pattern to that of the entire collections (e.g., melon shown in **Figure 1**).

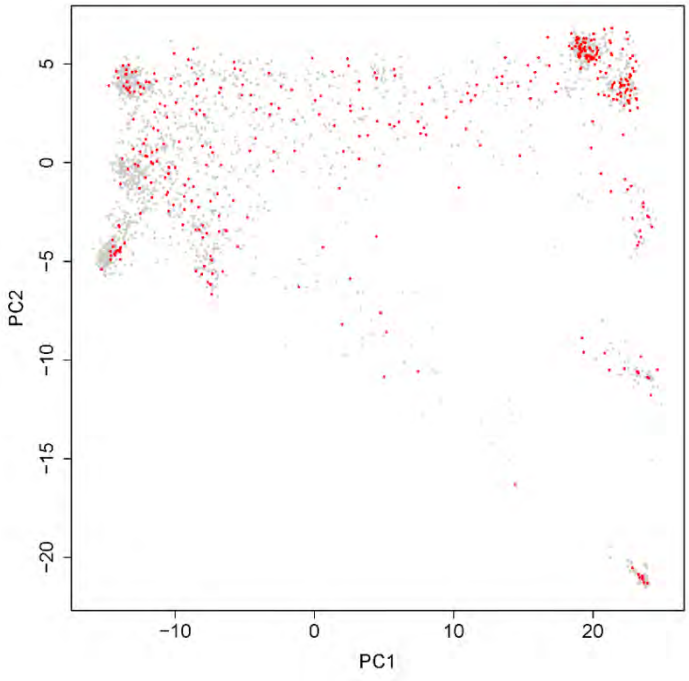


Figure 1. Principal component analysis of the melon core collection (red) and the entire collection (gray)

The melon core S_1 production is well underway. About 150 members were selfed in 2019. The balance of the 384-member group is being selfed through summer 2020 with fruit from about 110 accessions harvested as of May 28, 2020.

1.2.2. Population genomics and GWAS analyses

Using SNPs called from the GBS data, we have performed population genomic analyses for cucumber, watermelon and melon accessions. Phylogenetic, PCA and population structure analyses have been done for accessions of cucumber, watermelon and melon. The results from these analyses for watermelon accessions are shown in **Figure 2** as an example. Linkage disequilibrium (LD) decay patterns and population differentiation have also been investigated for these species.

We have collected historical phenotype data from the USDA National Plant Germplasm System for cucumber, watermelon and melon accessions. GWAS have been performed to identify SNPs

and regions that are significantly associated with important agronomic traits. GWAS for watermelon resistance to powdery mildew race 2 is shown in **Figure 3** as an example.

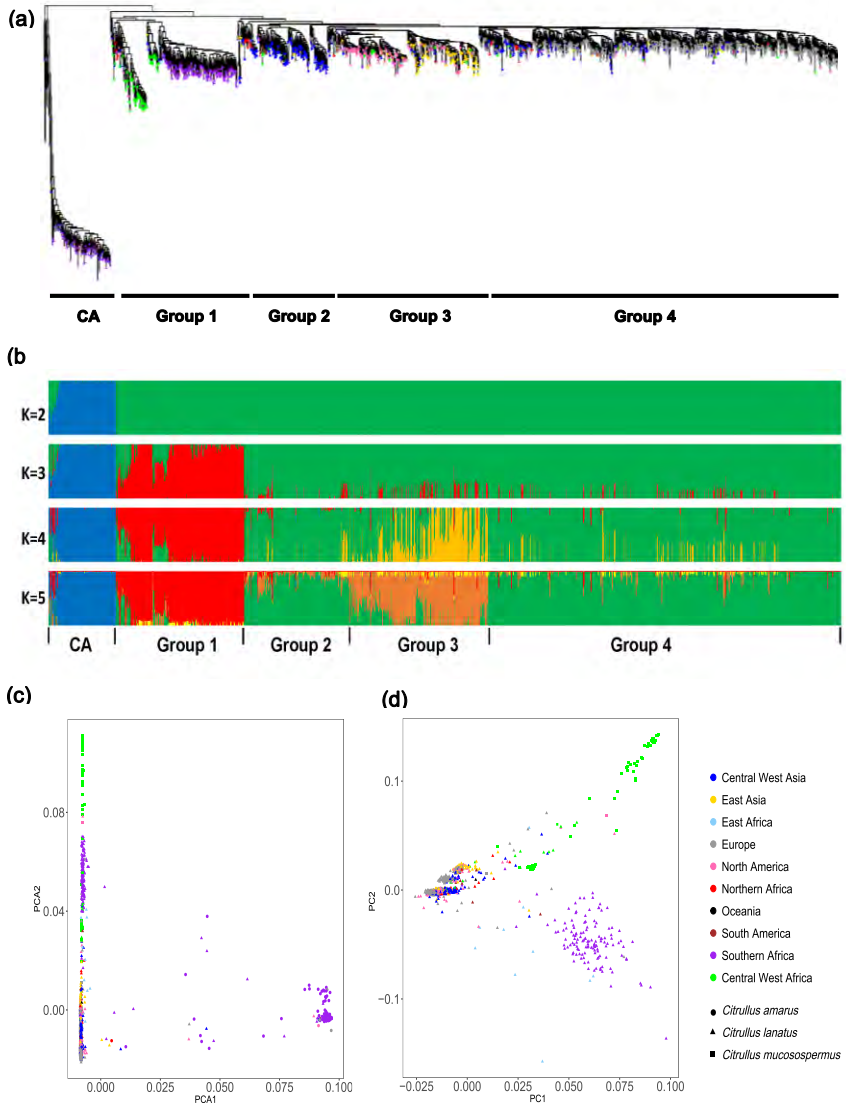


Figure 2. Phylogenetic relationship and population structure of *Citrullus* spp. accessions. (a) Maximum-likelihood tree of 1,367 *Citrullus* spp. accessions. (b) Model-based clustering analysis with *K* from 2 to 5. Each accession is represented by a vertical bar. Each color represents one ancestral population, and the length of each colored segment in each vertical bar represents the proportion contributed by ancestral populations. (c) Principal component analysis of 1,367 watermelon accessions with PC1 and PC2 explaining 63.7%, and 2.1% of variance. (d) Principal component analysis of *C. lanatus* and *C. mucuosospermus* accessions with PC1 and PC2 explaining 4.6% and 2.3% of variance.

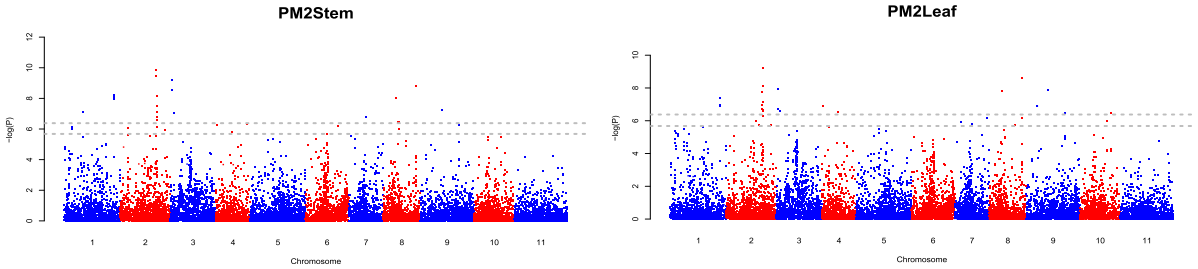


Figure 3. Genome-wide association studies (GWAS) of resistance to powdery mildew race 2 in stem (left) and leaf (right) of watermelon.

Manuscripts reporting the results from population genomics and GWAS analyses as well as core collection development has been published for cucumber (Wang et al., 2018, Horticulture Research 5:64) and watermelon (Wu et al., 2019, Plant Biotechnol J 17:2246), and for melon and *Cucurbita* spp. are under preparation.

1.2.3 Genomic resequencing of core collections

We have compared cost-effective services for Illumina genomic library construction to accommodate our budget for genome resequencing of the core collections, and selected the "Nextera skim sequencing WGS library preps (1/3 concentration)' service provided by Cornell Biotechnology Resource Center (http://www.biotech.cornell.edu/brc/genomics/services/price-list#ht), which charges $1,152 per full plate (96 samples) and additional $900 for pooling and Blue pippin size selection ($2,052 in total; $21.4 per sample).

We have sent 21 *C. pepo* samples (one Illumina lane) and two plates of cucumber samples (192 samples; 6 lanes per plate) in the core collection for library construction. The constructed libraries have been sequenced at GENEWIZ (~$1,500 per lane, which generates ~120 Gb paired-end sequence data). We have obtained cleaned sequence data of >10× depth of the coverage for most of the accessions (**Figure 4**).

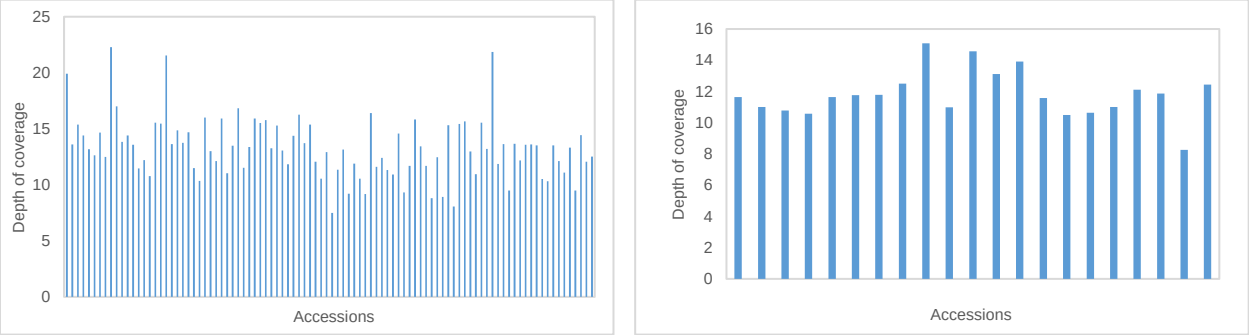


Figure 4. Sequencing depth (based on the final cleaned data) of 96 cucumber accessions (one plate; left) and 21 C. pepo accessions (right).

WATERMELON TEAM

Cucurbit CAP Grant 2016-2019
Watermelon Team Progress Report for 2019-2020.
Overall objectives: Identifying quantitative trait loci (QTL) associated with resistance to major and emerging diseases, developing useful molecular markers and utilizing the genomic tools to incorporate resistance into watermelon cultivars.
Major diseases: Fusarium wilt, Gummy stem blight, Powdery mildew, Phytophthora fruit rot, Papaya ringspot virus (PRSV) and Cucumber green motile mosaic virus (CGMMV).
Team Members: Amnon Levi, Patrick Wechter, Shaker Kousik, Kai-shu Ling (USDA, ARS). Todd Wehner (NCSU) and Cecilia McGregor (UGA)

SCRI CuCAP grant, progress report Year 2018-19

Objective	**Personnel/Institution**	**Year**			
	(initials as in Table 3)	1	2	3	4
(a) Obj. 1. Develop common genomic approaches and tools for cucurbits					
1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)					
1.2.1. GBS of cucurbit species, establish molecular-informed core populations	ZF (BTI), RG (MSU)	X	X		
- watermelon	AL (ARS-SC), TW (NCSU)	X	X		
1.2.2 Population genetics and GWAS analysis	UR (WSVU), ZF (BTI)		x	x	x
- watermelon	AL (ARS-SC)				
(b) Obj. 2. Genomic assisted breeding for disease resistance					
2.1 QTL map resistances:	Screen for resistance (**Sc**), develop populations **(P)**, phenotype (**F**), sequence **(S)**, QTL map **(Q)**				
2.1.1. Watermelon					
- CGMMV	KSL (ARS-SC), AL (ARS-SC)	Sc	Sc,P	P,F,S	S,Q
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	FSQ	Q		
race 2	PW (ARS-SC), AL (ARS-SC)	PFS	PFSQ	FSQ	
- gummy stem blight	CM (UGA), TW (NCSU)	P	PFSQ	FQ	
- Phytophthora	SK (ARS-SC)	PFS	SQ		
- powdery mildew	SK (ARS-SC)	PFS	SQ		
- PRSV-W	AL (ARS-SC), KSL (ARS-SC)	PF	FSQ	FSQ	
2.2 Marker development and verification:	Refine map (**R**) develop marker **(M)**, verify **(V)**				
2.2.1. Watermelon					
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	R	RV	V	V
2.3. Introgress resistance into advanced breeding lines:	Develop breeding lines **(B),** introgress into cultivated **(I)**, advanced lines **(A)**, release to breeders **(R)**				
2.3.1. Watermelon					
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	B	I	IA	AR
race 2	PW (ARS-SC), AL (ARS-SC)	B	B	I	I
- gummy stem blight	CM (UGA), TW (NCSU)	B	B	I	I
- Phytophthora	SK (ARS-SC)	B	I	I	A
- powdery mildew	SK (ARS-SC)	B	I	I	A
- PRSV-W	AL (ARS-SC), KSL (ARS-SC)		B	I	I

Pat Wechter, Sandra Branham, and Amnon Levi, USDA, ARS, U.S. Vegetable Laboratory (USVL), Charleston, SC

Genetic mapping of QTL associated with resistance to Fusarium wilt race 2- A genetic population of 220 $F_{2:3}$ families derived from the cross USVL-252^{FR} x PI 244019-PRSV-R($_{S3}$) was constructed in

collaboration with Dr. Nihat Guner and team at Sakata Seeds. The 220 families were evaluated for resistance to Fusarium wilt (FW) race 2 resistance (Figure 1) in two separate experiments at the U.S. Vegetable Laboratory. The distribution of FW race 2 resistance in the population indicates polygenic inheritance (Figure 2). Genotyping-by-sequencing (GBS) of the $F_{2:3}$ population identified a major QTL on Chromosome 1 of USVL-252^{FR2} associated with resistance to FW race 2. This QTL co-locates on the same genomic region conferring resistance to *Fon* race 1 identified in our previous studies (Lamble et al. 2014; Branham et al. 2018). Also, a major QTL (*qFon1-9*) associated with resistance to *Fon* race 1 was identified on chromosome 9 of USVL246-FR2. These discoveries provide host-resistance source of resistance to *Fon* races 1 and 2 in watermelon and as it co-locates with the QTL for *Fon* race 2 resistance in the same population, may provide non-race specific resistance (Branham et al. 2017, 2019). Sixty KASP markers were constructed and are being validated for utility of incorporating resistance to *Fon* races 1 and 2 into the genetic background of watermelon cultivars.

Figure 1. Two-hundred and twenty $F_{2:3}$ families derived from the cross USVL-252^{FR} x PI 244019-PRSV-R($_{S3}$) being evaluated for Fusarium wilt race 2 in a greenhouse at the U.S. Vegetable Laboratory (Summer, 2017).

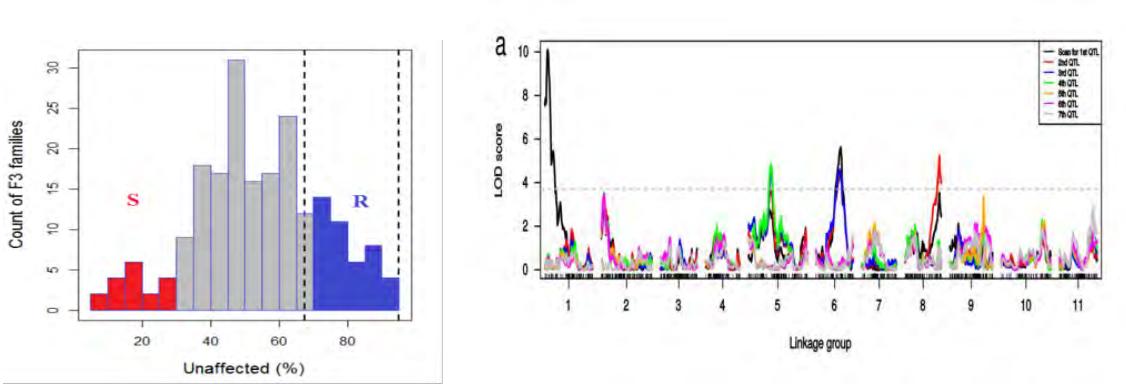


Figure 2. Distribution of $F_{2:3}$ families derived from the cross USVL-252^{FR} x PI 244019-PRSV-R($_{S3}$) for resistance to Fusarium wilt (FW) race 2 (Left). A major QTL associated with FW race 2 resistance on Chromosome 1 of USVL-252^{FR} (right).

Converting QTL to Kompetitive Allele Specific PCR **KASP markers tightly linked to Fusarium wilt race 1 resistance**- DNA of the resistant and susceptible parents (*C. lanatus*) and the F_2 parental plants of the most resistant versus the most susceptible $F_{2:3}$ families (Lambel et al. 2014) were used for a QTL-seq analysis. QTL-seq narrowed the *Fon* race 1 QTL interval on chromosome 1 of watermelon (Lambel et al. 2014) by 500 kb (Branham et al. 2018). SNPs from the interval were converted to KASP primers. The KASP primers were used in genetic mapping of the same population used for the initial mapping of QTL associated with FW race 1 resistance (Lambel et al. 2014). QTL mapping yielded several KASP markers tightly linked to race 1 resistance and narrowed the QTL interval further from 1.56 Mb to 315 kb (Figure 3). In collaboration with the HM.Clause team in Davis, California we conducted QTL-seq and developed KASP markers tightly linked to FW race 1 resistance (Figure 3; Branham et al. 2018). We have developed KASP markers for *Fon* races 1 and 2 QTL in *C. amarus*. The FW races 1 and 2 resistant lines USVL246-FR2 and USVL252-FR2 were crossed with Charleston Gray, Calhoun Gray and Sugar Baby to generate F_1, F_2, BC_1 and BC_2F_2. The KASP markers will be used to incorporate resistance to *Fon* races 1 and 2 into the genome background of watermelon cultivars.

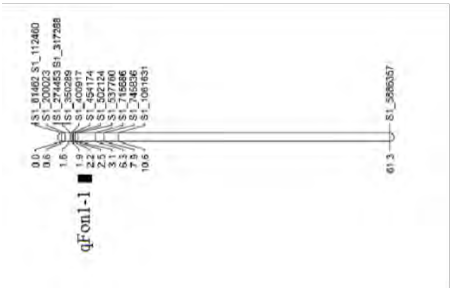


Figure 3. KASP markers tightly linked to Fusarium wilt race 1 resistance (qFon1-1) on chromosome 1 of watermelon (Branham et al. 2018).

Downy mildew resistance

Pat Wechter and Dennis Katuuramu

C. amarus PI have been assayed for downy mildew resistance and GWAS performed, identifying two putative QTL. Assays will be performed during 2020-2021 of the USVL246 x USVL114 RILs which segregate for Downy mildew resistance, and QTL analysis performed (Katuuramu et al. 2020). Assays will be performed during 2020-2021 of the USVL246 x USVL114 RILs which segregate for Downy mildew resistance, and QTL analysis performed.

What do you plan to do during the next reporting period to accomplish the goals?

Following the Covid19 aftermath we should complete development and validation of KASP markers. We have crossed USVL246-FR2 ^{FR} and USVL-252^{FR} with watermelon cultivars and constructing genetic populations that will be used in marker assisted selection (MAS) to incorporate *Fon* races 1 and 2 resistance into the genomic background of watermelon cultivars (Wechter, Branham and Levi). BC1F1 have been generated for USVL246 x sugar baby, USVL246 x Charleston Grey, and USVL246 x Calhoun grey. BC2F1 and BC2 F2 will be generated during 2020-2021. Similar populations have also been constructed with USVL 252 and will be used for incorporating the *Fon* 1 and 2 resistance gene loci into watermelon cultivars.

Other Products

Product Type

BC_2F_2 and BC_3F_2 lines with resistance to FW races 1 and 2.

Students and Post-docs: A Post-doctoral researcher shared with Pat Wechter

Amnon Levi, Kai-shu Ling, and Sandra Branham, USDA, ARS, U.S. vegetable Laboratory (USVL), Charleston, SC

Identifying QTL associated with Papaya ringspot virus (PRSV) and with Zucchini yellow mosaic virus (ZYMV) resistance- Several F_2 and BC_1 populations derived from the cross USVL-252^{FR} x PI 244019-PRSV-R($_{S3}$) and a BC3F2 population [(PI 595203 x Charleston Gray) Charleston Gray] were analyzed in collaboration with Dr. Nihat Guner and team at Sakata Seeds. The genetic populations were evaluated for resistance to PRSV-resistance at the U.S. Vegetable Laboratory and at Sakata Seeds Station in Ft. Meyers, FL. The genetic analyses of PRSV-resistance in the population confirmed inheritance by a single homozygous recessive gene in PI 244019 (Guner, 2004; Guner and Wehner, 2008). Genotyping-by-sequencing (GBS) of an F_2 population identified a major QTL on Chromosome 3 of PI 244019 associated with PRSV-resistance (Figure 4). A QTL-seq analysis of the BC3F2 population also identified a major QTL associated with ZYMV-resistance on chromosome 3 of PI 595203, counterparts to the genomic region on chromosome 3 of PI 244019. The major QTL interval comprises several ribosomal genes, among them the eukaryotic elongation factor eIF4E known to be associated with resistance to potyviruses in cucurbit crops (Ling et al. 2009). KASP markers are being developed and will be used for incorporating the resistance into the genomic background of watermelon cultivars.

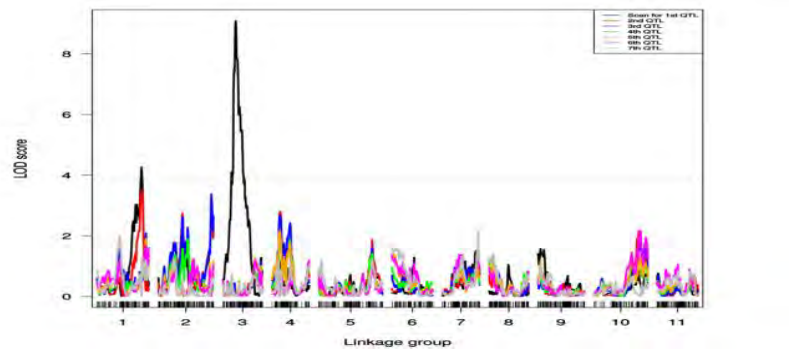


Figure 4. A major QTL associated with PRSV resistance identified on chromosome 3 of *Citrullus amarus* PI 482019 using GBS-SNP data analysis.

What do you plan to do during the next reporting period to accomplish the goals?
-Complete development of KASP markers tightly linked to PRSV-resistance in PI 244019-PRSV-R($_{S3}$) and to ZYMV resistance in PI 595203 and use them to incorporate resistance into genome background of watermelon cultivars (Levi, Ling, Branham).

Other Products
Product Type
BC_1F_2 and BC_2F_2 lines with resistance to PRSV [(244019 x Charleston Gray) Charleston Gray]
BC_6F_2 and BC_7F_2 lines with resistance to ZYMV [(595203 x Crimson Sweet) Crimson Sweet].
Students and Post-docs: A Post-doctoral researcher.

Shaker Kousik, Patrick Wechter, Sandra Barnham, Amnon Levi; USDA, ARS, U.S. vegetable Laboratory (USVL), Charleston, SC

Powdery mildew of watermelon.
Powdery mildew (PM) of watermelon (*Citrullus lanatus*) and other cucurbits caused by *Podosphaera xanthii* is a major factor limiting production in greenhouses and open fields. In recent years, occurrence of PM has been increasing on watermelon across the United States, and commercial watermelon cultivars with resistance are rare. The disease continues to be a constant problem throughout the southeast. Our survey of watermelon researchers also indicated that powdery mildew was considered an important priority for research across the U.S.A.
Prior to the start of the project, not much information on PM of watermelon was available and not many highly developed sources of resistance were available. In 2018 we released four red fleshed lines with high levels of resistance to powdery mildew (USVL608-PMR, USVL313-PMR, USVL585-PMR and USVL225-PMR). A paper documenting the release of these four PM resistant watermelon lines was published in HortScience in 2018. Several of these lines will be used in the extension part of CucCAP2. We also released USVL531-MDR which is resistant to powdery mildew and Phytophthora fruit rot. Seeds of these resistant germplasm lines have been provided to most seed companies upon request (e.g. Syngenta, HM Clause, Sakata, Seminis).

Relative susceptibility of commercial watermelon varieties to powdery mildew
This study was conducted in 2014, 2015 and 2016 to determine the relative susceptibility of twenty six watermelon varieties (seeded and seedless) and three pollenizers to *P. xanthii* populations prevailing in Charleston, South Carolina. USVL677-PMS, which is highly susceptible to PM and USVL531-MDR, which is resistant to PM were included as controls. A randomized complete block design with three replications was used each year and plants were rated on a 0-10 scale of increasing disease severity. During all three years, USVL677-PMS was the most susceptible line with the highest values for area under disease progress curve (AUDPC). Disease severity at the last ratings for USVL677-PMS ranged from 61-82%. In comparison USVL531-MDR was very resistant to PM based on disease severity (1-3%) and AUDPC. The commercial pollenizers, SP5, SP6 and Lion were all resistant to PM (1-5% disease severity) and had significantly lower AUDPC compared to USVL677-PMS and most other varieties evaluated. Among the red fleshed varieties, Suprema (seedless variety), was relatively resistant compared to other seeded and seedless varieties. Most of the seeded varieties evaluated (e.g. Malali, Black Mama, Mickey Lee) were highly susceptible to PM, however, some were relatively less susceptible (e.g. Declaration) under field conditions. Currently very few to no varieties with high levels of resistance to PM are available and there is a need to develop newer resistant varieties as PM is occurring more frequently during the growing season.

Identifying and developing multiple disease resistant lines from accessions
Majority of the watermelon plant introductions (PI) considered as resistant or tolerant display varying levels of disease resistance. Hence it is important to screen and select for several generations to develop highly resistant lines from these PI. We have developed 36 lines with high levels of resistance to powdery mildew from various PI. Of these 13 are also resistant to Phytophthora fruit rot and can be considered as multiple disease resistant (MDR). These lines were evaluated for resistance to powdery mildew and Phytophthora fruit rot in the field and displayed high levels of resistance compared to

susceptible lines including Mickey Lee and USVL677-PMS. These lines will serve as useful sources of resistance for future studies.

Inheritance studies on USVL531-MDR x USVL677-PMS

We completed inheritance studies on the egusi type watermelon (*C. mucosospermus*) line USVL531-MDR. This line was found to be resistant to 11 PM isolates from across the U.S.A. and was released by USDA ARS in 2018. This line was used as the female parent (P_1) and crossed with USVL677-PMS which is highly susceptible (P_2). The parents, F_1, backcrosses to both parents (BC_1, BC_2) and a large F_2 population were inoculated with a local isolate of PM and assessed for disease severity on a 0-10 scale of increasing disease severity. The susceptible parent (USVL677-PMS) had mean disease severity of 8.14 on the 0-10 scale, whereas it was 1.17 for the resistant parent. Segregation patterns point to single gene inheritance, but also indicated another gene is inherited maternally. Chi-square analyses of observed segregation of phenotypes for the F_2 populations fit models for these gene models and were further supported by segregation patterns in the backcross populations. QTL-seq analysis on the extremes from the F_2 populations and RNA-seq analysis of the parents during PM infection are being conducted to identify the chromosomal regions involved in resistance. USVL531-MDR will serve as a useful source to incorporate PM resistance into commercial cultivars. We have developed several red fleshed resistant lines (at F_8) using USVL531-MDR as the source of resistance as described below.

Identification of an NBS-LLR R gene *ClaPMR2* and development of CAPS markers

To gain a better understanding of the innate and activated molecular defense mechanisms involved during compatible and incompatible PM-watermelon interactions, we inoculated PM susceptible (USVL677-PMS) and resistant (USVL531-MDR) watermelon plants with 10^5 conidia ml^{-1} of *P.xanthii*. RNA-seq profiling was done on leaf samples collected at 0, 1, 3, and 8 days post inoculation (DPI). A total of 2566 unique differentially expressed genes (DEGs) were identified between compatible and incompatible interactions with *P. xanthii*. The compatible interactions resulted in distinct plant gene activation (>2 fold unique transcripts, 335:191:1762 :: 1:3:8 DPI) as compared to incompatible interaction (>2 fold unique transcripts, 314:681:487 :: 1:3:8 DPI). Further, comparative whole-genome resequencing analysis of USVL531-PMR, USVL677-PMS and four introgressed PM resistant recombinant inbred lines (RIL, USVL531-PMR x USVL677-PMS) were performed to identify the region of PM resistance introgressed break points along with other traits inherent by USVL531-PMR by comparing the SNPs and InDels. Based on SNPs identification and CAPS markers, the resistance gene was identified as *ClaPMR2, Citrullus lanatus PM Resistance gene 2* {Chr2 : 26750001 .. 26753327 (-)}, a NBS-LRR resistance protein (R) with homology to the *Arabidopsis thaliana* PM resistance protein, RPW8 (Figure 1). The CAPS marker was validated using the parents, four PM resistant RILs and susceptible and resistant F2 populations using DNA gel electrophoresis (Figure 2). The transcriptome data also revealed a complex regulatory network associated with the introgressed junctions mediated by PM resistance R proteins (R genes) that may involve multiple signal regulators and transducers, carbohydrate metabolism, cell wall modifications and the hormone-signaling pathway. The information presented here has been accepted for publication in Scientific Reports and the manuscript is entitled “Elucidation of Resistance Signaling and Identification of Powdery Mildew Resistant Mapping Loci (ClaPMR2) during Watermelon-Podosphaera xanthii interaction using RNA-Seq and Whole-Genome Resequencing Approach.”

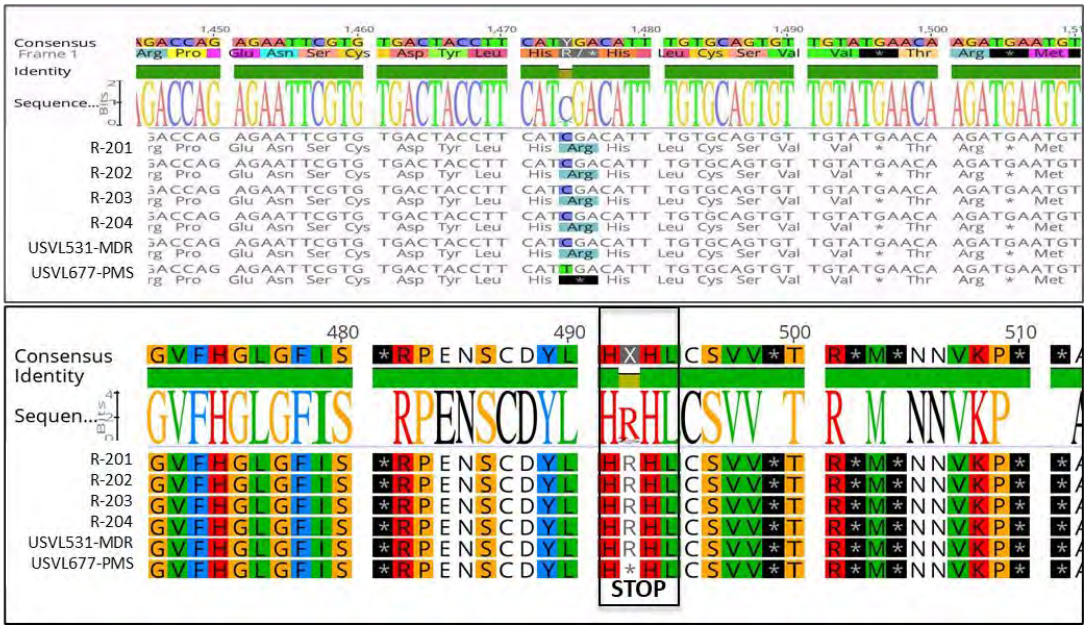


Figure 1. Comparative nucleotide and amino acid consensus sequences with predicted secondary structure alignment of *ClaPMR2* encoding protein in Parent (USVL531-MDR & USVL677-PMS), and Recombinant inbred lines (RIL:R-201, R-202, R-203, R-204). Black line rectangle area HRH (C-T; Arg-STOP), represents the location of SNP region with substitution position Arg-STOP codon in (*ClaPMR2, Citrullus lanatus PM Resistance gene*).

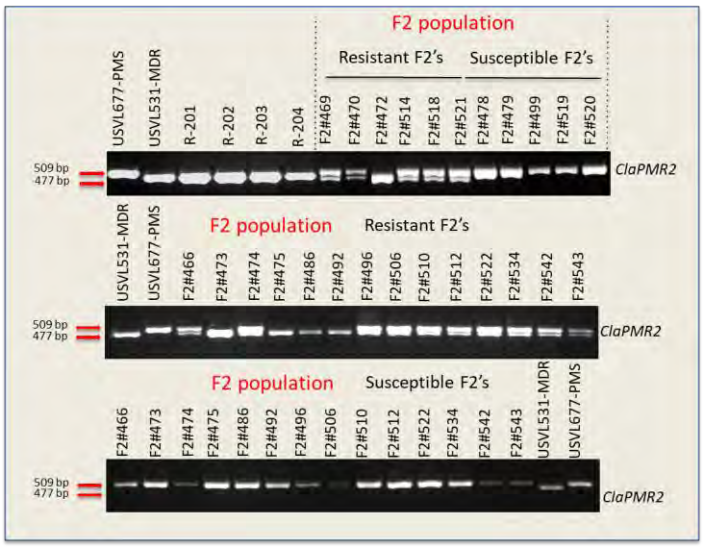


Figure 2. Single Nucleotide Polymorphisms (SNP) derived CAPS validation on Parent, Recombinant Inbred line (RIL) introgressed watermelon lines and resistant and susceptible F2 popluations. DNA gel electrophoresis showing the CAPS marker analysis on *ClaPMR2 gene* in parent lines: USVL531-MDR & USVL677-PMS; RILs: R-201, R-202, R-203 and R-204 and F_2 populations. Since the resistant phenotype is dominant, we observed *ClaPMR2 loci*, co-segregated with the resistant locus in heterozygous and homozygous individuals with PM resistant F_2.

Advancing Powdery mildew resistant inbred lines.
Fruit from F_2 plants from a cross of USVL531-MDR and USVL677-PMS with powdery mildew resistance, uniform red flesh and decent brix (>7) were collected and have been advanced till F_7 and further advancement to F_8 is in progress. We are currently evaluating 12 red fleshed F_8 lines that were homozygous for resistance. We completed assessment of fruit quality from F_6 and F_7 progenies that were homozygous for resistance to PM and had red flesh and brix >7 in 2019.

Inheritance of PM resistance, identification of QTL and RNAseq in USVL608-PMR
USVL608-PMR (S_6), a red fleshed watermelon line with high levels of resistance to PM was used as the female parent (P_1) and crossed with USVL677-PMS which is highly susceptible (P_2). The parents, F_1, backcrosses to both parents (BC_1, BC_2) and a large F_2 population were inoculated with a local isolate of PM and assessed for disease severity on a 0-10 scale of increasing disease severity. All susceptible parent (USVL677-PMS) plants were rated >7 [mean disease severity (DS) = 94%], whereas most resistant parent (USVL608-PMR) plants were rated as 1 (DS=2.5%). Majority of the BC_1 plants were rated ≤2 and considered as resistant. Of the 466 F_2 plants, 221 were rated ≤2 (DS=3.1%). Of the 76 BC_2 plants, 23 were rated ≤2 (DS=2.9%). Chi-square analyses of the observed segregation of phenotypes for the F_2 plants indicated that two genes control PM resistance with a good fit for a 7:9 resistance to susceptibility ratio. The proposed model for this ratio is two genes with one recessive for high resistance and one dominant for high resistance. This is supported by a backcrossing segregation ratio of 1:3. We have observed some highly and moderately resistant plants in the F_2 indicating the cumulative effect of the two genes. QTL-seq analysis on the most resistant and most susceptible DNA bulks from the F_2 populations identified a major QTL in chromosome 2.

We have also completed RNA-seq analysis of the parents during PM infection. Plants of the resistant line USVL608-PMR and the susceptible line USVL677-PMS were with inoculated with 10^5 conidia^{-ml} of *P.xanthii*. Symptom development was observed every day. In addition, leaf samples were collected for microscopy and for RNA extraction. RNA-seq profiling was done on leaf samples collected at 0, 1, 4, and 9 days post inoculation (DAI). Powdery mildew symptoms were visible on USVL677-PMS 4 DAI whereas leaves of USVL608-PMR were clean. We have completed RNA-seq on all these samples. Data analysis is in progress. A quick analysis of the differentially expressed genes (DEG) indicated several resistance genes in chromosome 2.

Phytophthora fruit rot of watermelon
Shaker Kousik; USDA, ARS, U.S. vegetable Laboratory, Charleston, SC
Phytophthora fruit rot of watermelon has been a major problem in watermelon growing areas in the Southeastern U.S. (FL, GA, SC, NC and VA). In recent years it has also become a problem in watermelon growing areas in Maryland (MD), Delaware (DE) and Indiana (IN). The National Watermelon Association considered Phytophthora fruit rot as its top research priority in 2017 as well. At the U.S. Vegetable Laboratory (USDA, ARS) in Charleston we have developed several germplasm lines with high levels of resistance to Phytophthora fruit rot. In these studies we used the germplasm line USVL531-MDR which was resistant to 20 different *P. capsici* isolates from across the U.S.A. Studies to determine inheritance of resistance to Phytophthora fruit rot using the same population described for powdery mildew (USVL531-MDR X USVL677-PMS) were conducted as USVL531 is resistant to both these diseases. However, based on this study it was difficult to assess the number of genes controlling resistance and hence we are in the process of developing a recombinant inbred line (RIL) population and are currently at the F_8 stage.
We are completed phenotyping the populations from USVL003-MDR x USVL677-PMS for resistance to Phytophthora fruit rot in 2019. DNA was extracted from the most susceptible and resistant F2 plants and bulked. Bulked DNA was sequenced by Novogene. Data analysis is in progress.

We completed experiments to determine the transcriptomic profile during *P. capsici* infection of resistant and susceptible genotypes. Advanced germplasm lines of USVL531-MDR, USVL0020-PFR, Charleston, Gray and Sugar Baby were grown in the field and fruit were harvested when mature. Fruit of each of these lines was inoculated with 10^4 zoospores/ml and maintained in a humid chamber (26 ± 1 °C >95%RH). Fruit rind samples were collected from individual fruit after 12h, 24h, 48h, 72h, and 96h after inoculation and immersed in liquid nitrogen to quench all the metabolomics processes. Rind samples were then processed for extraction of RNA and sent to Duke University Genomic center for RNA sequencing. Sequencing has been completed and we are currently analyzing the RNA-seq data.
We have identified three red fleshed (plants) with tolerance to Phytophthora fruit rot and high level of resistance to Powdery mildew. These will be screened for resistance to both the diseases and advanced further. Once we have adequate seeds of these we hope to use these in CucCAP2.

Project metrics (timeline) for research on Phytophthora fruit rot and powdery mildew of watermelon

- Develop germplasm lines with resistance to Phytophthora fruit rot and powdery mildew for watermelon: Completed.
- Develop populations for phenotyping resistance to Phytophthora fruit rot and powdery mildew of watermelon: Completed
- Sequence and map Powdery mildew resistance QTL in watermelon: Completed
- Sequence and map Phytophthora fruit rot QTL in watermelon: In progress.
- Introgress powdery mildew resistance into cultivated type watermelon: Completed
- Introgress Phytophthora fruit rot resistance into cultivated type watermelon: In Progress
- Develop markers for powdery mildew resistance: Completed
- Develop and Validate Markers for Phytophthora fruit rot: In Progress.
- Participation in outreach to stakeholder groups per year via industry events and field days. Completed

Todd C. Wehner
Increasing watermelon PI accessions and preparing leaf samples for DNA isolation and genome wide association study (GWAS)

Work in progress and plans
1.2. Perform GBS analysis of PI collections, establish core populations of the four species, and provide community resource for genome wide association studies (GWAS) (Takshay Patel and Todd C. Wehner)

1.2.1. GBS of cucurbit species, establish molecular-informed core populations
i. Objective*:* Develop molecular markers for high resistance to gummy stem blight (GSB) using genome-wide association studies (GWAS) in the USDA watermelon germplasm collection, and introgress GSB resistance into watermelon cultivars.
We are collecting and increasing *Citrullus* PI accessions, heirloom cultivars, and gene mutant type-lines. Seed increase of the 2000 PI accessions is being accomplished by seed companies, USDA scientists, and university researchers. Each is increasing 1 to 10 accessions per year using controlled pollination in greenhouse or field.

Association analysis: Collected phenotypic and genotypic data will be analyzed using R packages: SNPassoc, snpMatrix, GenABEL and pbatR. The result of the analysis will allow us to locate and identify SNP markers associated with GSB resistance.

PI accessions

Of the 1408 accessions of Citrullus that are available, we were able to grow 1365, which were sampled for leaf tissue, and shipped to Michigan State University for DNA sequence. A core collection was developed, consisting of 420 PI accessions that had traits of interest to researchers. Of those, 250 germinated and were increased by self pollination. Seeds from self pollination and leaf tissue of those core accessions were sent to Michigan State University.

Gene type lines

Collection and seed increase of the watermelon gene type-lines will include all cultivars, breeding lines, and PI accessions in the gene mutant list at Cucurbit Genetics Cooperative. Examples include:
PI 189225 (*db, Ar-2-1*), NC-517 (*C*), PI 482261 (*Ctr*), Bush Charleston Gray (*dw-1*), PI 595203 (*zym-CH, zym-FL*).

Watermelon gummy stem blight resistance (Luis Rivera and Todd C. Wehner)

Objective: a) Evaluate a RIL population of watermelon (*Citrullus lanatus* × *C. amarus*) for resistance to gummy stem blight and fruit quality traits and b) Map GSB resistant genes through genome-wide association studies (GWAS).

Phenotyping: A watermelon GSB population was developed by intercrossing the most resistant accessions of *Citrullus* four times (I_4), followed by crossing with elite cultivars of watermelon (I_4F_1), followed by intercrossing without selection, while maintaining wild and elite types in the populations ($I_4F_1I_4$), followed by self-pollinations of plant at random ($I_4F_1I_4S_1$). The 300 RILs and 20 controls (10 PIs and 10 commercial cultivars) were evaluated for resistance to gummy stem blight in greenhouses at North Carolina State University in Raleigh, North Carolina (Figure 1 and 2), and in the field at the Horticultural Crops Research Station at Clinton, North Carolina (Figure 3). We inoculated plants with *Stagonosporopsis cucurbitacearum* at a concentration of 5×10^5 spores/ml (Figure 4). To evaluate disease severity, we adopted an ordinal disease assessment scale (Gusmini et al. 2002). Plants were rated four times, in an experiment with, 2 locations, and 10 replications (at greenhouse and field). We also evaluated fruit quality in the gummy stem blight field trial. We also collected data of fruit shape, rind pattern and toughness, seed size and color, flesh color and intensity and hollow heart. We will identify RILs with high yield of excellent fruit quality.

Additionally, genomic DNA of gummy stem blight isolates collected from field outbreaks was extracted, and a PCR-based marker test for distinguishing the three morphologically identical, but genetically distinct species causing gummy stem blight was performed (Figure 4). We used three sets of primers, including *Db05* that produces a 216 to 224-bp fragment in all three species, *Db06* that produces a 283- to 289-bp in *S. citrulli* and a 268-bp and slightly fainter fragment in *S. cucurbitacearum*, and *Db01* that produces a 256- to 364-bp fragment in *S. citrulli* (Brewer et al. 2015). Two of the isolates were *S. cucurbitacearum* (syn. *Didymella bryoniae*) and one isolate was *S. caricae*.

Figure 1. Greenhouse test for resistance to gummy stem blight

Figure 2. Gummy stem blight symptoms during the greenhouse test

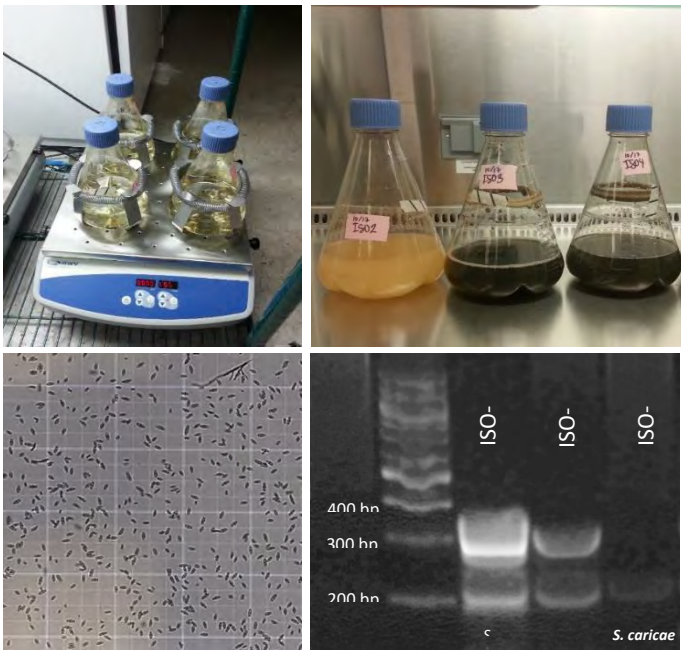


Figure 4. Gummy stem blight spore mass production and identification through PCR and electrophoresis

Genotyping: The 300 RILs were planted in spring 2018, at greenhouses of NC State, to sample leaf tissue for DNA extraction. The DNA was sent for SNP discovery through genotyping by sequencing (GBS) method at Cornell University. We expect to get several thousand of SNPs for association analysis (GWAS). Resistance to GSB and fruit quality are being evaluated in 3 years (2017, 2018, 2019), 2 locations (field, greenhouse), and 10 replications on 300 lines ($I_4F_1I_4$) at the S_4, along with 20 controls (10 PIs and 10 commercial cultivars).

Association analysis: The phenotypic and genotypic data is being analyzed using R packages: GWASTools, GWASdata, SNPassoc, snpMatrix, GenABEL and pbatR. The result of the analysis will allow us to locate and identify SNP markers associated with GSB resistance.

Figure 3. Field test for resistance to gummy stem blight

Watermelon Breeding Lines

NC-524 – Breeder: T.C. Wehner, L. Rivera and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: S7 inbred selected from NC GSB resistant population. The population was developed as follows: 5 *Citrullus amarus* PI accessions (189225, 482283, 482342, 482374, 526233) intercrossed 4 generations, then crossed with 7 cultivars (Allsweet, Calhoun Gray, Charleston Gray, Crimson Sweet, Mickylee, Minilee, Petite Sweet) and the progeny intercrossed 4 generations before self pollinating. Previous number 18GH-031@ (RIL-039). Characteristics: monoecious watermelon with **GSB resistance**, elongate fruit shape, wide-stripe pattern, tough rind, coral red flesh, sweetness of 11 °brix, medium-size black seeds, high quality (8 on 1-9 scale), hollowheart resistant. Resistance: gummy stem blight. Similar: Allsweet. Adaptation: southern U.S. 2020.

NC-527 – Breeder: T.C. Wehner, L. Rivera and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: S7 inbred selected from NC GSB resistant population. The population was developed as follows: 5 *Citrullus amarus* PI accessions (189225, 482283, 482342, 482374, 526233) intercrossed 4 generations, then crossed with 7 cultivars (Allsweet, Calhoun Gray, Charleston Gray, Crimson Sweet, Mickylee, Minilee, Petite Sweet) and the progeny intercrossed 4 generations before self pollinating. Previous number 18GH-221@ (RIL-267). Characteristics: monoecious watermelon with **GSB resistance**, round fruit shape, solid light green pattern, tough rind, coral red flesh, sweetness of 10 °brix, medium-size black seeds, high quality (8 on 1-9 scale), hollowheart resistant. Resistance: gummy stem blight. Similar: King & Queen. Adaptation: southern U.S. 2020.

NC-528 – Breeder: T.C. Wehner, L. Rivera and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: S7 inbred selected from NC GSB resistant population. The population was developed as follows: 5 *Citrullus amarus* PI accessions (189225, 482283, 482342, 482374, 526233) intercrossed 4 generations, then crossed with 7 cultivars (Allsweet, Calhoun Gray, Charleston Gray,

Crimson Sweet, Mickylee, Minilee, Petite Sweet) and the progeny intercrossed 4 generations before self pollinating. Previous number 18GH-222@ (RIL-268). Characteristics: monoecious watermelon with **GSB resistance**, round fruit shape, solid light green pattern, tough rind, coral red flesh, sweetness of 11 °brix, medium-size black seeds, high quality (8 on 1-9 scale), hollowheart resistant. Resistance: gummy stem blight. Similar: King & Queen. Adaptation: southern U.S. 2020.

NC-530 – Breeder: T.C. Wehner, L. Rivera and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: S7 inbred selected from NC GSB resistant population. The population was developed as follows: 5 *Citrullus amarus* PI accessions (189225, 482283, 482342, 482374, 526233) intercrossed 4 generations, then crossed with 7 cultivars (Allsweet, Calhoun Gray, Charleston Gray, Crimson Sweet, Mickylee, Minilee, Petite Sweet) and the progeny intercrossed 4 generations before self pollinating. Previous number 18GH-049@ (RIL-066). Characteristics: monoecious watermelon with **GSB resistance**, round fruit shape, solid light green pattern, tough rind, coral red flesh, sweetness of 9 °brix, small black seeds, high quality (8 on 1-9 scale), hollowheart resistant. Resistance: gummy stem blight. Similar: King & Queen. Adaptation: southern U.S. 2020.

NC-531 – Breeder: T.C. Wehner, L. Rivera and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: S7 inbred selected from NC GSB resistant population. The population was developed as follows: 5 *Citrullus amarus* PI accessions (189225, 482283, 482342, 482374, 526233) intercrossed 4 generations, then crossed with 7 cultivars (Allsweet, Calhoun Gray, Charleston Gray, Crimson Sweet, Mickylee, Minilee, Petite Sweet) and the progeny intercrossed 4 generations before self pollinating. Previous number 18GH-099@ (RIL-125). Characteristics: monoecious watermelon with **GSB resistance**, oval fruit shape, medium-wide stripe pattern, tough rind, scarlet red flesh, sweetness of 11 °brix, large black seeds, high quality (8 on 1-9 scale), hollowheart resistant. Resistance: gummy stem blight. Similar: Crimson Sweet. Adaptation: southern U.S. 2020.

NC-532 – Breeder: T.C. Wehner, L. Rivera and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: S7 inbred selected from NC GSB resistant population. The population was developed as follows: 5 *Citrullus amarus* PI accessions (189225, 482283, 482342, 482374, 526233) intercrossed 4 generations, then crossed with 7 cultivars (Allsweet, Calhoun Gray, Charleston Gray, Crimson Sweet, Mickylee, Minilee, Petite Sweet) and the progeny intercrossed 4 generations before self pollinating. Previous number 17GH-154@ (RIL-131). Characteristics: monoecious watermelon with **GSB resistance**, round fruit shape, solid light green pattern, tough rind, coral red flesh, sweetness of 12 °brix, large black seeds, high quality (8 on 1-9 scale), hollowheart resistant. Resistance: gummy stem blight. Similar: King & Queen. Adaptation: southern U.S. 2020.

CuCecilia McGregor, University of Georgia, Athens, GA

QTL Mapping, Marker Validation and Trait introgression of Gummy Stem Blight resistance in Watermelon.

1. Progress for 2019-2020


1.1 Phenotyping

- WPop GSB1: PI 482276 x Crimson Sweet population of 178 F2:3 lines (2 x 3 reps x 4 plants/rep x 178 lines = 4,272 plants) was phenotyped in a humidity tunnel in the greenhouse using C. citrilli isolate 12178A (GA). Disease symptoms for each seedling were scored on a 0 – 5 scale and disease severity calculated. Parents and F1 and 4 other control genotypes were also included. **Complete**
- WPop GSB2: PI 189225 x Sugar Baby. PI 189225 x Sugar Baby population of 114 F2:3 lines (4 x 4 plants/rep x 114 lines = 1,824 plants) was phenotyped in a humidity tunnel in the greenhouse using C. citrilli isolate 12178A (GA). Disease symptoms for each seedling were scored on a 0 – 9 scale and disease severity calculated. Parents and F1 and 4 other control genotypes were also included. **Complete**

1.3 Genotyping and QTL mapping

- WPop GSB1: PI 482276 x Crimson Sweet population of 178 F2 plants were genotyped by GBS. The reads were aligned (Fei lab) to the C. lanatus Charleston Gray reference genome. A genetic map consisting of 1,525 SNP markers with a 1.2 cM average distance between markers and a total length of 1,744 cM was created. Three QTL for GSB resistance was identified: ClGSB3.1 (R2=14.1%; 76-79.1 cM); ClGSB5.1 (R2=10.2%; 135.3-147.1 cM) and ClGSB7.1 (R2=21.1%; 103.1-116.3 cM) (Fig.1a). **Complete**
- WPop GSB2: DNA from the 12 most resistance and most susceptible F2 lines of PI 189225 x Sugar Baby population was bulked to form a R-bulk and S-bulk, respectively. The bulks sequenced and aligned to the 97103_V2 watermelon genome. Δ-SNP index was then used with a 1Mb sliding window to calculate a smoothed Δ-SNP index as well as 95% and 99% confidence using the R package QTLseqr (Mansfeld & Grumet, 2018). Four significant (95% confidence interval) QTL were detected: ClGSB2.1, ClGSB5.1, ClGSB9.1, ClGSB11.1 (Fig.1b). The QTL on chromosome 5 of the two populations partially overlap. Complete
- KASP markers have been developed for selection of QTL. **In progress**

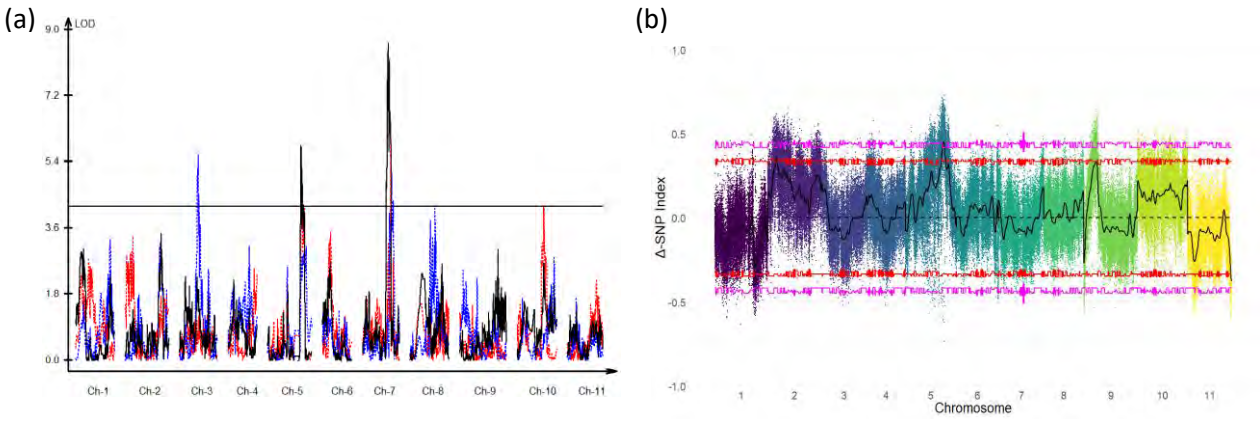


Fig. 1 (a) QTL associated with GSB resistance in experiment 1 (red), experiment 2 (blue) and joint data (black) in the Crimson Sweet (CS) × UGA1081 F2:3 watermelon population (N=178) inoculated with *Stagonosporopsis citrulli*, and (b) ΔSNP index comparing the R-bulk and S-bulk from PI 189225 x Sugar Baby F2:3 population inoculated with *Stagonosporopsis citrulli*. The red and pink lines are the 95% and 99% confidence intervals, respectively.

2. Remaining Goals

- Validate KASP makers for selection and start introgression of QTL in elite backgrounds.

Kai-shu Ling and Amnon Levi USDA, ARS, U.S. Vegetable Laboratory, Charleston, SC

Cucumber green mottle mosaic virus –

- We have completed the initial screening of USDA watermelon germplasm (~1,400 accessions). In the repeated test, several selected lines showed promising level of tolerance to CGMMV (without visible symptom). However none of them was immune to CGMMV, the virus titer were detectable in the tolerant plants using ELISA tests.
- We made single plant selection of the promising lines and are developing segregating populations through crossing. S2 seeds have been generated from one of the most promising *Citrullus colocynthis* line.
- Seeds from seven PI lines with potential for resistance (tolerance) to CGMMV have been sent to the collaborator to generate plant tissue for support the re-sequencing efforts under the CucCAP project.
- We submitted a release notice 'Virus-resistant desert watermelon (*Citrullus colocynthis*) germplasm line 'USVL18-157VR' useful for enhancing CGMMV-resistance in watermelon cultivars. The release notice is currently in the process of review and approval by USDA, ARS, National Program Leaders (NPL).
-

Table 1. Selected lines with potential tolerance to CGMMV were selected for re-sequencing

Test Item Number	Taxon	Seed
138	*Citrullus colocynthis*	30
145	*Citrullus colocynthis*	30
151	*Citrullus colocynthis*	30
157	*Citrullus colocynthis*	30
565	*Citrullus lanatus*	30
570	*Citrullus lanatus*	30
714	*Citrullus lanatus*	30

Remaining goals

- Cucumber green mottle mosaic virus. We are advancing through single plant selection of the most promising *Citrullus colocynthis* line to S3. Those seeds will be provided with the sponsoring seed companies to make crosses to your elite materials. In addition, once the F2, BC1 seeds are generated, materials from segregating populations will be used for Genotyping-by-sequencing or similar study to identify SNPs in association with the tolerance to CGMMV.

Other Products

Product Type

A germplasm line tolerant to CGMMV.

Description

Research materials.

Advanced S2 single plant selection from the most promising tolerance line have been generated, are being advanced to S3 and S4 generation before sharing with seed companies. Several F1 progenies for CGMMV resistance in watermelon will be advanced to segregating F2 and BC1 populations.

Watermelon Core Collection

Our group is increasing 384 *Citrullus* spp. lines (including 300 *C. lanatus* and *C. mucosuspermus*, 50 *C. amarus* and 10 *C. colocynthis* PIs and 24 heirloom cultivars) to S2 generation. Leaves for DNA extraction have been collected from the S1 plants and stored for shipment to Michigan State University. Fifteen S1 seeds of each of the 384 PIs are being collected and tested for presence of bacterial fruit blotch (BFB) using real time PCR (Wechter's Lab). The S2 seeds of each PI will be increased to 1000 S3 seeds by HM.CLAUSE.

Melon Team

Team members:
Jim McCreight (USDA, ARS)
Shaker Kousik (USDA, ARS)
Michael Mazourek (Cornell Univ.)
Pat Wechter (USDA, ARS)
Bill Wintermantel (USDA, ARS)

Table 4. TIMELINE CHART					
Objective	**Personnel/Institution**	**Year**			
	(initials as in Table 3)	1	2	3	4
(a) Obj. 1. Develop common genomic approaches and tools for cucurbits					
1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)					
1.2.1. GBS of cucurbit species, establish molecular-informed core populations - melon	JM (ARS-CA)	X	X		
1.2.2 Population genetics and GWAS analyses - melon	UR (WVSU), ZF (BTI) JM (ARS-CA)		x	x	x

1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)

1.2.1. GBS of cucurbit species, establish molecular-informed core populations

Melon

- GBS analysis of the melon collection (2083 USDA, NPGS accessions, and selected heirloom and lines) emphasized correctly identifying their respective classification with regard to the division of the species into two subspecies, *agrestis* and *melo*, based upon the difference in ovary hair (Pitrat *et al.* 2000. Some comments on infraspecific classification of cultivars of melon. Acta Hort. 510:29–36.). For example, 73 (3.5 %) accessions had no subspecies designations in NPGS passport data. Other accessions were identified as ssp. *melo*, but were known in the literature to be ssp. *agrestis*, e.g., PI 414723.
  - A manuscript is in preparation to report results of population structure analyses using a suite of tools, LD decay, Core collection selections, and GWAS using historical and project-generated data.
- The melon core S_1 production is well underway. About 150 members were selfed in 2019. The balance of the 384 member group is being selfed through summer 2020 with fruit from about 110 accessions harvested as of May 28. Seeds of each member will be increased by an industry partner for deposit in USDA, NPGS.
- Endorna virus (CmEV) analysis of 42 *C. melo* ssp. *agrestis* var. *texanus* was continued in crosses with 'Top Mark' and among selected *texanus* accessions.

(b) Obj. 2. Genomic assisted breeding for disease resistance		Y1	Y2	Y3	Y4
2.1 QTL map resistances:	Screen for resistance (**Sc**), develop populations **(P)**, phenotype (**F**), sequence **(S)**, QTL map **(Q)**				
2.1.2. Melon - powdery mildew - Fusarium - CYSDV - CMV	 SK,PW (ARS-SC), JM (ARS-CA) PW (ARS-SC) JM (ARS-CA), WW (ARS-CA) JM (ARS-CA), MM (CU)	 PF PFS F P	 PFS PFS FS F	 FQ PFSQ FSQ SQ	 FQ

2.1.2. Melon

Powdery Mildew (*Podosphaera xanthii*) resistance in MR-1 x AY (Ananas Yoqne'am) RIL

- Awaiting growth chamber space availability for Race 2 test.

Powdery Mildew (*Podosphaera xanthii*) resistance in Top Mark x PI 313970 $F_{2:3}$

- Resistance to race S was reported to be controlled by the single gene *pm–S*. The Race S isolate used in this test was previously identified/confirmed in a growth chamber test of powdery mildew race differentials. Due to the large number of $F_{2:3}$ families evaluated (161), only one replication was completed. The susceptible check, Védrantais, was fully susceptible, while PI 313970 was about equally split between plants free of infection and those showing limited mycelial growth and sporulation (Table 2.1.2–1). A characteristic reaction of some PI 313970 individuals to powdery mildew is similar to the category 5 rating, but typically more restricted. Even if the 1 and 5 categories are considered resistant, the $F_{2:3}$ segregation was an unacceptable fit to a 3 susceptible: 1 resistant ratio (F = 24.5983, $P_{<0.001}$). The isolate may have become a mixture of races S and SD, which infects PI 313970. The four resistant families are starting points for introgression of race S/SD resistance to western U.S. shipper type cantaloupe.

Table 2.1.2–1. Reaction of Top Mark x PI 313970 $F_{2:3}$ to cucurbit powdery mildew race S in a growth chamber experiment.

		Disease severity[z]		
Entry	n	1	5	9
$F_{2:3}$	161	4	9	148
PI 313970	7	4	3	0
Védrantais	9	0	0	9

[z]rated on a three-point scale where 1=no disease, 5= limited mycelial growth and sporulation, and 9=abundant growth and sporulation.

- Two replicated field tests of cucurbit powdery mildew race differentials subjected to natural infection were planted in Imperial Valley. University of California, Desert Research and Extension Center, Holtville; watered 8 March 2018. The race present was similar to race 3 (Table 2.1.2–2).

Table 2.1.2–1. Reactions of 13 cucurbit powdery mildew race differentialsto natural infection in Imperial Valley at the University of California, Desert Research and Extension Center, Holtville;

Entry	Mean disease reaction[z]	Summary reaction
Iran H	9	S
Top Mark	6.5	S
Vedrantais	9	S
PMR 45	6.17	S
PMR 5	4.67	S
PMR 6	5.33	S
WMR 29	4.5	S
Edisto 47	2.5	R
PI 414723	1.67	R
MR-1	1	R
PI 124111	1	R
PI 124112	2.83	R
PI 313970	1	R

[z]rated on a 1 (no disease) to 9 (>75 % of adaxial surface covered with hyphae and spores, numerous or coalesced colonies on the abaxial surface).

- A second field test was planted in the Central Valley at University of California, Westside (Westside Research and Extension Center, Five Points; Planted 25 June 2018; insufficient infection to evaluate.

Downy mildew

- Phenotyped resistance in the MR1 x AY RIL.

CYSDV

- PI 313970 x Top Mark–Evaluated 200 F2:F3 progenies for phenotypic reaction and relative virus titer analyses in two field tests in Imperial Valley at the University of California, Desert Research and Extension Center, Holtville. The Spring test was watered 7 March and the Fall test was watered 16 August 2018. Infection by two viruses was confirmed: CYSDV and *Cucurbit chlorotic yellows virus* (CCYV). Their symptoms on melon are nearly identical. As a result virtually all plants exhibited foliar yellowing symptoms typical of CYSDV and CCYV. Phenotypic data (foliar yellowing) were not useful for mapping CYSDV resistance QTL, as plants resistant to CYSDV in the $F_{2:3}$ population exhibited yellowing symptoms from CCYV infection. QTL analysis in both tests of the relative titer of CYSDV calculated from RT-qPCR data identified one locus on chromosome 5 that explained 34-38% of the variation in CYSDV titer. Our result confirmed the previous report of a CYSDV resistance QTL on chromosome 5 in TGR 1551 (PI 482420) based on yellowing symptoms and virus titer. Markers flanking this QTL can be utilized in marker assisted breeding of CYSDV-resistant melons.

CMV

- Selfed progenies from four of the 25 advanced Cornell University CMV-resistant melon lines developed by M. Kyle-Jahn and H.M. Munger were evaluated in a field in a field planting at University of California, Westside Research and Extension Center (WSREC), 5-Points. Aphid-borne viruses such as CMV and WMV are common in this area. The test was watered June 25. The test was relatively free of melon aphid, which caused extensive damage in 2018, so the test provided a much better opportunity to evaluate the horticultural qualities of the lines (Table 2.1.2–3). CMV and WMV were present throughout the field. The border rows of 'Golden Beauty Casaba' exhibited pronounced symptoms from co-infection by CMV and WMV. The Cornell lines exhibited mild mosaic symptoms, and were negative for CMV and positive for WMV. Progenies from lines 17-4068-1 and 17-4069-2.2 yielded fruit closer to western U.S. shipper type than the other two lines. The canopy of 17-4069-2.2 was noticeably more open, than the other three lines, 'Top Mark' and 'Greenflesh Honeydew'.

Table 2.1.2–3. Field notes of seven progenies from four Cornell University CMV-resistant melon breeding lines, 2019, University of California, Westside Research and Extension Center (WSREC), 5-Points, and comparison of fruit characters with U.S. western shipper type cantaloupe ('Top Mark') and 'Greenflesh Honeydew'. Note: all Cornell lines exhibited mild virus mosaic symptoms determined to have resulted from infection by *Watermelon mosaic virus* (WMV).

			Plant		Fruit							Flesh color
Line	ARS pedigree	n plants	Size[z]	Cover[y]	Earliness	Shape	Net	Vein tracts	Ribbing	Slip	Blossom scar	
17-4068-1	38379	5	7			globular	light warts	smooth	intermediate superficial	no	large	orange
	38381	1	3			globular		smooth				
17-4069-2.2	38382	2	6	open	early	globular	light	smooth	intermediate	no	medium	green
	38383	5	6	open	early	elliptical	light	variable	intermediate superficial			
	38384	1	6	open		globular	light	variable				
17-4074-2	38387	2	9			flattened[y]	variable	smooth	intermediate	no	medium	orange
17-4075-21	38386	4	6			elliptical[y]	light	smooth	intermediate	?	medium	orange
Top Mark		–	–			elliptical	heavy	none	none	yes	small	orange
Greenflesh Honeydew		–	–			elliptical	none	none	none	no	small	green

[z] 1 to 9 scale, where 1 is very small and 9 covers a standard 80-inch bed.

[y]open canopy exposed fruit to possible sunburn

[x]segregated in 2018

2.2 Marker development and verification

2.2 Marker development and verification:	Refine map (**R**) develop marker **(M)**, verify **(V)**				
2.2.2. Melon					
- powdery mildew	SK (ARS-SC)			RM	V
- Fusarium	PW (ARS-SC)	M	RM	RM	V
- CYSDV	WW (ARS-CA), JM (ARS-CA)			RM	V
- CMV	JM (ARS-CA), MM (CU)			RM	V

Powdery and Downy Mildews

- QTL have been identified for powdery mildew race 1 derived from MR-1 in the MR-1 x AY (Ananas Yoqne'am) RIL. KASP primers have been developed and currently being validated in the RIL.
- QTL have been identified for sulfur tolerance derived from AY in the MR-1 x AY RIL. KASP primers have been developed and have been validated in the RIL. The top five KASP markers that both target and flank the genetic region of interests are being validated in an additional sulfur susceptible and resistant (tolerant) cultivars.
- Identified QTL for downy mildew resistance in the MR-1 x AY RIL population.
- In process of completing sequencing (short-read, long read, 10X genome and Pacbio) of MR-1 and Ananas Yoqne'am.

Fusarium wilt

- KASP primers have been developed and validated in the recombinant inbred lines (MR-1 x AY) for QTL associated with *Fusarium oxysporum* f. sp *melonis* race 1 and race 2. The top five KASP markers that both target and flank the genetic region of interests are being validated in an additional population derived from MR-1.

CYSDV

- A total of 1,038 GBS SNP markers were utilized to develop the genetic linkage mapping in MapDisto 2.0 (Lorieux, 2012) and the resulting maps contained 12 linkage groups corresponding 12 chromosomes of melon. Qgene 4.0 (Johanes and Nelson 2008) was used for QTL mapping analysis.

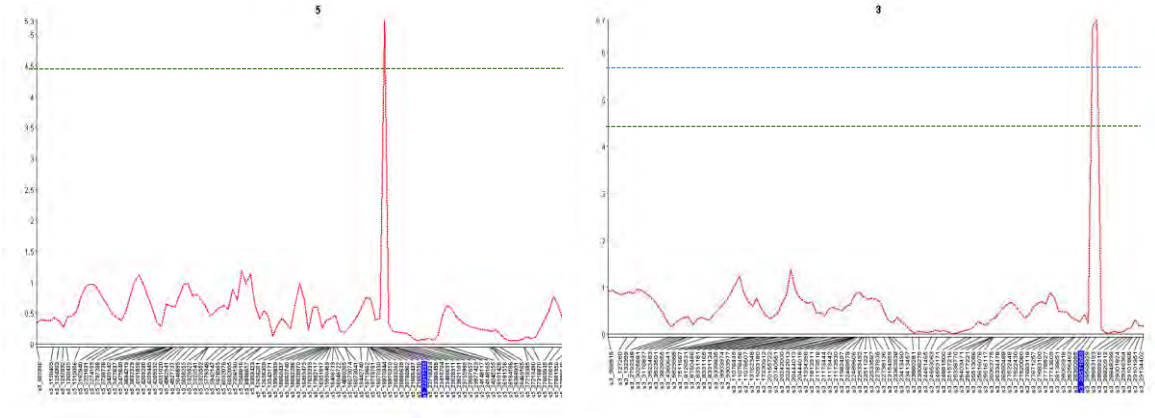
- One QTL on chromosome 5 located at physical position of 22217535 bp was identified that explained ~16% of the variation in CYSDV titer for Fall 2018 (Fig 2, Table 1). The significant marker associated with this QTL is S5-22217535 and was significant at only α level 0.05. Another QTL on chromosome 3 was also identified at a physical position of 28571859 (R^2 = 20%) which was significant at both α level 0.05 and 0.01 (Table 1).

Table 1: QTL identified in year 2018 and 2019.

Chromosome	Year	Significant markers	Interval (cM)	R^2	Physical position (bp)	LOD
5	2018	S5-22217535[z]	149.15 cM	16	22217535	5.3
	2019	S5-20880639[z,y]	145.31 cM	35	20880639	8.12
3	2018	S3-28571859[z,y]	218.81 cM	20	28571859	6.7

[z]Significant at alpha level 0.05.

[y]Significant at both alpha level 0.01.



A B

Fig 2. Composite interval mapping of chr 5 (A) and chr 3 (B) showing significant CYSDV resistance QTL in the Top Mark x PI 313970 F_2 populations in Fall of 2018. The x-axis represents the markers and the y-axis represents the LOD values. LOD threshold was calculated by 1000 permutations at a 0.05 and 0.01 significance level and are denoted by blue and green lines, respectively. The LOD and R^2 values for chromosome 5 QTL are 5.74 and 16%. The LOD and R^2 values for chr 3 QTL are 6.7 and 20%. The significant marker on chr 5 was S5-22217535 which is located at physical position of 20880639 bp and the significant marker on chr 3 was S3-28571859 located at physical position of 28571859.

- Similarly, with 2019 fall data, a QTL was identified on the same chromosome 5 in proximity of 2018 QTL which is approximately 1.3 mb away from S5-22217535 (Fig 3, Table 1). The significant marker associated with this QTL is S5-20880639 that explained ~35% of the variation in CYSDV titer for Fall 2019 (Fig 1, Table 1). This QTL was significant at both α level 0.05 and 0.01.

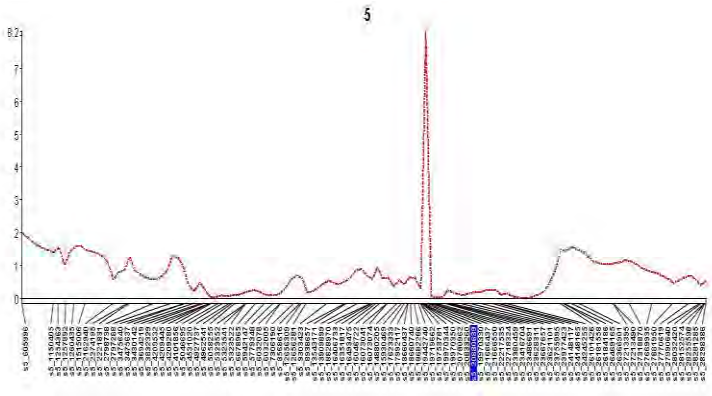


Fig 3. Composite interval mapping of chr 5 showing significant CYSDV resistance QTL in the Top Mark x PI 313970 F_2 populations in Fall of 2019. The x-axis represents the markers and the y-axis represents the LOD values. LOD threshold was calculated by 1000 permutations at a 0.05 significance level is denoted by blue line and at 0.01 is represented by green line. The LOD and R^2 values for chromosome 5 QTL was 8.12 and 35%. The significant marker S5-

20880639 is located at 20880639 bp on chr 5.

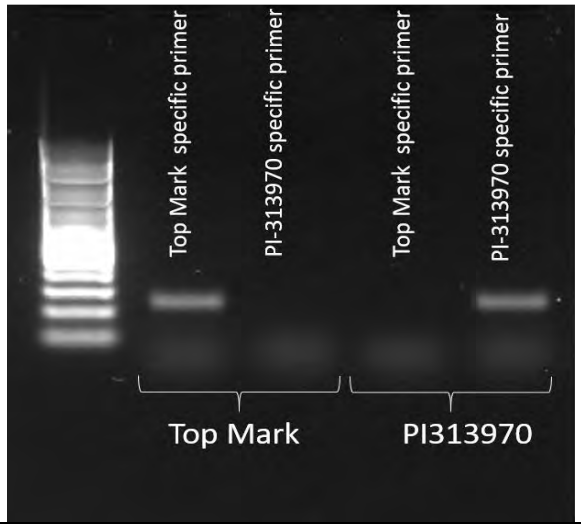
Our result confirmed the previous report of a CYSDV resistance QTL on chromosome 5 in TGR 1551 (PI 482420) based on yellowing symptoms and virus titer (Palomares-Ríus et al., 2016). Markers flanking this QTL can be utilized in marker assisted breeding of CYSDV-resistant melons. This study showed the utility of molecular data for genetic analysis of resistance to one specific virus when co-infection by a second virus induces identical symptoms.

We searched the S5-20880639 marker sequence along with some other markers in proximity from http://cucurbitgenomics.org/JBrowse/ and designed the marker based on the single nucleotide polymorphism between two parents 'Top Mark' and PI 313970 as shown below. Parent specific sequence tagged site (STS) markers were developed for SNP marker S5-20880639 which served as a co-dominant marker (Fig 4). The PCR reaction parameter were optimized by increasing the annealing temperature to increase the specificity of the STS primers. Other primers did not perform well to differentiate the two parental lines. We will have to find another marker that works better and will have two flanking markers delimiting the QTL region of our interest.

>chr5 chr5:20880052..20880995 (S5-20880639 sequence)
GAGGACAACAAACCACTGGTTGAAACAGAGGAAGAACCCCAACAGAAGGAAGAGGAGGTTGCTGAACCTC
CACCTCTAATAGAAACCCACGACCCAAGTGATCTTCTGGTAAGAATTTTCAGATAAAGCATTTTCTTGAC
ATGTTTAACTGCCTCTCCCATTACATCCAGTGATAATTTCCCATGCAGGGTCTGAATGAAATAAATCCCA
AAGCTGCAGAAATAGAAGAAAGCAATGCTTTAGCTCTTGCTATTGTTACAAATGGTAG/AGTTGTCCGCC
ATTTAGCTTTGTAATGTCTG/TAGCTTATTTTCCATGAAGAGTCTATCAGCGATGCAGCAACTACCTCTA
CTCTGGCACATCTTTTGTGTGTTTATTTGCAAATCATATAGACTGACAAAGTAAATTCAGAGTTGAAAAT
TCTATCTTTTCCAATTGTTGCAATTATAGAGGTTTTGTCCGTCCATTTATCTAATGAAGTTTTTAATTAT
ATGCAGGGAATGACCCATCTTCTTCAAATCGTGCATTGAGTGAAATTGGCGGTAGTGGTTGGGAGCTAGC
GCTTGTTACCACACCAAGCAATAATACTGGTCCTTCTGTCGAAGGCAGACTGGTTTTTCCCTTCCCCT

Top Mark-specific primer (G/G allele)
20880639-F1: CTGCCTCTCCCATTACATCCAG
20880639-TM-R1: CAAAGCTAAATGGCGGACAACC

PI 313970-specific primer (TT allele)
20880639-F1: CTGCCTCTCCCATTACATCCAG
20880639-PI313-R1: CAAAGCTAAATGGCGGACAACT

Fig 4. Genotyping of 'Top Mark' and PI 313970 with TM specific and PI 313970-specific primers. The 'Top Mark'-specific primers amplified only from TM and PI 313970 amplified only from PI 313970	

For further validation of this marker S5-20880639, we genotyped possible CYSDV resistance PI lines. The genotypes of PI 122847, PI 614213, PI 614185, PI 482431 and PI 116482 were all PI 313970-like (Table 2.2–1). However, the genotype AMES 26704, PI 123496, PI 116482 are 'Top Mark'-like.

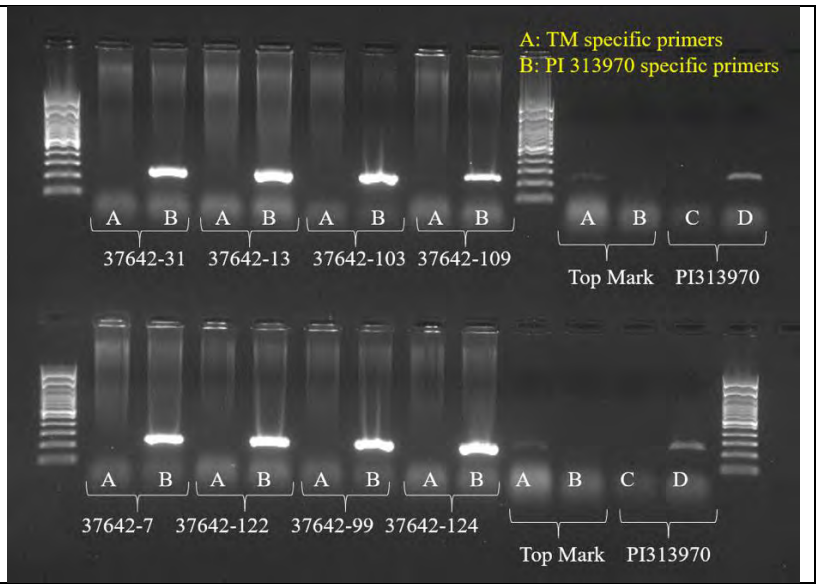
Since, the F_1 of Top Mark x PI 123496 is 'Top Mark'-like, PI 123496 must be in heterozygous state. We need to test more of the PI 123496 and F_1 lines made with this PI 123496.

Table 2.2–1. Assay of PI 313970 marker flanking CYSDV resistance QTL.

CYSDV resistance source or progeny	PI 313970-like marker[z]
F_1PI 145594 x Impac	+/-
F_1Top Mark x PI 123496	-
PI 116482	-
PI 122847	+
PI 123496	+/-
PI 145594	+
PI 482431	+
PI 614185	+
PI 614213	+

[z]+= presence of marker, -= absence of marker, and +/-= heterozygote

We genotyped with this new marker (S5-20880639) eight $F_{2:3}$ Top Mark x PI 313970 plants in eight different families that had low virus titer, and they were all PI 313970-like (Figure 5).

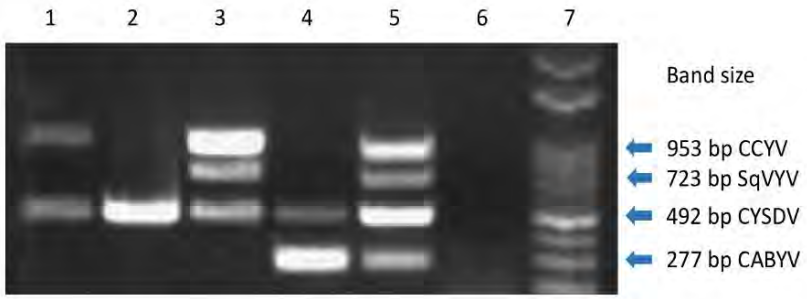
Figure 5. Eight individuals in eight $F_{2:3}$ with low CYSDV titers possessed S5-20880639 marker present in PI 313970 but not in 'Top Mark'. Conversley, 'Top Mark' primers were absent in the eight progeny plants.	

CMV

- No progress to date.

CCYV–*Cucurbit chlorotic yellows virus*

- During the summer of 2018, melon plants from a germplasm diversity study in the Imperial Valley, CA were found infected with *Cucurbit chlorotic yellows virus* (CCYV; genus *Crinivirus*, family Closteroviridae). Two melon plants were found exhibiting interveinal yellowing and chlorotic spot symptoms similar to those caused by a crinivirus, but varying from symptoms normally observed during infection by *Cucurbit yellow stunting disorder virus* (CYSDV; genus *Crinivirus*). Further studies will be necessary to evaluate epidemiology of CCYV in the southwestern U.S. desert production region, and to determine its impact on melon production and development of crinivirus-resistant cultivars. (See Wintermantel et al. 2019). To this end, a multiplex primer system has been developed to allow detection and differentiation of CCYV, CYSDV, SqVYV, and the aphid-transmitted polerovirus, *Cucurbit aphid-borne yellows virus* (CABYV) which produces symptoms identical to those of CCYV and CYSDV on cucurbit plants (**Figure 6**). Similarly, a RT-qPCR primer set was also developed for single reaction quantification of CCYV, CYSDV and SqVYV that allows determination of concentrations for each virus in melon breeding lines (not shown). These primer sets can also be used on other cucurbit crop plants for resistance evaluation.



Lane 1. Melon – CCYV + CYSDV mixed source
Lane 2. Melon – CYSDV source
Lane 3. Field melon – 2015
Lane 4. Field zucchini – 2017
Lane 5. Mixture of 4 viruses (pos. control)
Lane 6. Non-infected melon
Lane 7. DNA size marker

Figure 6. Single test detection of four major viruses impacting U.S. cucurbit production: CYSDV, CCYV, SqVYV, and CABYV.

- The multiplex RT-PCR system identified a prevalence of CCYV in limited Spring sampling in 2019, where all yellowed melons were positive for CCYV, and two had mixed infection of CCYV and CYSDV. No other whitefly-transmitted viruses were identified in the spring sampling. The purpose of the limited spring sampling was primarily to evaluate the performance of the new multiplex detection systems and therefore was limited in scope.

- Results of a more extensive fall sampling of melons crops throughout the western Arizona and southern California desert production regions demonstrated an abundance of CYSDV among the Fall melon samples from throughout the region with 76 % of plants sampled (26/34 plants) testing positive for CYSDV (**Table 3**). CCYV was also detected in 38 % of plants tested, and in 50 % (13/26) of the CYSDV infected plants. No single infections of CCYV were identified during the fall season. Some melon fields had nearly universal co-infection of both CYSDV and CCYV. SqVYV was detected in 24 % of plants tested (8/34), and in 31 % of the CYSDV infected plants. Like CCYV, all SqVYV infected plants were also co-infected with CYSDV. Interestingly, all SqVYV-infected plants were from Arizona; no SqVYV was detected in samples collected from California in the fall of 2019. CABYV is aphid-transmitted and symptoms resemble those of CYSDV and CCYV, which is why it was included in the multiplex detection system. CABYV is more prevalent in the Central Valley of California than in the Low Desert region, but we did identify four melon samples with CABYV infections, all from Arizona. Three of these melon plants were infected with CABYV alone, whereas one was co-infected with CYSDV. These results strongly suggest CYSDV remains the dominant virus in the region during the Fall season, and contrasted with the results of the more limited Spring sampling in which CCYV was by far the most prevalent of the viruses.

Table 3. Virus incidence among commercial fields and research plots[1] in low desert production regions of California and Arizona sampled in September 2019.

Sample #	CYSDV	CCYV	SqVYV	CABYV
1	+[2]	–	–	–
2	+	–	–	–
3	+	–	+	–
4	+	–	+	–
5	+	–	–	–
6	+	–	–	–
7	+	+	+	–
8	+	+	–	–
9	+	+	–	–
10	+	–	+	–
11	+	+	–	–
12	+	+	+	–
13	–	–	–	–
14	+	–	+	–
15	+	+	–	–
16	–	–	–	–
17	+	–	+	–
18	+	–	+	–
19	–	–	–	–
20	–	–	–	–
21	–	–	–	+
22	–	–	–	+
23	–	–	–	+
24	+	–	–	
25	+	–	–	+
26	–	–	–	–
27	+	+	–	–
28	+	–	–	–
29	+	+	–	–
30	+	+	–	–
31	+	+	–	–
32	+	+	–	–
33	+	+	–	–
34	+	+	–	–
CYSDV positive	+	–	–	–
CCYV positive	–	+	–	–
SqVYV positive	–	–	+	–
CABYV positive	–	–	–	+

[1] Not including results at DREC.

[2] + = positive, – = negative

2.3 Introgress resistance into advanced breeding lines

2.3. Introgress resistance into advanced breeding lines:	Develop breeding lines **(B),** introgress into cultivated **(I)**, advanced lines **(A)**, release to breeders **(R)**				
2.3.2. Melon					
- powdery mildew	SK (ARS-SC), JM (ARS-CA)	B	I	I	IA
- Fusarium	PW (ARS-SC)	B	B	I	IA
- CYSDV	JM (ARS-CA), WW (ARS-CA)	I	I	IA	IAR
- CMV	JM (ARS-CA)	I	I	I	IA

- Generated backcross populations of USVL206 (Majik Melon) x Top Mark and USVL206 (Majik Melon) x Charentais.
- Making selections from the backcross populations using KASP markers for a subsequent BC_2 populations.
- KASP selection will identify individuals with all resistant markers, as well as select resistances for use in developing additional KASP validation populations and fine mapping analysis.

Cucumber Team

Team members:
Yiqun Weng (USDA, ARS)
Rebecca Grumet (Michigan St. Univ.)
Todd Wehner (North Carolina St. Univ.)

Objectives	Personnel/Institution	2016	2017	2018	2019
1. Develop genomic approaches and tools					
1.2. GBS PI lines; establish GWAS core	ZF (BTI), RG (MSU) YW (ARS-WI)	X X	X X		
1.2.1. GBS of cucurbit species, establish molecular-informed core populations - cucumber	ZF (BTI), RG (MSU) YW (ARS-WI)	X X	X X		
1.2.2 Population genetics and GWAS analysis - cucumber	UR (WSVU), ZF (BTI) YW (ARS-WI), RG (MSU)		x	x	x
2. Genomic assisted breeding					
2.1 QTL map resistances	Sc=Screening, P=populations, F=phenotyping, S=sequence (S), Q=QTL				
2.1.3. Cucumber - downy mildew - Phytophthora	YW (ARS-WI), TW (NCSU) RG (MSU)	PFS PF	SQ PFS Q	SQ SQ	
2.2 Marker development and verification	R=Refining map, M=develop marker, V= verification				
2.2.3. Cucumber - downy mildew - Phytophthora	YW (ARS-WI), TW (NCSU) RG (MSU)	RM	RM	V RM	V V
2.3. Advanced breeding line development	B=breeding line**,** I=introgression, A=advanced, R=release				
2.3.3 Cucumber - DM - PFR	YW (ARS-WI), TW (NCSU) RG (MSU)	B B	I B	I I	R I

1.2 GBS of PI collection, establish GWAS core
Personnel: Weng (Wang Y, Tan J, Dymerski R), Grumet (Grumet R, Hammar S.) and Wehner (Wehner T., Silverman EJ) Labs

GBS of PI lines and GWAS panel selection

GBS has been completed for 1234 cucumber accessions including plant introduction (PI) lines and historical cultivars or landraces of cultivated (*Cucumis sativus* var. *sativus*) and wild (*C. sativus* var. *hardwickii*) cucumber lines. Data analysis was been performed by the bioinformatics team to identify SNPs, determine minor allele frequency, perform phylogenetic, population genomic, and linkage disequilibrium (LD) analysis. A core collection consisting of 392 lines was constructed which captures >95% of allelic diversity as well combined with representation of key disease resistance, fruit quality and agronomic features. This part of work was recently published in the journal, Horticultural Research (Wang et al., 2018).

Seed increase and selfing was started for the GWAS panel. Among the 390 lines, we requested fresh seeds from USDA collection for 119 lines. All 390 lines have undergone at least at least two-generation of selfing. Seed increase for 188 lines is underway. We have also re-sequenced one and half plates of samples 144 lines) at ~10× coverage.

Phenotyping of morphological traits and DM resistance in cucumber core population.

Three hundred cucumber lines were grown in the University of Wisconsin Hancock Agricultural Research Station (HARS) for collection of morphological data. Meanwhile, 100 cucumber lines (2 reps, 6 plants per rep) were planted in North Carolina State University experimental field in summer 2019. Data for responses to DM natural infestation were collected. Unfortunately, the data were incomplete due to weather-related inoculation failure.

2.1 and 2.2: QTL mapping, marker development for DM and PFR resistances

Downy mildew (DM) (Weng and Wehner Labs)

2019 progress

We aim to conduct QTL mapping of DM resistance from two resistant sources: PI 330628 (WI7120) and PI 197088. We previously identified two major-effect QTL *dm4.1* and *dm5.2* for DM resistance from WI7120 (Wang et al. 2016). Using the PI 197088×Coolgreen RIL population, we also identified 4 major- or moderate-effect QTL, *dm4.1, dm5.1, dm5.2*, and *dm5.3* for DM resistance in PI 190788; *dm5.3* is co-localized with *pm5.1* (syn. *CsMLO1* or *CsMLO8*, *pm-h*), which is a major-effect QTL for PM resistance in cucumber (Wang et al. 2017). We focused on three major-effect DMR QTL, *dm4.1*, *dm5.2* from WI7210 and *dm5.3* from PI 197088 for fine mapping.

F_2 and RIL plants carrying respective QTL regions were selected to backcross with the susceptible cucumber line 9930. Near isogenic lines (NILs) for each QTL were developed in the susceptible 9930 genetic background. We have completed marker-assisted development of NILs for *dm4.1* and *dm5.2*. Secondary F_2 populations from crosses between resistant and susceptible NILs were developed, which were genotyped for DM inoculation responses in both field and controlled environments. The development of NILs for *dm5.3* has been advanced to BC_2.

Through QTL analysis in the secondary F_2 populations, the *dm4.1* and *dm5.3* loci have been delimited to ~33 and 68 kb intervals on chromosomes 4 and 5, respectively. Growth chamber and field evaluation of DM resistance of the NILs was conducted in collaboration of industry collaborators.

Phytophthora capsici fruit rot resistance in cucumber (R Grumet lab – YC Lin, B Mansfeld)

Young fruit resistance to *P. capsici*

SNP-based linkage analyses are being performed to identify disease resistance QTL from crosses between the susceptible, sequenced pickling cucumber breeding line, Gy14, and two PI109483-derived breeding lines using three populations. Phenotyping of F2 populations exhibited normal distributions for disease scores. Individuals representing the most resistant and most susceptible plants were selected for bulk segregant QTL-seq analysis. There was good correspondence between peaks observed on chromosomes 5 and 6 from field grown plants in 2018 and 2019.

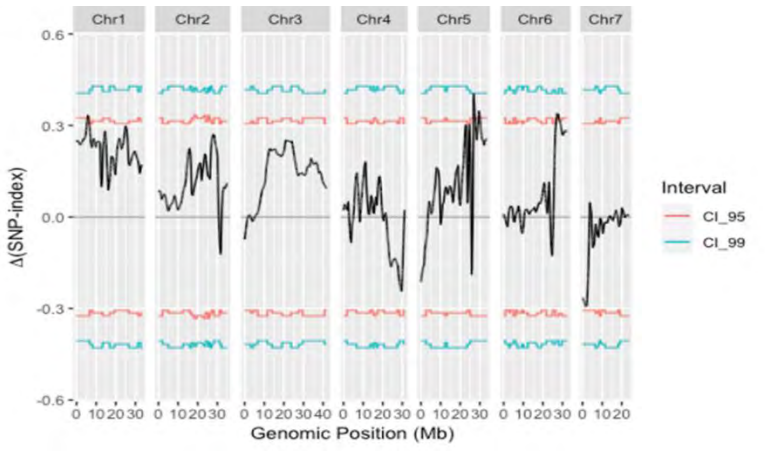
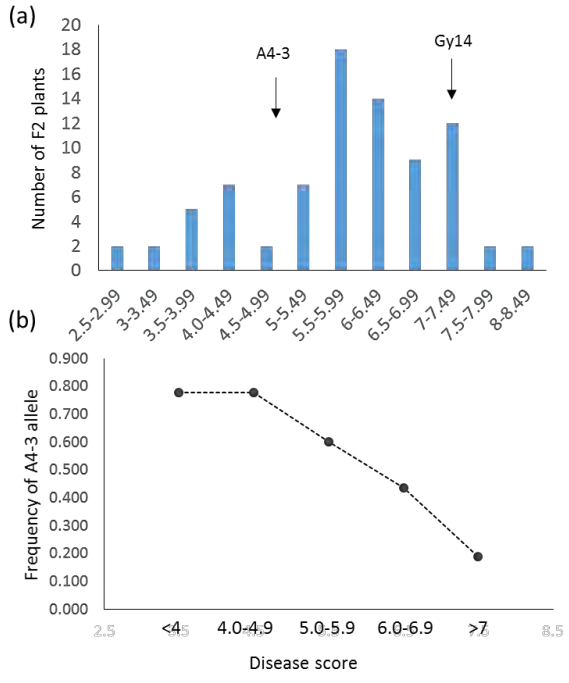


Figure 1. QTL-seq analysis of response of young cucumber fruit to *P. capsici* (data from field trial, summer 2018). Red and green lines - significance P,0.05, 0.01, respectively.

2019/2020. KASP markers flanking the putative QTL on chromosomes 5 and 6 were designed to verify QTL and narrow the QTL intervals. A population of F_2 seedlings (n=768) was planted in the greenhouse in late spring 2019. Non-recombinant individuals for the QTL on either chromosome 5 or 6 were selected for QTL verification. Plants were grown in the field in summer 2019 and



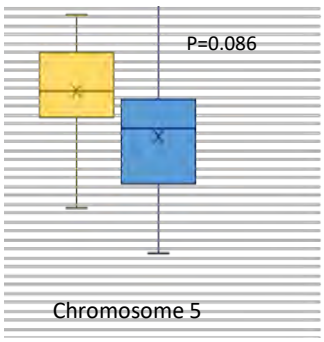


Figure 3. **(a)** Distribution of disease ratings of young fruit harvested from F_2 individuals [Gy14 x PI109483-53B] non-recombinant for the putative resistance QTL on chromosome 5 and/or 6. . Ratings at 5 days post inoculation. (Population size=82; 10-30 fruits were sampled from each plant over three harvests to provide replication in sampling dates). **(b)** Frequency of A4-3 allele at chromosome 5 QTL as a function of disease rating.

inoculated in the laboratory with *P capsisi*, isolate Bartley's 1.
Disease screening of F_2s supported a role for disease resistance for the QTL on chromosome 5. The frequency of the A4-3 was very high (0.78) in the most resistant plants and dropped to 0.19 in the most susceptible plants (Figure 3b). The QTL on chromosome 6 did not appear to correlate with resistance.

Recombinant individuals within the QTL regions were selected to refine the QTL interval. We are currently producing $F_{3:4}$ seed for testing this summer.

To enable future GWAS analysis, seed for 267 accessions of the cucumber core population that were available, were tested for response to *P. capsisi*. Three plants per line were grown in a completely randomized design in the field in summer 2019; 20-40 fruit were sampled per line. Depending on restrictions, the remainder of the population will be tested in 2020 or 2021.

Age-related resistance (ARR) to *P. capsici*

QTL mapping of ARR. F2 progeny and doubled haploid (DH) lines derived from F_1 progeny of 'Gy 14' (ARR-) X 'Poinsett 76' (ARR+) were tested for ARR for QTL-seq analysis. In both populations a single QTL was identified on chromosome 3.

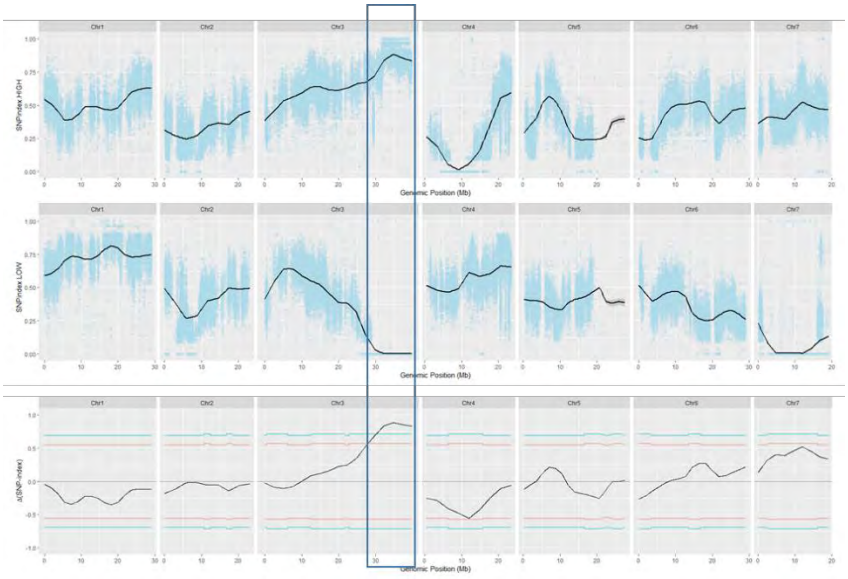


Figure 2. QTL-seq analysis of ARR of cucumber fruit to *P. capsici*. Red and green lines - significance, P,0.05, 0.01, respectively.

2019/2020. KASP markers flanking the QTL on chromosome 3 were designed to initiate fine mapping. A population of F_2 seedlings (n=768) was screened to identify recombinants in this region. $F_{3:4}$ seed has been produced from 41 recombinant families. These families will be genotyped with an expanded set of KASP markers to identify points of recombination. Pending permits, the F4 families will be phenotyped in the greenhouse in summer 2020.

Phenotyping the cucumber core collection.
Phenotyping of the core collection for fruit traits and resistance to *P. capsici* was initiated with 267 lines (S2 or S3 generation, 3 plants/accession) in summer 2019 as a prelude to future GWAS analysis. The core accessions exhibited variation for response to *P. capsici*.

Figure 3. Phenotypic analysis of the cucumber core collection for fruit morphology and resistance to *P. capsici*. 30-50 fruits were tested for *P. capsici*/PI

2.3 Advanced line development for downy mildew resistance
<u>Marker-assisted QTL pyramiding</u> *(Weng and Wehner Labs)*
Our objective is to develop a new version of the elite pickle cucumber inbred line Gy14 with improved DM resistance to the post-2014 DM strain. We focused on marker-assisted pyramiding of the two major-effect QTL (*dm4.1* and *dm5.2*) of DM resistance from WI7120 into Gy14 genetic background. Crosses were made between Gy14 and plants carrying *dm4.1* and *dm5.2* QTL from WI7120/PI 197088. In 2019, homozygous lines carrying *dm4.1*, *dm5.2* and both in Gy14 background (*dm1*) were selected from BC3F2. Plants of the three NILs (*dm4.1, dm5.2*, *dm4.1+dm5.2*) and control (Gy14) plants were gown in the University of Wisconsin Hancock Agricultural Research Station for evaluation of horticultural traits. These lines plants were also tested for DM inoculation responses in controlled environments (growth chambers, climate control rooms, artificial inoculation) and open fields (natural infection). Single aNIls and NIL carrying both QTL consistently performed better for downy mildew resistances in oth field and control environments. No linkage drag was found for either QTL on other horticultural traits. A

manuscript is being prepared for public release of the introgression lines carrying *dm4.*1, *dm5.2*, and *dm4.1+dm5.2* (in Gy14 background).

Breeding line development for DM resistance

(Wehner lab: T Wehner, EJ Silverman)

RIL development and evaluation of DM resistance.

The RILs population was developed in 2007 by a cross PI 197088 (HR) × Coolgreen (S). A total of 200 F_2 lines were generated and self pollinated in the greenhouse in 2009. The RILs have been tested in 7 years of field evaluations under high disease intensity. The 2017 population contains 146 lines; 71 at S12 generation, 35 at S11 generation, 32 at S10 generation, and 8 at the S9 generation. Several lines are being recovered and advanced for use in genetic studies.

In 2016, we evaluated for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white).

In 2017, we evaluated for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white). The RILs ranged from 2.0 to 8.0 for best rating (0-9 scale) for DM resistance. The RILs ranged from 2.7 to 8.0 for fruit quality rating (9-1). Five RILs had DM resistance of 2.0 to 4.3 and fruit quality of 5.0 to 8.0, making them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

In 2018, we evaluated the 127 sublines in S_8 to S_{13} for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Sublines were rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white). The RILs ranged from 2.0 to 7.7 for best rating (0-9 scale) for DM resistance. The RILs were tested for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

In 2019, we will evaluate sublines for high resistance to the new downy mildew in the field in North Carolina. The design will be a randomized complete block with 3 replications and 4 disease ratings. Sublines will be rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white). The RILs usually range from 2.0 to 8.0 for best rating (0-9 scale) for DM resistance. The RILs will be tested for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars. We will also advance nine sublines that had high resistance and good fruit quality for use by industry.

Inbreds with resistance and quality

The population PI 197088 (HR) × Poinsett 76 (MR) contains 72 lines. The plants have been self-pollinated in the greenhouse 8 generations and tested in the field for evaluation of yield, quality and resistance. We recovered 9 lines of the 72 that did not advance to S8 in the past greenhouse cycle. We were not able to recover 3 lines last greenhouse cycle and these lines are in the S7 generation. Lines in S6 and S7 are being tested in the field for yield, earliness and quality for release to the industry.

We selected and self-pollinated sub-lines from 41 lines that are at the S8 to S9 generation in the greenhouse in 2016. The lines were evaluated for high resistance to the new downy mildew, as well as fruit quality, in the field in North Carolina. The most resistant lines were crossed in the greenhouse using parents that had intermediate fruit quality, with the objective of improving fruit quality among the highly resistant lines.

In 2017, we evaluated sublines for high resistance to the new downy mildew as well as fruit quality in the field in North Carolina. A total of 38 sublines were evaluated in a randomized complete block with 3 replications and 4 disease ratings. The RILs ranged from 2.0 to 8.0 for best rating (0-9 scale) for DM resistance. The RILs ranged from 2.7 to 8.0 for fruit quality rating (9-1). Five RILs had DM resistance of 2.0 to 4.3 and fruit quality of 5.0 to 8.0, making them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

In 2018, we evaluated sublines for high resistance to the new downy mildew as well as fruit quality in the field in North Carolina. Lines were evaluated in a randomized complete block with 3 replications and 4 disease ratings. The RILs were selected for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

In 2019, we will evaluate sublines for high resistance to the new downy mildew as well as fruit quality in the field in North Carolina. Lines will be evaluated in a randomized complete block with 3 replications and 4 disease ratings. The RILs will be selected for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

Develop inbred cucumber populations. Three populations (PI 197088 × Gy14, NC-25, or Poinsett 76) are being developed for inbred development of pickling and slicing type. Eight to 10 lines each have been selected with yield, earliness, quality and resistance. They will be released to industry for use cultivar development. In 2016, we advanced the most resistant families that also had acceptable fruit quality by self pollination in the greenhouse. There were 3 populations of 8, 9 or 10 families each (S1 to S4 generation) to make 1 or 2 sublines each. The resulting 50 families were tested for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also evaluated for fruit quality. Lines were evaluated for fruit quality on a 1 to 9 scale (1=poor, 9=excellent). A total of 3 lines were selected based on field data collected in 2016. The selected lines were self pollinated and also cross pollinated in pairs in fall 2016 to develop more highly resistant cucumber populations with better fruit quality.

In 2017, 54 lines (including checks) from the three populations (PI 197088 x Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The design was a randomized complete block with 3 replications and 4 disease ratings. Of those, 4 lines from Gy14, 3 lines from NC-25, and 2 lines from Poinsett 76 were advanced since they had resistance of 3 to 5 and quality of 5 to 7.

In 2018, lines (including checks) from the three populations (PI 197088 x Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The design was a randomized complete block with 3 replications and 4 disease ratings. The most resistant lines with high fruit quality were advanced.

In 2019, lines (including checks) from the three populations (PI 197088 x Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The design was a randomized complete block with 3 replications and 4 disease ratings. The most resistant lines with high fruit quality and high yield were advanced. Those were 2 lines of Gy14, 4 lines of NC-25, and 2 lines of Poinsett 76.

Identify new sources of resistance.

A new population derived from PI 605996 (HR) × 'Poinsett 76' is being developed to provide new sources of high resistance to downy mildew. The F_2 progeny will be self-pollinated and the S1 lines tested in the field for high resistance to natural disease incidence of downy mildew at the Clinton, NC research station. In addition to resistance, lines will be selected for yield, earliness and quality.

In 2017, we produced sublines (S2) and backcross lines (BC1S1) from PI 605996 x Poinsett 76 that will be tested for high resistance to DM as well as fruit quality.

In 2018, we produced sublines (S4) and backcross lines (BC1S3) from PI 605996 x Poinsett 76 for testing for high resistance to DM, as well as fruit quality.

In 2019, we produced eight sublines (S4) and backcross lines (BC1S3) from PI 605996 x Poinsett 76 for testing for high resistance to DM, as well as fruit quality.

Cucumber Breeding Lines Developed

NC-148 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S11 generation from a family (PI 197088 x Coolgreen), previous number 17GHFL-950-1@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, medium-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with medium-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Clinton, M 17. Adaptation: southern U.S. 2020.

NC-149 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S10 generation from a family (PI 197088 x Coolgreen), previous number 17GHSP-602-1@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, medium-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with medium-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Clinton, M 17. Adaptation: southern U.S. 2020.

NC-154 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S2 generation from a family (PI 197088 x Poinsett 76), previous number 17GHFL-988-1@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, medium-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with medium-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Sumter. Adaptation: southern U.S. 2020.

NC-155 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S3 generation from a family (PI 605996 x Poinsett 76), previous number 17GHFL-1084-2@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, dark-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with dark-green color. Resistance:

anthracnose, new downy mildew (high resistance). Similar: Addis. Adaptation: southern U.S. 2020.

NC-156 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S3 generation from a family (PI 605996 x Poinsett 76), previous number 17GHFL-1088-1@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, dark-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with dark-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Sumter. Adaptation: southern U.S. 2020.

NC-165 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S10 generation from a family (PI 197088 x Coolgreen), previous number 18GHFL-200-1@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, medium-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with medium-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Clinton, M 17. Adaptation: southern U.S. 2020.

NC-177 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S10 generation from a family (PI 197088 x Poinsett 76), previous number 17GHFL-1010-2@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, medium-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with medium-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Clinton, M 17. Adaptation: southern U.S. 2020.

NC-178 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S10 generation from a family (PI 197088 x Poinsett 76), previous number 17GHFL-1043-3@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, medium-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with medium-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Clinton, M 17. Adaptation: southern U.S. 2020.

NC-179 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S10 generation from a family (PI 197088 x Poinsett 76), previous number 17GHFL-1089-3@. Characteristics: monoecious pickling cucumber, tall indeterminate vines, with rapid growth, medium-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with medium-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Clinton, M 17. Adaptation: southern U.S. 2020.

NC-180 – Breeder: T.C. Wehner and E.J. Silverman. Vendor: North Carolina State University, Raleigh. Parentage: inbred self-pollinated past the S10 generation from a family (PI 197088 x Coolgreen), previous number 18GHFL-214-1@. Characteristics: monoecious pickling

cucumber, tall indeterminate vines, with rapid growth, dark-green leaves, and early maturity; high resistance to downy mildew; medium quality fruit with dark-green color. Resistance: anthracnose, new downy mildew (high resistance). Similar: Clinton, M 17. Adaptation: southern U.S. 2020.

Squash Team

Team members:

Michael Mazourek (Cornell Univ.) Angel Linares (Univ. Puerto Rico)
Linda Beaver (Univ. Puerto Rico) Chris Smart (Cornell Univ.)

Objective	**Personnel/Institution**	**Year**			
	(initials as in Table 3)	1	2	3	4
(a) Obj. 1. Develop common genomic approaches and tools for cucurbits					
1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)					
1.2.1. GBS of cucurbit species, establish molecular-informed core populations - squash	ZF (BTI), RG (MSU) MM (CU)	X X	X X		
1.2.2. Population genetics and GWAS analysis - squash	UR (WVSU), ZF (BTI) MM (CU)		X	X	X
(b) Obj. 2. Genomic assisted breeding for disease resistance					
2.1 QTL map resistances:	Screen for resistance (**Sc**), develop populations **(P)**, phenotype (**F**), sequence **(S)**, QTL map **(Q)**				
2.1.4 Squash - Phytophthora - PRSV-W - CMV	 MM (CU), CS (CU) MM MM	 PF PFQ PFQ	 PF Q Q	 Q	
2.2 Marker development and verification:	Refine map (**R**) develop marker **(M)**, verify **(V)**				
2.2.4 Squash - powdery mildew - Phytophthora - PRSV-W - CMV	 MM(CU), LWB(UPR) MM (CU) MM(CU), LWB(UPR) MM(CU), LWB(UPR)	 RM	 V RM RM	 RM V V	 V
2.3. Introgress resistance into advanced breeding lines:	Develop breeding lines **(B),** introgress into cultivated **(I)**, advanced lines **(A)**, release to breeders **(R)**				
2.3.4 Squash - powdery mildew - Phytophthora - PRSV-W - CMV	 Already exists MM (CU), CS (CU) Already exists Already exists	 I	 I	 AR	 AR

Establish core GWAS populations

1.2.1. ,1.2.2 GBS of cucurbit species, establish molecular-informed core populations and 1.2.2. Population genetics and GWAS analysis

The core set of accessions representing *Cucurbita pepo* diversity in the NPGS has been self-pollinated and is being combined with heirloom cultivars to anchor market classes and enrich for cultivar genetics. Sources of resistance and other representatives of diversity in other species to extend the utility of the panel and being selfed pollinated. The goal of representatives from other species is both because squash improvement often involves crosses between species and to extend the benefits of CucCAP investments to those that work with other species such as *C. moschata* (Puerto Rico). The second round of self pollination will likely take place with a subcontractor. The process is now including strain purification within the stocks for accessions that do not match their descriptors for hull-less seeded accessions that aren't, we are creating new selfed stocks from hull-less segregants.

Three projects are already taking advantage of the GWAS population. For CucCAP, given the lack of phenotypic data, we have phenotyped the collection for qualitative traits of bush growth habit and hull-less seeds. Markers were created with this material validated in breeding populations to include as part of the MS. A separate study is mapping cotyledon cucurbitacin content to support results from biparental populations.

PI collection genomics

Roughly 4,000 - 40,000 quality SNPs were called in each of the species collections (*C. pepo*., *C. moschata* and *C. maxima*), which ranged in size from 314 to 829 accessions. Filtered SNPs were used for population structure analysis. Available geographical, phenotypic, and other metadata were retrieved from GRIN and were used to help interpret structure results. These data support five ancestral groups in each of the species. Population structure was driven mostly by geography, except in *C. pepo* where the presence of different subspecies was responsible for some of the structure. Filtering all available historical data from GRIN traits with at least 100 entries resulted in 21 traits for *C. pepo*, 5 for *C. moschata* and 16 for *C. maxima* for further analysis. Traits spanned fruit and agronomic-related characteristics, as well as pest resistances. Fruit traits included fruit width, length, surface color and texture, and flesh color and thickness. Agronomic data included plant vigor and vining habit, and several phenotypes related to maturity. Pest-related traits included susceptibility to cucumber beetle and squash bug in *C. pepo* and *Watermelon mosaic virus* (WMV) and powdery mildew (PM) in *C. maxima*. Marker-based narrow-sense heritability was calculated for each trait and ranged from 0.12 to close to 1. Most traits had moderate to high heritabilities (≥0.4). Regression of trait data on the Q matrix obtained from structure analysis was used to determine the amount of phenotypic variation explained by population structure. In *C. pepo*, traits related to fruit morphology tended to have high correlations with population structure. Genome wide association was conducted for all traits using standard mixed-model analysis. No significant signals were detected in *C. moschata*. A weak signal was detected in *C. maxima* for fruit set on chromosome 12 and fruit ribbing on chromosome 17. Three phenotypes were significantly associated with SNPs in C. pepo: bush/vine plant architecture on chromosome 10, fruit flesh color on chromosome 5, and fruit width on chromosome 3.

PRSV resistance - Construction of RIL population for mapping

Unlike powdery mildew resistance, virus resistance has not been as widely deployed in commercial breeding lines. Therefore, while we were able to implicate a genomic region in PRSV resistance using a cultivar based association mapping approach, this was not definitive. To validate this region, we created a biparental F2:3 mapping population from a cross between Whitaker (*C. pepo* subsp *pepo*) and Success PM (*C. pepo* subsp *ovifera*). One hundred three of these families were inoculated with PRSV and phenotyped for the recessive single gene resistance derived from *C. equadorensis*. We designed SNP markers that we can test in this population once campus reopens to research.

Insect resistance

Lauren Brzozowski, a recent PhD in the Mazourek group, used the genotype data from the C. pepo collection to do targeted phenotyping of cucurbitacin content. She currently has a paper in review describing the mapping and candidate genes for the *Bi-4* locus in squash which drives

striped cucumber beetle herbivory in cotyledons mediated by the presense of cucurbitacins (Brzozowski et al, in review). Striped cucumber beetles are a vector of *Squash mosaic virus*. There is no known genetic resistance to this virus in Cucurbita and the neonicotinoid chemistry used to control these insects have increased regulatory scrutiny in the US and usage restrictions in Europe.

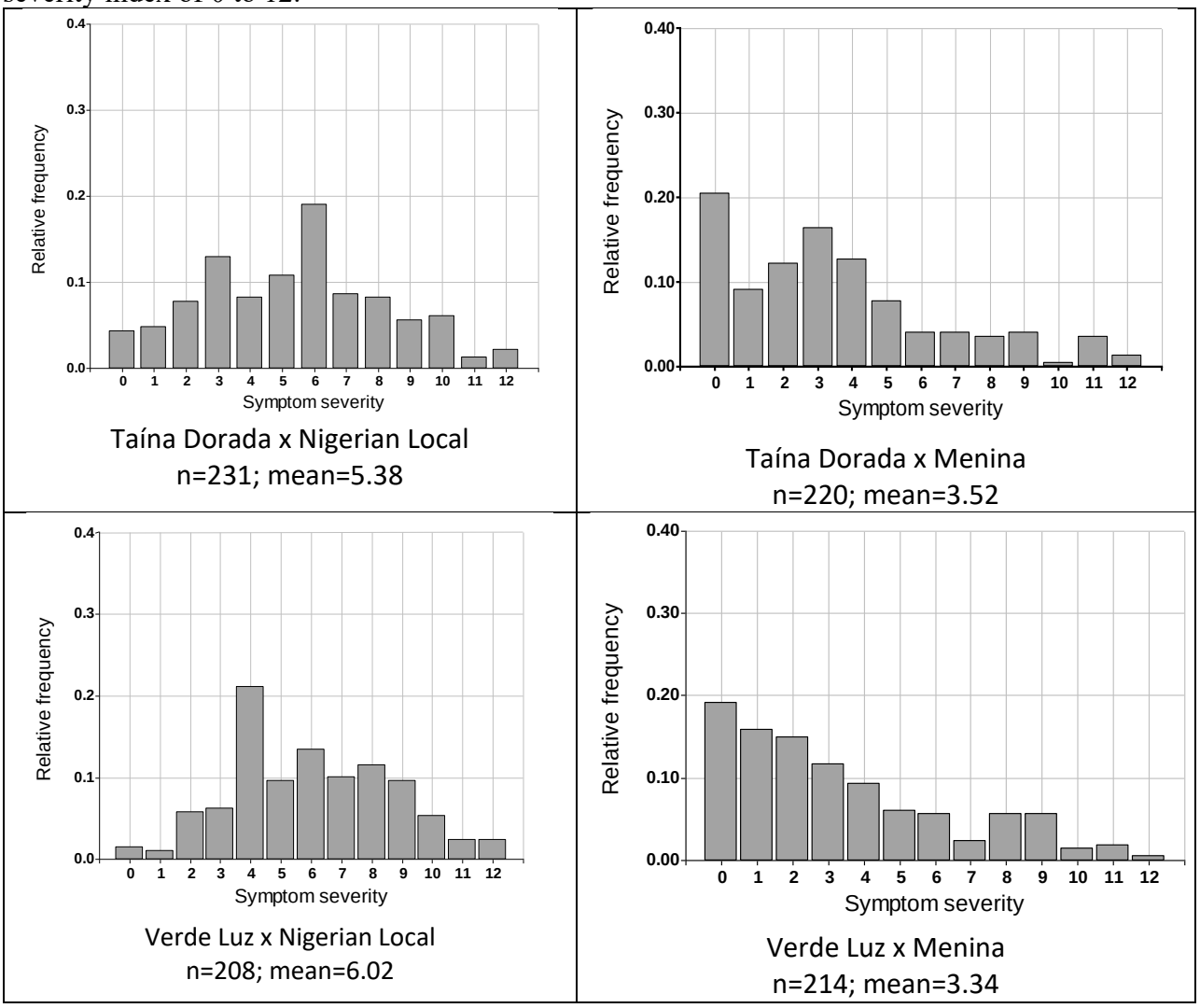
University of Puerto Rico, Mayagüez, Annual Report – May 2020

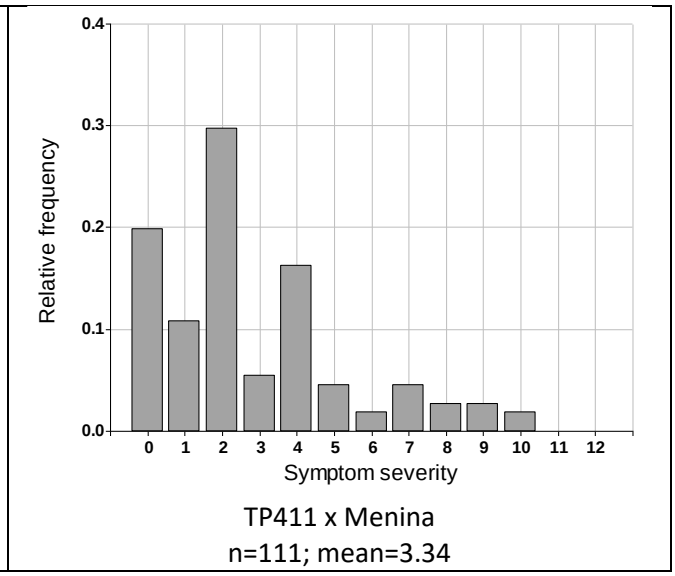
In this report we aggregate data from 2019-2020 with data collected in previous years concerning inheritance of resistance to *Papaya ringspot virus* (PRSV) in tropical pumpkin (*Cucurbita moschata*). Some of the conclusions made in previous annual reports have been modified after taking into consideration the additional data. For most of the populations, the new data doubles the number of individuals tested in F2 families resulting in much more robust inheritance tests.

Two sources of resistance are well known in *C. moschata*: 'Nigerian Local' and 'Menina'. The inheritance of resistance from 'Nigerian Local' has been previously studied, but prior to the CucCAP project inheritance studies have not been reported for 'Menina', nor was it known if resistance to PRSV in 'Nigerian Local' is allelic to that in 'Menina'. In our inheritance study susceptible genotypes were 'Verde Luz', 'Taina Dorada' and 'TP411'. The third to fifth leaf of inoculated seedlings were rated on a 0 to 4 scale for disease severity and scores were combined to convert to a 0 to 12 scale. Resistant x susceptible F_2 populations using 'Nigerian Local' as the source of resistance (distributions on the left-hand side of Figure 1) had somewhat normal distributions with an average disease severity of 5.385 (n=231) in Taína Dorada x Nigerian Local and 6.02 (n=208) in Verde Luz x Nigerian Local. In contrast, F_2 populations with 'Menina' (distributions on the right-hand side of Figure 1) were strongly skewed towards resistance with an average severity of 3.52 (n=220) in Taína Dorada x Menina, 3.34 (n=214) in Verde Luz x Menina and 2.80 (n=111) in TP411 x Menina. The Nigerian Local x Menina (resistant x resistant) F_2 population was very strongly skewed toward resistance, with an average combined severity of 0.840 (Figure 2).

To carry out chi-square goodness of fit tests, we grouped plants with an overall severity rating of $\leq$4 as resistant and plants with an overall severity rating of $\geq$5 as susceptible. The best fit for segregation in F_2 crosses made with 'Nigerian Local' was to a 7:9 (R:S) genetic model, although the fit was very poor for Verde Luz x Nigerian Local (Table 1). Goodness-of-fit to other two-class models were much worse than for 7:9. All three crosses using 'Menina' fit a 3:1 model. The resistant x resistant cross (Nigerian Local x Menina) fit a 15:1 model. These segregations suggest that at least two genes are involved in the inheritance of resistance to PRSV for 'Nigerian Local' while a single dominant gene might be responsible for the resistance of 'Menina'. The data clearly indicate that at least some of the genes for resistance in 'Nigerian Local' and 'Menina' are different. Our data indicates that the resistance to PRSV conferred by 'Menina' is superior to that of 'Nigerian Local'. If the resistance of 'Menina' is a single dominant gene as this data suggests, then it will likely be easier to identify resistance markers in 'Menina' than in 'Nigerian Local'.

Figure 1. (below) Distributions of symptom severity in F_2 populations of tropical pumpkin (*Cucurbita moschata*) inoculated with *Papaya ringspot* virus (PRSV). Populations developed with resistant parent 'Nigerian Local' are shown on the left; populations developed with resistant parent 'Menina' are shown on the right. For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12.





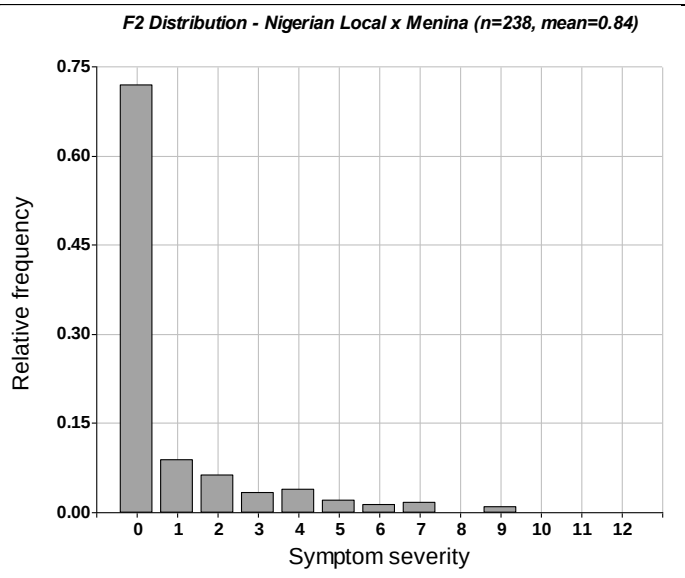


Figure 2. Distribution of combined severity ratings of plants (n=238) from the Nigerian Local x Menina F_2 population inoculated with *Papaya ringspot* virus (PRSV). For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12.

Table 1. Number of plants evaluated and observed segregations in parental, F1 and F2 populations. 'Nigerian Local' was the resistant parent in the F_1 and F_2 crosses. Goodness-of-fit in F_2 populations was tested with chi-square.

		Observed segregation			
Genotype	Population	R[1]	S[1]	$\chi 2$	Prob.
Nigerian Local	Res. parent	34	0		
Menina	Res. parent	50	0		
Taina Dorada	Sus. parent	2	18		
Verde Luz	Sus. parent	4	16		

TP411	Sus. parent	0	9			
Resistant x susceptible crosses with Nigerian Local as resistant parent:						
Taína Dorada x Nigerian Local	F_1	8	2			
Verde Luz x Nigerian Local	F_1	10	0			
Taína Dorada x Nigerian Local	F_2	88	143	7:9	3.019	0.0823
Verde Luz x Nigerian Local	F_2	74	134	7:9	5.646	0.0175
Resistant x susceptible crosses with Menina as resistant parent:						
Taína Dorada x Menina	F_1	10	0			
Verde Luz x Menina	F_1	10	0			
TP411 x Menina	F_1	10	0			
Taína Dorada x Menina	F_2	156	64	3:1	1.964	0.1611
Verde Luz x Menina	F_2	152	62	3:1	1.801	0.1796
TP411 x Menina	F_2	91	20	3:1	2.886	0.0894
Cross between two resistant parents:						
Nigerian Local x Menina	F_1	20	0			
Nigerian Local x Menina	F2	224	14	15:1	0.055	0.8147

Economics Team

Team members:
Marco Palma (Texas A&M Univ.)
Luis Ribera (Texas A&M Univ.)

(b) Obj. 3. Economic impact analyses, disease control information					
3.1 Perform economic analysis, cost of production/disease control					
3.1.1. Define, parameterize, simulate, validate production variables	LR (TAMU), MP (TAMU)	X	X		
3.1.2. Estimate the potential economic impacts to the cucurbit industry	LR (TAMU), MP (TAMU)			X	X

3.1 Perform economic analysis, cost of production/disease control

3.1.1. Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets

Completed:

- Macro and micro economic variables were collected to develop the economic model, such as interest rates, input costs, production windows and existing crop budgets.
- Graduate students were selected to work on the project and were trained on how to collect data to develop representative farms.
- Faculty and graduate students have IRB clearance to collect information from producers.
- Developed 11 representative farms in California (3 watermelon and 3 cantaloupe), Florida (3 watermelon) and Texas (1 watermelon and 1 cantaloupe)
- Estimated the economic impact of diseases to cantaloupes, fresh cucumbers, pickles, squash and watermelons.

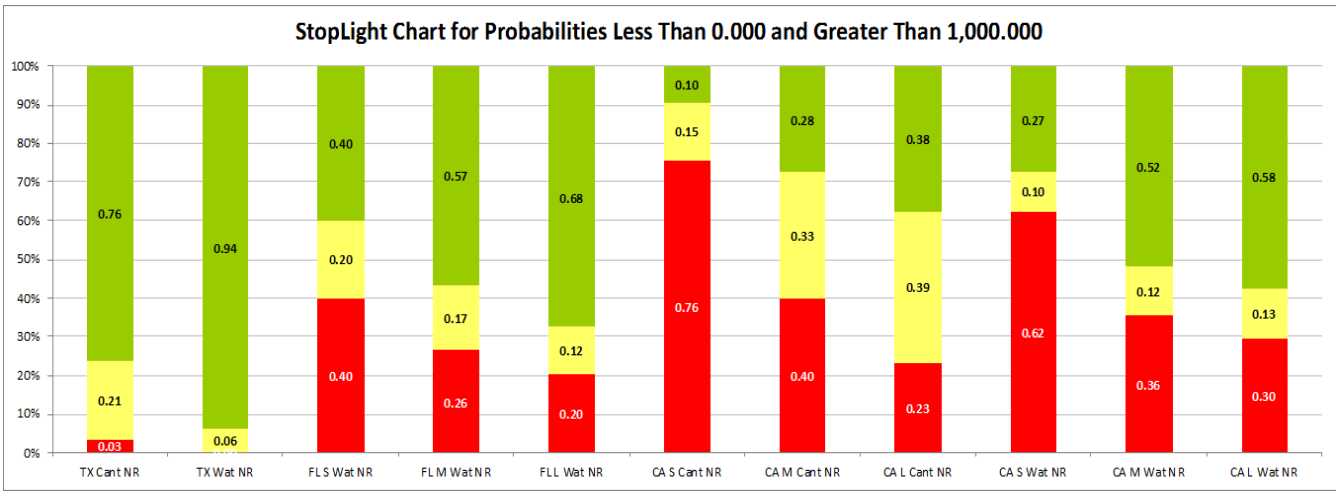
In progress:

- Identifying Extension budgets in the Northeast region
- Work with CucCap pathologists to estimate yield and quality changes due to CucCap work
- Validate economic impact of diseases
- Validate all representative farms

Publications

- Economic Impacts of Diseases on Cantaloupes. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-04. September 2018.
- Economic Impacts of Diseases on Fresh Cucumbers. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-01. September 2018.
- Economic Impacts of Diseases on Pickles. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-02. September 2018.
- Economic Impacts of Diseases on Squash. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-03. September 2018.
- Economic Impacts of Diseases on Watermelons. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-05. September 2018.

- Economic Impacts of Diseases on Selected Cucurbit Products. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Report 2018-01. September 2018.



Extension/Outreach Team

Team members:

Jonathan Schultheis (N. Carolina St.Univ.)
Mary Hausbeck (Michigan St. Univ.)
Angela Linares (Univ. Puerto Rico)
Jim McCreight (USDA, ARS)
Lina Quesada (N. Carolina St. Univ.)
Chris Smart (Cornell Univ.)
Linda Wessel Beaver (Univ. Puerto Rico)

(b) Obj. 3. Economic impact analyses, disease control information					
3.2 Provide readily accessible information to facilitate disease control					
3.2.1. Develop a centralized cucurbit disease website	JS (NCSU)	X	X		
3.2.2. Develop and post diagnostic resources and disease control information in English and Spanish; prepare diagnostic poster	LQ (NCSU), MH (MSU), CS (CU), ALR (UPR)	X	X X	X X	X
3.2.3 Provide disease alerts and forecasting tools		X	X	X	X
3.2.4 Provide diagnostic and disease management assistance.	LQ (NCSU), MH (MSU), CS (CU)	X	X	X	X
3.2.5. Field days and demonstration plots	Crop and extension teams	X	X	X	X

3.2.1 Develop a centralized cucurbit disease website.

The CucCAP website (http://cuccap.org) provides cucurbit disease management information, lists of CucCAP publications and presentations, a calendar of meetings and events, and pages detailing the breeding, genomic, pathology, and extension activities of the CucCAP teams. News about cucurbit disease outbreaks, current CucCAP research activities, recent CucCAP publications, and presentations by CucCAP researchers at scientific and cucurbit commodity meetings is posted on the website throughout the year. The CucCAP Chronicle, an email newsletter, was published 20 times since June 2017. The newsletter highlights recent news posts on the CucCAP website and is shared on social media with 109 followers on Facebook and 84 followers on Twitter. The CucCAP Chronicle has 120 subscribers. A link to previous installments of the CucCAP Chronicle is available in the footer of the website. Google Analytics was set up for the website on September 1, 2017 and 2 ¾ years of website visitor data has been collected.

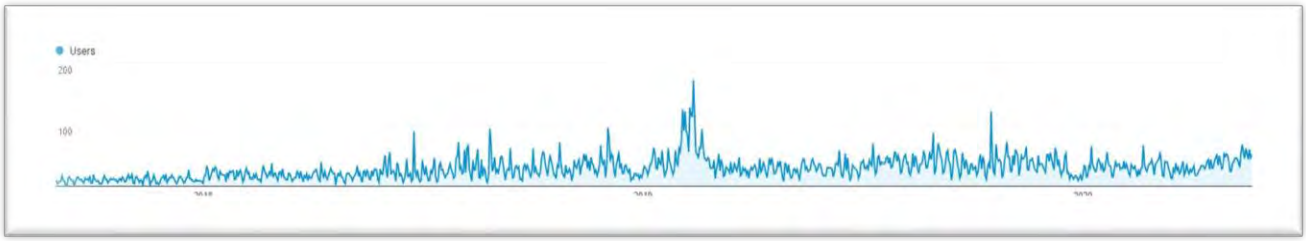


Figure 1. Site user and session data for the CucCAP website from Sept. 1, 2017 to May 21, 2020. (Users 23,255; new users 23,487; sessions 31,436; sessions per user 1.35; page views 63,744; pages / session 2.03; average session duration 2:15; bounce rate 74.65%). Peak use occurred in early February 2019 with over 550 site visits in a 10 day period during a series of Phytophthora and Downy Mildew Workshops for Vegetable Growers at MSU. Peak site visits in the 2019 to 2020 reporting period occured on October 17, 2019 with 121 site visits on the same day that an edition of the CucCAP Chronicle was published.

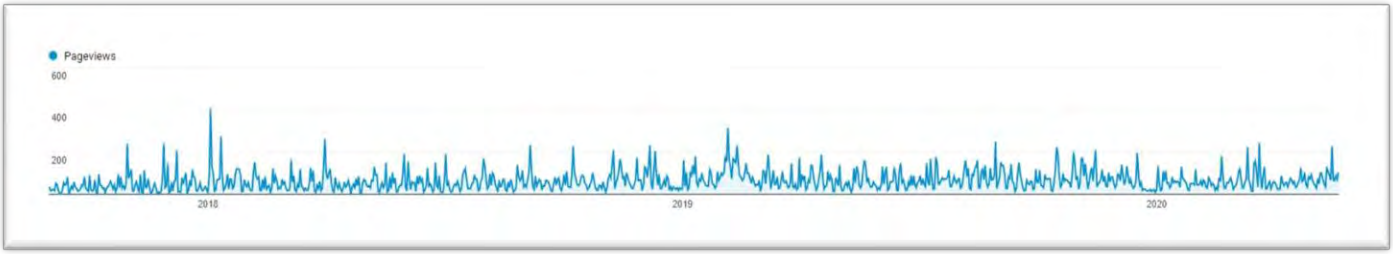


Figure 2. CucCAP website page views from Sept. 1, 2017 to May 21, 2020. (page views 63,744; unique page views 48,765; average time on page 2:11minutes; bounce rate 74.65%; exit rate 49.32%). Peak views in the 200 to 250 range coincided with the publication of the CucCAP Chronicle and when featured posts were shared on social media.

The top ten pages viewed on the CucCAP website from Sept. 1, 2017 to May 21, 2020.

Ranking	Page	Pageviews	% Pageviews
1	Homepage cuccap.org	10,576	16.59%
2	2019 Plant and Animal Genome Conference cucap.org/event/pag-xxvii/	1,726	2.71%
3	Breeding Watermelon for Disease Resistance cuccap.org/breeding/watermelon/	1,671	2.62%
4	Infección de Phytophthora cuccap.org/espanol/infeccion-de-phytophthora/	1,586	2.49%
5	Breeding Melon for Disease Resistance cuccap.org/breeding/melon/	1,532	2.40%
6	CucCAP Event Calendar cuccap.org/events/	1,271	1.99%
7	Project overview cuccap.org/about/overview/	1,126	1.77%
8	Manejo de Phytophthora en Cantalupe, Melón y Sandia cuccap.org/espanol/manejo-de-phytophthora-en-cantalupe-melon-y-sandia/	1,105	1.73%
9	Manejo de Phytophthora en Pepino https://cuccap.org/espanol/manejo-de-phytophthora-en-pepino/	1,051	1.65%
10	Research Publications cuccap.org/resources/research-publications/	994	1.56%

3.2.2. Develop and post diagnostic resources and disease control information

Cucurbit disease factsheets, crop production manuals, and integrated pest management resources for the Northeast, Southeast and the Midwest are maintained and updated on the website. Notices of regional commodity meetings and Extension education sessions are posted on the CucCAP website events calendar. News from CucCAP researchers is reported on the website and in the CucCAP Chronicle, the monthly newsletter. The CucCAP website shares weekly reports from The Cucurbit Downy Mildew Forecast and Melcast throughout the growing season.

3.2.3. Provide disease alerts and forecasting tools

Weekly conference calls, Cucurbit ipmPIPE (Hausbeck, Quesada, Smart): These calls begin in May and continue through August every year and include plant pathologists from the eastern US.

Hausbeck uses publications via her website and uses personal communication with MI growers and processors to report disease outbreaks and provide timely reports regarding disease management.

Quesada conducts weekly conference calls during the growing season with the NC State Vegetable Team. These calls occurred from May 28 to October 29 in 2019. The calls were cancelled in 2020 due to COVID-19 disruptions.

Smart has active Facebook and Twitter accounts, and is active in the Cornell Vegetable alerts blog (which sends messages to vegetable extension educators). As soon as diseases of cucurbits are first reported in NY, she alerts growers through these avenues. Additionally, any new advances made through CucCAP are shared through these methods.

3.2.4 Provide diagnostic and disease management assistance.

Hausbeck diagnosed 25 cucurbit samples and more than 45 via pictures sent via text or email. The diagnosis were primarily downy mildew and bacterial disease. Management recommendations occurred via phone to extension educators, processors, and growers.

Quesada provided diagnostics and disease management recommendations for 14 cucumber, 21 watermelon, 5 melon, 14 squash, and 6 pumpkin samples submitted to the NC State Plant Disease and Insect Clinic. Quesada has also been involved in providing disease management recommendations through oral presentations, social media (Twitter: 2,530 (lab) + 1,074 (Quesada) followers, Facebook: 847 followers, LinkedIn: 2,112 followers), and generating disease management resources such as the NC Agricultural and Chemicals Manual and the Southeastern US Vegetable Crop Handbook.

Smart diagnosed 32 cucurbit disease samples in addition to over 50 via email and text. The majority of cucurbit diseases in 2019 were bacterial. She continues to provide management recommendations through oral, written and virtual sessions. She works with conventional and organic growers in New York, including the plain sect community.

3.2.5. Field days and demonstration plots

Hausbeck has conducted cucurbit variety trials for Phytophthora blight to assist growers and processors in making control decisions. Powdery mildew trials were conducted for both pumpkin and processing squash in 2019.

Quesada evaluated commercial watermelon varieties for anthracnose resistance and supported demonstration plots to evaluate fungicides for disease control and combinations of tolerant varieties and fungicide applications.

1. Adams M., Collins H., Salcedo A., Purayannur S., Standish J., D'Arcangelo K., Stahr M., Parada C., Wong S., and Quesada-Ocampo L. M. Agent training on disease diagnostics and management in vegetable crops. Clayton and Raleigh, NC, July 2019.
2. Adams M., Collins H., Salcedo A., Purayannur S., Standish J., D'Arcangelo K., Stahr M., Parada C., Wong S., and Quesada-Ocampo L. M. Small Farms Tour: disease diagnostics and management in vegetable crops. Clayton, NC, June 2019.
3. Quesada-Ocampo L. M, Meadows I., Shew B., Eure E., Mauney C., Butler S., Adams M., Collins H., Rahman A., Salcedo A., Parada C., D'Arcangelo K., Stahr M., Wong S., and Scruggs A. Agent training on disease diagnostics and management in vegetable crops. Extension Conference. Raleigh, NC, November 2018.
4. Agent Training on Disease Management of Cucumber in the Greenhouse. Raleigh, NC, January 2020.

Schultheis conducted Variety trials on watermelon, melon, squash, and pumpkins in North Carolina in 2019.

Smart has yearly demonstration plots at the Phytophthora blight farm with variety trials for squash (winter squash and summer squash) and other vegetables; this may not be possible in 2020.

Cumulative CucCAP

PUBLICATIONS,

RESOURCE MATERIALS

and

PRESENTATIONS

REFEREED PUBLICATIONS, BOOK CHAPTERS, CONFERENCE PROCEEDINGS

A. REFEREED PUBLICATIONS (Total - 125 ; added this year - 30)

1. *Alzohairy SA, Hammerschmidt R, Hausbeck MK. 2019. Changes in winter squash fruit exocarp structure associated with age-related resistance to Phytophthora capsici. Phytopathology 110:447-455. https://apsjournals.apsnet.org/doi/10.1094/PHYTO-04-19-0128-R.*
2. Ando K, Carr KM, Colle M, Mansfeld BN, Grumet R. 2015. Exocarp properties and transcriptomic analysis of cucumber (*Cucumis sativus)* fruit expressing resistance to *Phytophthora capsici*. PloS One 10: e0142133, doi:10.1371/journal.pone.0142133.
3. Bertucci, M.B., K.M. Jenning, D.W. Monks, J.R. Schultheis, P. Perkins-Veazie, F.J. Louws, and D.L. Jordan. 2018. Early season growth, yield and fruit quality of standard and mini watermelon grafted onto several commercially available cucurbit rootstocks. HortTechnology 28(4):459-469.
4. *Bertucci, M.B, K.M. Jennings, D.W. Monks, J.R. Schultheis, F.J. Louws, and D.L. Jordan. 2019. Interference of (Amaranthus palmeri) density in grafted and nongrafted watermelon. Weed Sci. 67(2):229-238*.
5. Bertucci, M.B., D.H. Suchoff, K.M. Jennings, D.W. Monks, C.C. Gunter, J.R. Schultheis, and F.J. Louws. 2018. Comparison of root system morphology of cucurbit rootstocks for use in watermelon grafting. HortTechnology. 28(5):629-636
6. *Branham S.E., Daley J., Levi A., Hassell R., Wechter W.P. 2020. QTL mapping and marker development for tolerance to sulfur phytotoxicity in melon (Cucumis melo). Under review at Frontiers in Plant Science*.
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9. Branham SE, Levi A, Wechter WP. 2019a. QTL mapping identifies novel source of resistance to Fusarium wilt race 1 in *Citrullus amarus*. Plant Disease 103:984-989 https://doi.org/10.1094/PDIS-09-18-1677-*RE*.
10. Branham SE, Levi A, Katawczik ML, Wechter WP. 2019b. QTL mapping of resistance to bacterial fruit blotch in *Citrullus amarus*. Theoretical and Applied Genetics. https://doi.org/10.1007/s00122-019-03292-6.
11. Branham, S., L. Vexler, A. Meir, G. Tzuri, Z. Frieman, A. Levi, W.P. Wechter, Y. Tadmor and A. Gur. 2017. Genetic mapping of a major codominant QTL associated with β-carotene accumulation in watermelon. Mol. Breeding https://doi.org/10.1007/s11032-017-0747-0 .
12. Branham SE, Wechter WP, Lambel S, Massey L, Ma M, Fuave J, Farnham MW, Levi A. 2018b. QTL-seq and marker development for resistance to *Fusarium oxysporum* f. sp. *niveum* race 1 in cultivated watermelon. Molec Breed 38:139.
13. *Branham, S.E., Wechter, W.P., Ling, K.S., Chanda, B., Massey, L., Zhao, G., Guner, N., Bello, M., Kabelka, E., Fei, Z. and Levi, A., 2020. QTL mapping of resistance to Fusarium oxysporum f. sp. niveum race 2 and Papaya ringspot virus in Citrullus amarus. Theoretical and Applied Genetics, 133(2), pp.677-687.*
14. *Brzozowski, L. J., Gore, M.A., Agrawal, A.A., Mazourek, M. Divergence of defensive cucurbitacins in independent Cucurbita pepo domestication events leads to differences in specialist herbivore preference. Under review at Plant, Cell & Environment*

15. Carlson MO, Gazave E, Gore MA, Smart CD. 2017. Temporal genetic dynamics of an experimental, biparental field population of *Phytophthora capsici*. Frontiers in Genetics 8:26. Doi: 10.3389/fgene.2017.00026.
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20. Daley J, Branham S, Levi A, Hassell R, Wechter P. 2017. Mapping resistance to *Alternaria cucumerina* in *Cucumis melo*. Phytopathology 107:427-432.
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Webinars

Smart, C.D. Vegetable Diseases (for beginning growers), March 15, 2017. This was a 1.5 hour webinar.

Smart C.D. Managing cucurbit downy mildew in organic systems in the northeast. December 6, 2016. 1.5 hour webinar with 135 participants.

Smart, C. D. Vegetable Diseases (for beginning growers), March 16, 2016. 1 hour webinar.

SCIENTIFIC CONFERENCE and UNIVERSITY PRESENTATIONS

1. *Adams LF, McGregor CE. 2019. Utilizing watermelon wild relatives for gummy stem blight resistance. NAPB Annual Meeting, 2019, Pine Mountain, GA*.
2. *Adams L, McGregor C. 2019. Utilizing watermelon wild relatives for gummy stem blight resistance. IPBGG Retreat, Dawsonville, GA. Poster*
3. *Adams L, McGregor C. 2002. Bulked Segregant Analysis Identification of 5 Gummy Stem Blight Resistance Loci in Wild Watermelon Relatives. Southern Region American Society for Horticultural Science, Louisville, KY. Poster*
4. Alzohairy SA, Hammerschmidt R, Hausbeck MK. 2017. Characterization of the structural basis of winter squash fruit age-related resistance to *Phytophthora capsici*. American Phytopathological Society Annual Meeting, San Antonio, TX, 5-9 Aug. Poster
5. Alzohairy, S., and Hausbeck, M. 2015. Transcriptomic profiling of *Cucurbita* species to characterize the age-related resistance against *Phytophthora capsici*. Page 19 in: Proceedings of the 1st International Soilborne Oomycete Conference, Duck Key, FL, 8-10 Dec. Abstract.
6. Alzohairy, S.A., and Hausbeck, M.K. 2018. Characterization of winter squash age-related resistance to *Phytophthora capsici* through fruit peel transcriptome profiling and investigation of cell wall properties. Soilborne Oomycete International Conference, Islamorada, FL, 4-6 Dec. Abstract.
7. Alzohairy SA, Moore B, Hammerschmidt R, Shiu SH, Hausbeck MK. 2018. Characterization of winter squash age-related resistance to *Phytophthora capsici* through fruit peel transcriptome profiling. Phytopathology 108(10 Supplement):S1.41-1.42.
8. Ando, K. and McCreight, J.D. 2018. Potential for Production of Turkmen Melons in California, Nation Association of Plant Breeders annual meeting, Davis, CA, 7-10 August 7-10 2018.
9. Ando K, Wang X, Fei Z, Wintermantel W, McCreight J. 2018. Where in The New Melon Classification Schemes Does *Cucumis melo* ssp. *agrestis* var. *texanus* Belong? *Cucurbitaceae* 2018, Univ. California, Davis, 12-15 November 2018.
10. Ando K, Wang X, Reddy U, Fei Z, McCreight J. 2018. Exploring Genetic Diversity in the U.S. National Melon Collection. *Cucurbitaceae* 2018, Univ. California, Davis, 12-15 November 2018
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12. Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2017. Critical period for weed control in grafted vs nongrafted watermelon. Southern Weed Science Society Annual meeting. Birmingham, AL. January 24.
13. Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R., Smith S.C., Basinger N.A., Waldschmidt M.D. 2017. Influence of grafting on the critical period for weed control in watermelon. Southern Region American Society for Horticultural Science Annual meeting. Mobile, AL. February 4.
14. Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2017. Influence of grafting on the critical period for weed control in watermelon. Weed Science Society of America Annual meeting. Tuscon, AZ. February 7.
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19. Branham SE, Levi A, Katawczik M, Fei Z, Wechter WP. 2018. Construction of a High-Density Genome-Anchored Genetic Map for Melon (*Cucumis melo L.*) and Identification of *Fusarium oxysporum* f. sp. *melonis* Race 1 Resistance QTL. Plant and Animal Genome Conference (poster).
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26. Daley J, Branham S, Levi A, Hassell R, Wechter P. 2017. Mapping resistance to *Alternaria cucumerina* in muskmelon. Plant & Animal Genome XXV Conference. https://pag.confex.com/pag/xxv/meetingapp.cgi/Paper/25467
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53. Grumet R. et al., 2018. Genomic analysis of cucurbit PI collections. American Society for Horticultural Science, Washington DC
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56. *Grumet R. 2019. Leveraging applied genomics to increase disease resistance in cucurbit crops. CEPLAS Transatlantic Summer School – Frontiers in Plant Science. Cologne, Germany.*
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61. Guo, Y., Krasnow, C., and Hausbeck, M.K. 2018. Population structure, virulence and resistance to mefenoxam of *Phytophthora capsici* in Michigan. Phytopathology 108(10 Supplement):S1.87.
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65. *Jeon, S., Krasnow, C.S., Bhalsod, G.D., Harlan, B.R., Hausbeck, M.K., Safferman, S.I., and Zhange, W. 2019. Control of Phytophthora capsici diseases in greenhouse squash by fast-flow filtration. GreenSys 2019, International Symposium on Advanced Technologies and Management for Innovative Greenhouses, Angers, France, 17 Jun.*
66. *Katuuramu, D. Branham, S, Levi, A., Wechter WP 2020. Phenotypic variability and genome-wide association analysis of downy mildew (Pseudoperonospora cubensis) resistance in a pre-breeding watermelon (Citrullus amarus) collection. Plant and Animal Genome Conference. PE0692*
67. Kaur N, Chen W, Fei Z, Wintermantel WM. 2017. Transcriptome changes occurred in the whitefly, B. tabaci MEAM1 in response to feeding on melon infected with the crinivirus, CYSDV. 3rd Hemipteran-Plant Interactions Symposium, Madrid. Spain. June 4-8, 2017.
68. Kaur N, Chen W, Fei Z, Wintermantel WM. 2017. Transcriptome changes occurred in the whitefly, B. tabaci MEAM1 in response to feeding on CYSDV-infected melon. American Phytopathological Society annual meeting, San Antonio, TX, August 5-9, 2017.
69. Kousik CS. 2016. Breeding rootstocks of cucurbit vegetable crops for resistance to biotic and abiotic stress. (Invited presentation). Platinum Jubilee Celebrations, Indian Horticultural Congress. November 15, 2016. (>300 attendees at the talk).
70. Kousik CS. 2017. Progress and challenges in managing watermelon diseases. Dept of Plant Pathology, University of Georgia Athens, GA, Aug. 2017. >50 attendees
71. Kousik CS. 2018. Progress and Challenges in managing Phytophthora fruit rot of cucurbits. Keynote address presented at the 2nd International Soilborne Oomycete Conference, Keys, FL. December 2018. Proceedings of the 2nd International Soilborne Oomycete conference, Page 13.

72. *Kousik CS. 2019. Developing sustainable strategies for managing watermelon diseases in southeastern United States. Abstracts of the National Symposium on Recent challenges and opportunities in sustainable plant health management at the 71st Annual Meeting of the Indian Phytopathological Society. February 26-28, 2019. BHU, Varanasi, India. Pp. 267.*
73. *Kousik CS. 2020. Developing Sustainable Strategies for Managing Watermelon Diseases in Southeastern United States. Invited Presentation at the Indian Phytopathological Society Annual Symposium in Varanasi, India from February 26-28.*
74. Kousik CS, Egel D, Ji P, Quesada-Ocampo LM. 2016. Fungicide rotation schemes and Melcast for managing Phytophthora fruit rot of watermelon in Southeastern United States. Phytopathology. 106: S4.68.
75. Kousik CS, Ikerd JL. 2015. Reaction of Phytophthora fruit rot resistant germplasm lines to a broad range of *Phytophthora capsici* isolates from across United States of America. International soilborne Oomycete conference, Duck Key, FL. December
76. Kousik CS, Ikerd JL, Mandal MK. 2016. Breadth of resistance of Phytophthora fruit rot resistant watermelon germplasm to *Phytophthora capsici* isolates from across United States of America. Phytopathology S4.40 (Abstract)
77. Kousik CS, Ikerd JL, Mandal MK. 2017. Long term monitoring of cucurbit powdery mildew (*Podosphaera xanthii*) races in Charleston, South Carolina. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 503-P
78. Kousik CS, Ikerd JL, Mandal MK. 2018. Relative susceptibility of commercial watermelon varieties to powdery mildew. Presented at the Annual meeting of the Southern Division, American Phytopathological Society (SD-APS).Fayetteville, AR Feb 16-18, 2018.
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80. Kousik CS, Ikerd JL, Mandal MK. 2018. Relative Susceptibility of Commercial Watermelon Varieties to Powdery Mildew and Phytophthora Fruit Rot. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 51.
81. Kousik CS, Ikerd JL, Mandal MK. 2018. New Sources of Resistance to Phytophthora Crown and Root Rot in *Cucurbita moschata*. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 54.
82. Kousik CS, Ikerd JL, Mandal MK, Wadl P. 2018. Genetics of Resistance to Powdery Mildew in Watermelon Line USVL608-PMR. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 55.
83. Kousik CS, Mandal MK, Ikerd JL, Adkins S, Turechek W. 2018**.** Powdery mildew resistant watermelon germplasm lines USVL608-PMR, USVL278-PMR, USVL313-PMR and USVL585-PMR. Presented at the Watermelon Research and Development Group (WRDG) meeting. Southern Region ASHS, Jacksonville, FL. February 2018.
84. Kousik CS, Mandal MK, Ikerd JL, Adkins S, Turechek W. 2018. Broad resistance to U.S. powdery mildew isolates in newly developed watermelon germplasm lines. (Abstr.) Phytopathology 108:S1.173. https://doi.org/10.1094/PHYTO-108-10-S1.1
85. Kousik CS, Pingsheng J, Quesada-Ocampo LM 2015. Fungicide rotation schemes for managing Phytophthora fruit rot of watermelon across Southeastern United States (NC, SC, GA). International soilborne Oomycete conference, Duck Key, FL. December
86. Krasnow C, Hausbeck M. 2015. Using directed fungicide applications to manage Phytophthora fruit rot of processing squash. Page 23 in: Proceedings of the 1st International Soilborne Oomycete Conference, Duck Key, FL, 8-10 Dec. Abstract.

87. Krasnow CS, Hausbeck MK. 2016**.** Age-related resistance of Cucurbita spp. fruit to *Phytophthora capsici*. Abstr. Phytopathology 106 (Suppl.):S1.5.
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89. Lebeda A, Kristkova E, Sedlakova B, McCreight JD, Kosman E. 2018. Application of a new approach for study of virulence variation in cucurbit powdery mildew populations. International Congress of Plant Pathology, Boston, 30 July-2 August 2018
90. Lebeda A, Sedlakova B, Kristkova E, McCreight J, Kosman E. 2018. Cucurbit powdery mildew population virulence variation–complex view from a global perspective. Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018.
91. Levi, A. S. Steck, M. Horry, R.L. Jarret, P. Wechter, S. Kousik, B. Ward, G. Miller, R. Hassell, and A. Keinath. 2017. An overall small root system in watermelon cultivars indicates a need to improve their lateral fibrous root capacity. Presented at the Watermelon Research and Development Group meeting. Mobile, AL. February 2017
92. Lin YC, Grumet R. 2018. Genetic analysis of young fruit resistance to *Phytophthora capsici* in cucumber. American Society for Horticultural Sciences http://ashs.confex.com/ashs/2018/meetingsapp.cgi/paper/28744.
93. Lin YC, Grumet R. 2018. Genetic analysis of young fruit resistance to *Phytophthora capsici* in cucumber. National Association of Plant Breeders, Guelph Ontario.
94. Lin YC, Grumet R. 2018. QTL-seq of young fruit resistance to *Phytophthora capsici* in cucumber. Cucurbitaceae 2018, Davis CA 29.
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96. Lin YC, Rett-Cadman S, Weng Y, Fei Z, Grumet R. 2020. Phenotypic analysis of the US cucumber PI core collection for diversity of fruit morphological traits. Poster presentation at Plant and Animal Genome Conference XXVIII (Jan 13-18, 2020, San Diego, CA).
97. Linares-Ramirez AM. 2016. Cucurbits: From to the field to the lab. Agricultural Experimental Station, University of Puerto Rico.
98. Ling K-S. 2017. Presented invited seminars on "Developing genome-guided strategies to manage viral diseases of cucurbit crops" in four institutions throughout China, including:
    a. Zhengzhou Fruit Research Institute,
    b. Beijing Vegetable Research Center (China, Israel, and the U.S. Workshop on Cucurbit Research), Zhejiang Academy of Agricultural Sciences
    c. Fujian Agricultural and Forestry University.
99. Ling K, Sui X, Li R, Gilliard AC, Levi A, Wu Z. 2018. Cucumber green mottle mosaic virus: Seed transmissibility, seed health assays and screening watermelon germplasm for disease resistance. Cucurbitaceae 2018. http://cucurbit2018.ucdavis.edu/wp-content/uploads/2018/10/2018_Cucurbits_Abstracts_Book.pdf Abstracts P9.
100. Liu HQ, Zhao JY, Weng Y. 2020. A modified protocol improves efficiency of Agrobacterium-mediated transformation in cucumber (Cucumis sativus L.). Poster presentation at Plant and Animal Genome Conference XXVIII (Jan 13-18, 2020, San Diego, CA).
101. Lonnee M, Gimode WR, McGregor CE. 2018. Evaluation of Gummy Stem Blight Resistance in Watermelon Using *Stagonosporopsis* spp. Isolates. CAES, Young Scholars Athens, GA.
102. Mandal, M.K., Ikerd, J.L., Shrestha, S. Battiste, A., Boroujerdi, A., Ward, B., Kousik, C.S. 2017. 1H NMR and HPLC-based metabolite profiling of watermelon varieties. Presented at the Watermelon Research and Development Group meeting. Mobile, AL. February 2017.
103. Mandal MK, Ikerd JL, Soorni A, Kousik CS. 2016. Molecular dissection of resistance signaling in watermelon fruit through transcriptomic approach. Phytopathology S4.153 (Abstract)

104. Mandal MK, Ikerd J, Wadl PA, Williams L, Quesada-Ocampo L, Kousik CS. 2019. Comprehensive disease survey on USDA-ARS cucumber germplasm collections in search for downy mildew resistance. Charleston SC. APS Annual Meeting at Cleveland, OH.

105. Mandal MK, Ikerd JL, Wallace EC, Rebbeca G, Turechek W, Quesada-Ocampo LM, Kousik CS. 2017. Population biology of the downy mildew pathogen on tolerant and susceptible cucumber in the southeastern United States. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 563-P.

106. Mandal, M.K., Kousik, C.S. and Ward, B. 2016. Molecular dissection of resistance signaling in watermelon fruit through metabolomics and transriptomic approach. Watermelon Research and Development Group meeting. San Antonio, TX. Feb

107. Mandal, M.K., Suren, H., Ikerd, J.L., and Kousik, C.S. 2018. Molecular Dissection of Resistance Signaling during Compatible and Incompatible Watermelon- Powdery Mildew (Podosphaera xanthii) Interactions using RNA-Seq Approach. Presented at the Watermelon Research and Development Group (WRDG) meeting. Southern Region ASHS, Jacksonville, FL. February 2018.

108. Mandal MK, Suren H, Kousik CS. 2017. Transcriptomic profiling of watermelon-powdery mildew (*Podosphaera xanthii*) interactions. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 361-P.

109. Mandal MK, Suren H, Ward B, Boroujerdi A, Kousik CS. 2018. Role of Antioxidant Molecule Melatonin in Plant-Host Resistance and Pathogen Suppression in Cucurbits. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 11.

110. Mansfeld B, Colle M, Grumet R. 2017. Genome-wide SNP discovery and identification of age-related resistance loci in cucumber by QTL-seq. PAG XXV, San Diego CA.

111. Mansfeld B, Grumet R. 2018. QTLseqr: An R package for bulk segregant analysis with next generation sequencing. PAG XXVI, San Diego CA.

112. Mansfeld B, Grumet R. 2018. Differential transcriptomic responses to infection associated with cucumber age-related resistance to *Phytophthora capsici*. American Society for Plant Biology https://www.eventscribe.com/2018/ASPB/postertitles.sap?gotoLetter=D&pfp=postertitles.

113. Mansfeld B, Grumet R. 2018. QTLseqr: An R package for bulk segregant analysis with next generation sequencing. National Association for Plant Breeders, Guelph Ontario

114. Mansfeld B, Grumet R. 2018. Inhibitory effects of cucumber fruit age-related resistance to *Phytophthora capsici* manifest within 24 hours of infection. Cucurbitaceae 2018, Davis CA

115. Mansfeld B, Grumet R. 2019. Gene expression dynamics of age-related resistance of cucumber to *Phytophthora capsici*. PAG XXVII https://plan.core-apps.com/pag_2019/abstract/918bc1c1-89c4-4b70-acc5-21223f797605

116. Mantooth, K.L., Ikerd, J.L., Mandal, M.K. and Kousik, C.S. 2017. Potential sources of resistance to Phytophthora crown rot in *Cucurbita maxima* and *Cucurbita moschata*. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 291-P

117. Mazourek M, Holdsworth WL, Hernandez C, LaPlant KE. 2016. Making up for lost time in Cucurbita molecular breeding. Plant and Animal Genome Conference. San Diego, CA.

118. McCreight JD, Coffey M, Ando K, Kousik CS. 2018. Cucurbit powdery mildew races on melon: current status in the U.S., American Society for Horticultural Science 2018 Annual Conference, Washington, D.C., July 30–August 3.

119. McCreight JD, Wintermantel WM, Natwick ET. 2015. Evaluations of melon germplasm reported to exhibit host plant resistance to sweetpotato whitefly. Entomological Society of America, Annual Meeting, Minneapolis, MN, Nov. abstract

120. McCreight JD, Wintermantel WM, Natwick ET. 2016. Expression of Host Plant Resistance in Melon to Sweetpotato Whitefly in the Desert Southwest United States. XXV International Congress of Entomology, Orlando, FL, Sep. abstract

121. McCreight, J.D., W.M. Wintermantel, A.I. Lopez-Sese, and M.L. Gomez-Guillamon. 2019. Allelism of resistance to Cucurbit yellow stunting disorder virus in melon accessions PI 313970 and TGR 1551. Annual meeting, Amer. Soc. Hort. Sci. 22-25 July, Las Vegas, NV.

122. McGregor CE. 2019. Out of Africa: The Story of Watermelon Disease Resistance. Georgia Association of Plant Pathologists annual meeting. Savannah, GA.

123. McGregor C. 2020. Breeding for Disease Resistance and Fruit Quality. Southeast Regional Fruit & Vegetable Conference, Savannah, GA

124. McGregor, C., Gimode, W., Adams, L. & J. Reyes (2020) Is There Light at the End of the Gummy Stem Blight Tunnel? Watermelon Research & Development Group, SR-ASHS, Louisville, KY

125. Miller, N., M. Adams, and L.M. Quesada-Ocampo. 2017. Evaluation of a new fungicide for management of Fusarium wilt of watermelon. Watermelon Res Group. Mobile, AL, Feb 2017

126. Miller NF, Quesada-Ocampo LM. 2016. Evaluation of fungicides for management of Fusarium wilt of watermelon. Phytopathology. 106:S4.2

127. Miranda-Vélez ML, Wessel-Beaver L, Rodrigues JCV, Seda-Martínez W. 2016. Effect of leaf position on the assessment of resistance to *Papaya ringspot virus* and *Zucchini yellow mosaic virus* in tropical pumpkin. Proceedings of the 41st meeting of the Sociedad Puertorriqueña de Ciencias Agrícolas, November 18, 2016, Corozal, Puerto Rico. p. 57. (abstract)

128. Miranda-Vélez M, Wessel-Beaver L, Rodrigues JCV. 2018. Disease assessment, inference on virus movement, and seedling development in tropical pumpkin (*Cucurbita moschata*) infected with *Papaya ringspot virus* and *Zucchini yellow mosaic virus*. Reunión Científica de Estudiantes Graduados, Sociedad Puertorriqueña de Ciencias Agrícolas. May 18, 2018. College of Agriculture, University of Puerto Rico, Mayaguez.

129. Miranda-Vélez M, Wessel-Beaver L, Rodrigues JCV. 2018. Disease assessment in seedlings of tropical pumpkin infected with PRSV and ZYMV. Cucurbitaceae 2018. November 12-15, 2018. Davis, CA.

130. Natwick ET, Wintermantel WM, Gilbertson RL, Blanco SG, McCreight JD. 2017. "Evaluation of potential new sources of melon host plant Resistance to the whitefly, *Bemisia tabaci*," 3rd Hemipteran-Plant Interactions Symposium, Madrid. Spain. June 4-8, 2017.

131. Noel N, Quesada-Ocampo LM. 2016. Fungicide resistance and host susceptibility of *Colletotrichum orbiculare* infecting cucurbit crops in North Carolina. Phytopathology. 106:S4.36

132. Noel N, Quesada-Ocampo LM. 2017. Characterizing *Colletotrichum orbiculare*, the causal agent of cucurbit anthracnose, for fungicide efficacy and host susceptibility in North Carolina. Phytopathology 107: S5.77.

133. Parada-Rojas CH, Quesada-Ocampo LM. 2017. Population structure of the oomycete soilborne pathogen *Phytophthora capsici* in North Carolina. Phytopathology 107: S5.21.

134. Patel T, Wehner T. 2017. Identification of new resistance sources and SNPs markers in watermelon for anthracnose (*Colletotrichum orbiculare*). Abstract and Poster. National Association of Plant Breeders, Davis, CA.

135. Quesada-Ocampo LM. 2017. From the field to the lab and back: translational strategies to improve disease control in vegetable crops. VIB-PSB-NC State Plant Sciences Workshop, Ghent, Belgium

136. Quesada-Ocampo LM. 2017. Next generation sequencing to develop molecular diagnostics for *Pseudoperonospora cubensis*. Phytopathology 107: S5.150.

137. Quesada-Ocampo LM. 2019. Harnessing new technologies to improve management of cucurbit downy mildew. American Phytopathological Society Annual Meeting. Plant Health. Schroth Faces of the Future in Mycology Symposium. Cleveland OH, August 2019.

138. Quesada-Ocampo LM, Rahman A, Martin F, Miles T. 2018. Biosurveillance for improved management of cucurbit downy mildew caused by *Pseudoperonospora cubensis*. Plant Pathology Society of North Carolina Meeting, Raleigh, NC, October.

139. Quesada-Ocampo LM, Rahman A, Martin F, Miles T. 2018. Tracking a cucurbit killer: developing biosurveillance tools for improved management of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. 6th International Oomycetes Workshop, International Congress of Plant Pathology, Boston, MA, July.
140. Rahman A, Quesada-Ocampo LM. 2016. Early detection and quantification of *Pseudoperonospora cubensis* airborne sporangia using real-time PCR. Phytopathology. 106:S4.16
141. Rahman A, Quesada-Ocampo LM. 2018. Biosurveillance for precision disease management of *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. Phytopathology 108:S1.52
142. Rahman A, Martin F, Shands A, Miles T, Quesada-Ocampo LM. 2017. Using comparative genomics to develop biosurveillance tools for the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Oomycete Molecular Genetics Network Meet, Pacific Grove, CA.
143. Rahman A, Wallace E, Crouch J, Martin F, Quesada-Ocampo LM. 2017. Unravelling historical shifts in *Pseudoperonospora cubensis* populations in the U.S. that resulted in the 2004 cucurbit downy mildew epidemic. Phytopathology 107: S5.22.
144. Reddy U, Patel T, Wehner T, Nimmahayala P, Kousik CS, Wechter WP, Branham S, Ling K, Jarret RL, Levi A, Ortiz C, Abbiro V, Bao K, Wang X, Rivera-Burgos L, Fei Z. 2018. Genome-wide diversity for worldwide watermelon collections: Analysis of population structure, haplotype networks, selective sweeps and LD decay to characterize domestication signals. Cucurbitaceae Proceedings. http://cucurbit.ucdavis.edu/wp-content/uploads/2018/10/2018_Cucurbits_Abstracts_Book.pdf Abstracts Book, Page 87
145. Reyes J, Villari C, Brewer M, McGregor CE. 2018. Detection of the Gummy Stem Blight-Causing Pathogens (*Stagonosporopsis spp*.) in Watermelon Using Species-Specific LAMP Assays. Cucurbitaceae 2018, University of California, Davis-CA.
146. Reyes J, Villari C, Brewer M, McGregor C. 2019. Detection of the Gummy Stem Blight-causing Pathogens (Stagonosporopsis spp.) in Watermelon Using Species-specific LAMP Assays In Southern Region American Society for Horticultural Science, Birmingham, AL.
147. Reyes, J.A., Villari, C., Brewer, M.T., Dutta, B., and McGregor, C.E. (2020) Detection of the Gummy Stem Blight Pathogens in Watermelon Using Quick Field-Adapted Technologies. Southern Region American Society for Horticultural Science, Louisville, KY.
148. Rivera-Burgos L. Wehner T. 2017. Evaluation of gummy stem blight resistance in a recombinant inbred line watermelon population. Abstract and Poster. Nat Assoc Plant Breeders, Davis, CA
149. Schultheis, J.R. and K.D. Starke. 2017. Pollenizer placement considerations effects on watermelon (*Citrullus lanatus*) yield and quality. Watermelon Res Group, Mobile, AL. Feb. 2017
150. Schultheis JR, Starke KD. 2018. Standard size watermelon cultivar yield and quality results, North Carolina, 2017. HortScience 53(9):S502 (abstr.).
151. Schultheis, J. and K. Starke. 2018. Standard size watermelon cultivar yield and quality results, North Carolina, 2017. 2018 Watermelon Research and Development Group Annual meeting, Jacksonville, FL, February.
152. Schultheis, J.R. and K.D. Starke. 2018. Pollenizer placement considerations effects on watermelon (*Citrullus lanatus*) yield and quality over two growing seasons. Watermelon Research and Development Group Annual meeting, Jacksonville, FL, February.
153. Schultheis, J.R. and K.D. Starke. 2019. 2018 North Carolina triploid watermelon (standard size) cultivar evaluation trial. ASHS-SR, Birmingham, AL, Feb. HortScience (abstr.).
154. Schultheis JR, Thompson WB. 2016. Watermelon cultivar yield and quality trial results, North Carolina, 2015. HortScience. 51(9):S37
155. Schultheis, J.R. and W.B. Thompson. 2016. Watermelon yield and fruit size response to grafted versus non-grafted transplants in plasticulture and bare ground production systems. Watermelon Research Group, San Antonio, TX, Feb.

156. Schultheis, J.R. and W. B. Thompson. 2016, Watermelon cultivar yield and quality trial results, North Carolina, 2015. 2016. Watermelon Research Group, San Antonio, TX, Feb.
157. Schultheis JR, Thompson WB. 2016. Watermelon yield and fruit size response to grafted versus non-grafted transplants in plasticulture and bare ground production systems. HortScience. 51(9):S38
158. Seda-Martínez W, Miranda-Vélez M, Wessel-Beaver L, Linares-Ramirez AM. 2017. Approaches to Phenotyping PRSV and ZYMV Resistance in Tropical Pumpkin. HortScience 45(8):S234.
159. Seda-Martínez W, Wessel-Beaver L, Linares-Ramírez A. 2018. Effect of two Potyviruses on development and yield of tropical pumpkin. Cucurbitaceae 2018. November 12-15, 2018. Davis, California.
160. Seda-Martínez W, Wessel-Beaver L, Rodriguez JCV, Linares-Ramírez A. 2019. Inheritance of *Papaya ringspot virus* resistance from two distinct sources in tropical pumpkin (*Cucurbita moschata*). HortScience 54(9):S207.
161. Shan W, Gao L, Branham SE, Wechter PW, Kousik C, Levi A, Xu Y, Fei Z. 2020. De novo genome assembly of sweet watermelon relatives and a pan-genome of Citrullus species. Plant and Animal Genome Conference W322.
162. Silverman EJ, Rivera-Burgos L, Wehner TC. 2018. Improving gummy stem blight resistance and fruit quality in watermelon. Abstract. Cucurbitaceae annual conf. Davis, CA (abstract).
163. Silverman EJ, Wehner TC. 2018. NC State cucumber lines developed for downy mildew resistance. Abstract and Poster. Cucurbitaceae annual conf. Davis, CA (abstract).
164. Simmons AM, Jarret RL, Cantrell CL, Levi A. 2018. Enhancing watermelon with resistance against whiteflies. 3rd International Whitefly Symposium, September 16-19, 2018, Perth, Australia p.14.
165. Smart CD. 2017. A tale of two *Phytophthora*: life with and without sex. Michigan State University, East Lansing, MI, March 2.
166. Smart CD. 2016. Multiplex detection for vegetable diseases. National Plant Diagnostic Network National meeting. Crystal City, VA, March 10.
167. Smart CD. 2017. SUNY Potsdam November, Potsdam NY. Genomic approaches to understand and manage plant disease epidemics.
168. Smart CD. 2017. James Hutton Institute, August 10, Dundee Scotland. Comparing sexual and asexual *Phytophthora* species.
169. Starke KD, Schultheis JR. 2018. Mini watermelon cultivar yield and quality results, North Carolina, 2017. HortScience 53 (9):S502 (abstr.).
170. Starke, K.D., B. Thompson, C. Jiang, and J. Schultheis. 2016. Planting density influences mini-watermelon yield and quality. VII International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops, Pretoria, South Africa, September 2016.
171. Sui X, Li R, Wu Z, Ling K-S. 2018. Seed Transmissibility of Cucumber Green Mottle Mosaic Virus in Cucurbits and Seed Health Assays. Presented at the Watermelon Research and Development Group (WRDG) meeting. Southern Region ASHS, Jacksonville, FL. February 2018.
172. Thammapichai P, Pan JS, Koo D-H, Han YH, Jiang JM, Weng Y. 2018. Genomics-aided development and characterization of *Cucumis hystrix* introgression lines in cucumber. Cucurbitaceae 2018 International Meeting Abstracts (November 12-15, 2018, Davis, California)
173. Taylor J, Boroujerdi A, Pikes T, Harris R, Kousik CS, Mandal MK. 2020. Environmental bacteria as potent biocontrol agents for controlling Phytophthora fruit rot on watermelon. Southern division APS Annual Meeting Charleston, SC.
174. Trandel, M., P. Perkins-Veazie, and J. Schultheis. 2018. Tissue firmness and hollow heart development in 2012, 2013, and 2014 triploid watermelon variety trials.

175. Trandel, M.A., P. Perkins-Veazie, and J. Schultheis. 2019. Tissue firmness and hollow heart development in 2012, 2013, and 2014 triploid watermelon variety trials. ASHS-SR, Birmingham, AL, Feb. HortScience 54(9):S399 (abstr.).

176. Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and S. Johanningsmeier. 2019. Exploring cell wall chemistry to understand a watermelon fruit disorder. North Carolina State Graduate Student Symposium. Mar.

177. Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and S. Johanningsmeier. 2019. Exploring cell wall polysaccharide composition in a watermelon variety susceptible to hollow heart. ASHS-SR, Birmingham, AL, Feb.HortScience 54(9):S373 (abstr.).

178. Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and E. Johannes. 2018. Hollow heart formation in grafted and non-grafted watermelon. SR-ASHS, Jacksonville, FL, February.

179. Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and E. Johannes. 2018. Hollow heart formation in grafted and nongrafted watermelons. HortScience 53(9):S461 (abstr.).

180. Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and E. Johannes. 2018. Hollow heart formation in grafted and nongrafted watermelons. HortScience 53(9):S503 (abstr.).Cucurbitaceae 2018. Production and Quality session. Davis, CA (abstr.).

181. Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and S. Johanningsmeier. 2019. Understanding hollow heart formation in 'Liberty' watermelon. . ASHS, Las Vegas, NV, Jul. HortScience 54(9):S107 (abstr.).

182. Vogel G, LaPlant K, Reeves E, Mazourek M, Gore M, Smart CD. 2017. Evaluation of *Cucurbita pepo* breeding lines with reduced susceptibility to root and crown rot caused by *Phytophthora capsici*. American Phytopathological Society. San Antonio TX August 2017

183. Vogel G, LaPlant K, Mazourek M, Gore M, Smart CD. 2018. Bulked segregant analysis with whole-genome resequencing to map QTL involved in *Phytophthora* crown and root rot resistance in *Cucurbita pepo*. (Abstr.) Phytopathology 108:S1.1. https://doi.org/10.1094/PHYTO-108-10-S1.1.

184. Vogel G, LaPlant K, Mazourek M, Gore M, Smart C. 2018. Next-Generation Sequencing Bulk Segregant Analysis Reveals Multiple Loci Involved in *Phytophthora* Root and Crown Rot Resistance in Squash. Cucurbitaceae 2018. November 12-15, 2018. Davis, California.

185. Wallace EC, Quesada-Ocampo LM. 2016. *Pseudoperonospora cubensis* on commercial and non-commercial cucurbits in North Carolina: population structure determine by simple sequence repeats (SSRs). Phytopathology. 106:S4.12

186. Wallace EC, Quesada-Ocampo LM. 2016. Genetic structure of *Pseudoperonospora cubensis* populations infecting commercial and non-commercial cucurbits in North Carolina. XIth Eucarpia Cucurbitaceae Proceedings

187. Wallace EC, Quesada-Ocampo LM. 2017. Examining the population structure of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*, by host, location, and time. Phytopathology 107: S4.6.

188. Wang X. 2018. The USDA Cucumber (*Cucumis sativus L.)* Collection: Genetic Diversity, Population Structure, Genome-Wide Association Studies and Core Collection Development. Cucurbitaceae 2018.

189. Wang YH, Tan JY, Wu ZM, VandenLangenberg K, Wehner TC, Wen CL, Zheng XY, Owens K, Thornton A, Bang HH, Hoeft E, Kraan PAG, Suelmann J, Pan JS, Weng Y. 2018. A loss-of-susceptibility *mutation* in the STAYGREEN gene (CsSGR) provides durable, broad-spectrum disease resistances for US cucumber production. . Cucurbitaceae 2018 International Meeting Abstracts (November 12-15, 2018, Davis, California)

190. Wang YH, VandenLangenberg K, Wehner TC, Weng Y. 2018. Genetic architecture of downy mildew resistance in cucumber. Abstract for Plant and Animal Genome Meeting XXVII (Jan 12-16, 2018, San Diego, CA)
191. Wechter P. 2017. "Identification of quantitative trait loci associated with resistance to race 1 Fusarium wilt in *Cucumis melo*," American Phytopathological Society annual meeting, San Antonio, TX, August 5-9, 2017
192. Wechter P. 2017. Mapping Resistance to *Alternaria cucumerina* in Muskmelon. Plant and Animal Genome meeting, San Diego, CA, January 14-18, 2017.
193. Wechter WP. 2019. Cucurbit and Brassica pathology research at the U.S. Vegetable Laboratory: Old-school and New-school approaches to plant disease resistance. Invited seminar at the Department of Plant Pathology, University of California-Davis.
194. Wechter P, Branham S, Levi A. 2017. 66-P: GBS-SNP-based linkage mapping and QTL associated with resistance to race 1 Fusarium wilt in *Cucumis melo*. Amer Phytopathological Soc. 266-P
195. Wechter, WP, Levi, A., Hassell, R. 2019. Carolina Strongback: Fusarium wilt and root knot nematode resistant Citrullus amarus rootstock for watermelon production. ISHS II International Symposium on Vegetable Grafting, Charlotte, NC USA S7-3.
196. Weng Y. 2017. Improve QTL detection power: cucumber downy mildew resistance. An invited talk at China Agricultural University (Beijing, China, July 111, 2017)
197. Weng Y. et al. 2018. Genetic architecture of downy mildew resistance in cucumber. Cucurbit workshop. Plant and Animal Genome Conference (Jan 9-13, 2018, San Diego, CA).
198. Weng Y. 2018. Genetic basis of downy mildew resistance in cucumber. Cucurbitaceae 2018 International Meeting Abstracts (November 12-15, 2018, Davis, California)
199. Weng Y. 2018. 'Molecular Breeding Infrastructure Development in the USDA-ARS/University of Wisconsin Cucumber Improvement Program'. Invited talk at First China National Conference on Molecular Breeding in Horticulture Crops (July 29, 2018, Harbin, China)
200. Weng Y. 2018. 'Cucumber Molecular Breeding- current status and perspectives'. Invited talk in the Institute of Vegetable Research, Sichuan Academy of Agricultural Sciences (May 7, 2018, Chengdu, China)
201. Weng Y. 2018. Genetic makeup of the legendary Gy14 and WI 2757 cucumbers. Oral presentation at Cucurbitaceae 2018 (Nov 13-16, 20118, Davis, CA)
202. Weng Y. 2020. Genetic basis of fruit size and shape variation in cucurbits. Oral presentation at Plant and Animal Genome Conference XXVIII (Jan 13-18, 2020, San Diego, CA).
203. Wintermantel WM, McCreight JD, Natwick ET. 2016. Epidemiology of *Cucurbit yellow stunting disorder virus* (CYSDV) and associated whitefly-transmitted viruses in the US Southwest and development of CYSDV resistant melon. Paper presentation at 2nd International Whitefly Symposium, February 14-19, Arusha, Tanzania.
204. Wintermantel WM, McCreight JD, Natwick ET. 2016. Reservoir hosts of *Cucurbit yellow stunting disorder virus* and development of resistant melon. 13th International Plant Virus Epidemiology Symposium. Avignon, France, June 6-10, 2016.
205. Wu S, Zhong Y, Grumet R, Levi A, Weng Y, Mazourek M, McCreight J, Katzir N, Garcia-Mas J, Fei Z. 2017. Cucurbit genomics database. Sol/Cuc Conference, Valencia, Spain
206. Wu S. 2018. Cucurbita genome sequences provide insights into polyploid genome evolution and heterosis in interspecific hybrid. PAG. January.
207. Wu S. 2018. Pan-genomes of the *Citrullus* Species. Cucurbitaceae 2018. Davis CA
208. Wu S. 2020. De novo genome assembly of sweet watermelon relatives and construction of Citrullus pan-genome. Plant & Animal Genomes XXVIII Conference. January

209. Zhang C, Mansfeld BM, Grumet R. 2018. Development of a real-time fluorescence based microplate assay for pathogen growth on plant tissue: *Phytophthora capsici* infection of cucumber fruit. Cucurbitaceae 2018, Davis CA

210. Zhao JZ, Bo KL, Pan YP, Gu XF, Weng Y. 2020. CsphyB and CsGA20ox-2 coordinate hypocotyl elongation in cucumber. Poster presentation at Plant and Animal Genome Conference XXVIII (Jan 13-18, 2020, San Diego, CA).

211. Zheng Y. 2018. Cucurbit Genomics Database: Integration genetic and genomics resources for cucurbit breeding. PAG. January

EXTENSION PRESENTATIONS

1. Adams ML, Quesada-Ocampo LM. 2016. Managing fungal diseases in cucurbits. NC Watermelon Convention. Wrightsville Beach, SC, Mar.
2. Adams ML, Quesada-Ocampo LM. 2015. Managing fungal foliar diseases in cucurbits. 30th Annual
3. Adams M, Quesada-Ocampo LM. 2018. Control options for cucurbit powdery mildew. 33rd Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, December.
4. Adams M, Quesada-Ocampo LM. 2018. Phytophthora fruit rot of watermelon. NC Watermelon Convention. Wrightsville Beach, NC, March 2018.
5. Bertucci, M., K. Jennings, D. Monks, D. Jordan, F. Louws, and J. Schultheis. 2015. Competitiveness of grafted watermelon plants versus nongrafted watermelon plants at various times of weedy and weed-free intervals. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC. Dec.
6. Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2015. Critical period for weed control in grafted and nongrafted triploid watermelon (Poster). North Carolina Crop Protection School. December 2, Cary, NC.
7. Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2016. Effect of grafting on the critical period of weed control of triploid watermelon (Poster). North Carolina Crop Protection School. December 6, Cary, NC.
8. Birdsell T, Schultheis J, Perkins-Veazie P. 2017. Butternut squash cultivar production, harvest, and enterprise budget considerations. Annual North Carolina Vegetable Growers Association. Myrtle Beach, SC, November.
9. Chacko NJ, Mou B, Coffey MD. 2016. Powdery mildew race variation in California. California Melon Research Board, Annual Meeting, San Diego, CA, Jan.
10. D'Arcangelo K, Adams M, Quesada-Ocampo LM. 2018. Controlling downy mildew in cucumber. NC Pickle Grower Meeting. Wilson, NC, March 2018.
11. Druffel A, Adams M, Quesada-Ocampo LM. 2018. Management options for watermelon Fusarium wilt. NC Watermelon Convention. Wrightsville Beach, NC, March 2018.
12. Grumet R. 2015. Update on resistance to *Phytophthora capsici* in cucumber. PPI Annual Meeting October 30, 2015, Fort Worth, TX
13. Grumet R. 2015. Introduction to CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
14. Grumet R. 2016. Introduction to CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops. California Melon Research Board, Annual Meeting, San Diego, CA, Jan.
15. Grumet R. 2017. CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops. PPI Spring Meeting, April 19, Milwaukee WI
16. *Grumet R. 2019. Resistance of cucumber fruit to Phytophthora capsici. (Oral Presentation, Pickling Cucumber Research Committee, December 5, 2019, Grand Rapids, MI)*
17. *Grumet R. 2019. Resistance of cucumber fruit to Phytophthora capsici. Oral presentation in Pickle Packer International (PPI) Annual meeting (November 11, 2019, St Petersburg, FL)*
18. Grumet R, Colle M. 2015. Development of genetic stocks for cucumber fruit resistance to *Phytophthora capsici*. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
19. Grumet R, Lin YC, Mansfeld B. 2017. Resistance of cucumber fruit to *Phytophthora capsici*. PCIC/PPI, Nov. 1, Chicago IL
20. Grumet R, Lin YC. 2017**.** Resistance to Phytophthora fruit rot in cucumber. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.

21. Grumet R, Lin YC. 2018. Resistance to Phytophthora fruit rot in cucumber. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
22. Grumet R, Mansfeld B, Lin Y-C. 2016. Genetic characterization and development of breeding materials for resistance of young cucumber fruit to infection by *Phythophthora capsici.* Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
23. Harlan B, Hausbeck M. 2017. Vegetable diseases and control strategies. Michigan Agribusiness Association Meeting, Lansing, MI, 11 Jan. 60 attendees.
24. Hausbeck M. 2015. The downy mildew report. Pickling Cucumber Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. 144 attendees.
25. Hausbeck M. 2015. Downy mildew research. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec. 30 attendees.
26. Hausbeck MK. 2015. Ten years of downy mildew in Michigan. Pickle Packers International Inc Annual Meeting, Fort Worth TX, Oct. 30 attendees.
27. Hausbeck M. 2016. The downy mildew report. Syngenta Meeting, Lansing, MI, Feb. 120 attendees.
28. Hausbeck M. 2016. Soilborne *Phytophthora capsici* on vine crops: Update and implications, Extension Specialist Breakfast Meeting via Zoom videoconference, East Lansing, 16 Jun. 15 attendees
29. Hausbeck M. 2016. *Phytophthora capsici*: Pathogen biology. *Phytophthora capsici* Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, Dec. 25 attendees.
30. Hausbeck M. 2017. A smorgasbord of vegetable diseases is on today's menu. MSU Extension and AgBioResearch State Council Meeting, Lansing, MI, Mar. 30 attendees.
31. Hausbeck M. 2017. Managing Phytophthora crown and fruit rot in cucurbit crops. Vegetable Growers' Meeting, East Aurora, NY, 15 Feb. 40 attendees.
32. Hausbeck M. 2017. Managing Phytophthora crown and fruit rot in cucurbit crops. Syngenta Meeting, East Lansing, MI, 9 Feb. 75 attendees.
33. Hausbeck M. 2017. Managing Phytophthora crown and fruit rot in cucurbit crops. Wisconsin Fresh Fruit and Vegetable Conference, Wisconsin Dells, WI, 23 Jan. 40 attendees.
34. Hausbeck M. 2018. Phytophthora management for winter squash, cucumber and pepper. Grower Meeting, Hudsonville, MI, 21 Feb. 30 attendees.
35. Hausbeck M. 2018. Fungicides & Diseases: Managing Diseases for Higher Profitability and a Safer Environment at MSU Agriculture Innovation Day: Focus on Fruit and Vegetable Technologies. June 28.
36. *Hausbeck M. 2018.* Organic Management Field Day: Phytophthora management in squash production, Kellogg Biological Station, Hickory Corners, MI, 19 Sep, 40 attendees
37. Hausbeck M. 2018. Managing Cucumber Diseases in 2019 at the Great Lakes Expo and Michigan Greenhouse Growers Expo. Dec. 4
38. Hausbeck M. 2019. *Phytophthora* and Downy Mildew Workshop at the Southwest Michigan Research and Education Center. Jan. 23
39. Hausbeck M. 2019. Real-world management strategies for *Phytophthora*-infested ground. Oceana County MSU Extension, Hart, MI. Jan 31.
40. Hausbeck M. 2019. Biology and control of Cucurbit Downy Mildew and *Phytophthora capsici* in Pickling Cucumbers. Saginaw Valley Research and Extension Center in Frankenmuth, MI. Feb. 14
41. Hausbeck M. 2019. Managing Phytophthora Crown and Fruit Rot. Ontario Fruit and Vegetable Convention. Feb 20-21.
42. Hausbeck M. 2019. Disease Management. SE Michigan Winter Vegetable Meeting. March 12.
43. *Hausbeck M. 2019, Phytophthora and Cucurbit Downy Mildew Workshop, Muskegon, MI, 5 Mar, 48 attendees.*

44. *Hausbeck M. 2019. Phytophthora capsici – what you need to know about its biology and management. Phytophthora and Cucurbit Downy Mildew Meeting, Hart, 5 Mar.*
45. *Hausbeck M. 2019. Managing Phytophthora crown and fruit rot in cucurbit crops. Phytophthora and Cucurbit Downy Mildew Meeting, Hart, 5 Mar.*
46. *Hausbeck, M.K. 2020. Phytophthora capsici-What You Need to Know About Its Biology and management. Northern Growers and Marketers Conference, St Cloud, MN, 16-17 Jan. Zoom presentation.*
47. Hausbeck MK, Cook A. 2015. The downy mildew report. Pages 9-14 in: Pickling Cucumber Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. Online.
48. Hausbeck MK, Goldenhar K. 2016. Downy mildew prevention and control. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
49. Hausbeck MK, Golenhar K, Bello JR. 2016. Downy mildew: What's next? Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
50. Hausbeck MK, Perla D, Linderman S. 2018. Diagnosing and managing Phytophthora on squash. Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, Dec. Poster presentation.
51. *Higgins, D.S., and Hausbeck, M.K. 2020. Managing vegetable diseases: tomatoes and cucurbits. Thumb Vegetable Meeting, Attica, MI, 6 Feb. 21 attendees.*
52. *Higgins, D.S., and Hausbeck, M.K. 2020. Managing vegetable diseases: cucurbits and cabbage. Southeast Vegetable Meeting, Dundee, MI, 4 Feb. 33 attendees.*
53. Indermaur, E., K. Starke, and J. Schultheis. 2017. Galia and canary melon cultivar evaluation. Annual North Carolina Vegetable Growers Association. Myrtle Beach, SC, November.
54. Kousik CS. 2016. Progress and challenges in managing Phytophthora fruit rot of watermelon. Indiana Horticultural Congress, Indianapolis, IN. January. 45 attendees (at the talk)
55. Kousik CS. 2016. Managing Phytophthora fruit rot of watermelon. Georgia Watermelon Association, St. Simmons, GA. January. Over 100 attendees
56. Kousik CS. 2016. Progress and challenges in managing Phytophthora fruit rot of watermelon. U.S. Vegetable Laboratory Seminar. Charleston, SC. March
57. Kousik CS. 2017. Presented information on Phytophthora fruit rot and powdery mildew of watermelon to the U.S. Secretary of Agriculture, Dr. Sonny Purdue and his team when they visited the U.S. Vegetable Laboratory, USDA, ARS in Charleston, SC. August 21, 2017.
58. Kousik CS. 2017. Provided information to Sarah Mock, Washington D.C. Bureau Chief for RFD-TV on research being conducted on watermelon at the U.S. Vegetable Laboratory and details of the visit of Dr. Sonny Purdue to USVL. August, 21, 2017. The interview was aired by RFD-TV and is located at website: https://youtu.be/R4tHGZSJqRI
59. Kousik CS. 2018. Best practices to reduce impact of *Phytophthora*. Southeast Regional Fruit and Vegetable Conference, Savannah, GA, January 2018. >100 attendees at the talk.
60. Krasnow C, Hausbeck M. 2016. Progress in cucumber downy mildew control. Southwest Hort Days, Benton Harbor, MI, Feb. 35 attendees.
61. Krasnow C, Hausbeck M. 2016. *Phytophthora capsici*: Fungicide programs and crop resistance. *Phytophthora capsici* Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, Dec. 55 attendees.
62. Krasnow C, Hausbeck M. 2016. Orondis: a new tool for controlling Phytophthora blight on pepper and squash. Syngenta Meeting, Lansing, MI, Feb. 75 attendees.
63. Krasnow C, Hausbeck M. 2016. Phytophthora blight: management strategies for pepper and squash. Southwest Hort Days, Benton Harbor, MI, Feb. 35 attendees.
64. Krasnow C, Hausbeck M. 2016. Rots and blights of vegetables. Bay Area Growers Extension Meeting, Bay City, MI, Jan. 40 attendees.

65. Mandal MK, Kousik CS. 2017. Resistance Signaling in Watermelon using Genomics and Metabolomics. Invited talk to seed industry HM. Clause, (HARRIS MORAN and CLAUSE, LIMAGRAIN), Sacramento, Davis CA. June, 2017. >30 attendees.
66. Mandal MK, Kousik CS. 2018. Multidimensional approaches to study host-resistance signaling in cucurbits against diseases: from epidemiology to omics. Invited talk to USVL USDA-ARS, Charleston SC. January, 2018. >40 attendees
67. McCreight JD. 2015. Melon host plant resistance to powdery mildew and CYSDV. Fall Desert Crops workshop, sponsored by the University of California ANR Cooperative Extension, Imperial County, and University of Arizona Cooperative Extension, Yuma County. El Centro, CA, Oct.
68. McCreight JD. 2017. "Agricultural Research Technology Center," Western Regional Seed Physiology Research Group, University of California, Davis, January 24, 2017.
69. McCreight JD. 2017. AgKnowledge class annual visit to U.S. Agricultural Research Station, Grower-Shipper Association of Central California, Salinas, CA, June 2017.
70. McCreight JD. 2017. Assisted Seed Central (http://www.seedcentral.org) hosting 100 persons from ag related companies with research updates and provided laboratory and greenhouse tours, Salinas, CA, April.
71. McCreight JD. 2017. U.S. Plant Breeding: Lettuce, Spinach, Melon, and Sugar beet. Seed Central (an initiative of the Seed Biotechnology Center at the University of California Davis, and Seed Quest), Salinas CA, April.
72. McCreight JD. 2017. Melon powdery mildew race variation in California. University of California, Cooperative Extension, Imperial County, 28th Annual Fall Desert Crops Workshop, Imperial, CA, December.
73. McCreight JD. 2018. Lettuce and melon breeding for resistance to diseases and insects. University of California, Plant Breeding Retreat, Monterey, CA, 17-18 December 2018.
74. McCreight JD, Natwick ET. 2016. Evaluation and breeding of new sources of host plant resistance to CYSDV and sweetpotato whitefly biotype B. California Melon Research Board, Annual Meeting, San Diego, CA, Jan.
75. McCreight JD, Natwick ET. 2017. Evaluation and breeding of new sources of host plant resistance to CYSDV and sweetpotato whitefly biotype B. California Melon Research Board, Annual Meeting, San Diego, CA, Jan. 4, 2017.
76. McCreight JD, Natwick ET. 2018. Evaluation and breeding of new sources of host plant resistance to CYSDV and sweet potato whitefly biotype. California Melon Research Board, Annual Meeting, San Diego, 4 January 2018.
77. McGregor CE. 2016. Advances in Watermelon Breeding. Southeast Regional Fruit & Vegetable Conference, 8-10 January 2016, Savannah, GA .
78. Meadows I, Mauney C, Quesada-Ocampo LM. 2016. Agent training on disease diagnostics and management in vegetable crops. Extension Conference. Raleigh, NC, November 2016.
79. Miller NF, Quesada-Ocampo LM. 2015. New control options for Fusarium wilt in watermelon. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, Dec.
80. Miller NF, Quesada-Ocampo LM. 2016. Evaluation of a new fungicide for management of Fusarium wilt of watermelon. NCSU Masters Symposium, Raleigh, NC, November 2016.
81. Miller N, Druffel A, Adams M, Quesada-Ocampo LM. 2017. Control options for Fusarium wilt of watermelon. 32nd Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December
82. Noel N, Adams M, Quesada-Ocampo LM. 2018. Fungicides and host resistance for control of watermelon anthracnose. NC Watermelon Convention. Wrightsville Beach, NC, March 2018.
83. Quesada-Ocampo LM. 2015. Diagnostics and management of cucurbit downy mildew. Pickle Packers International Annual Meeting. Fort Worth, TX, Oct.

84. Quesada-Ocampo LM. 2016. Downy mildew and *Phytophthora* control in cucurbits. NC Watermelon Convention. Wrightsville Beach, NC, Mar.
85. Quesada-Ocampo LM. 2016. Cucurbit downy mildew management, diagnostics, and pathogen populations. Pickle Packers International Spring Meeting. Raleigh, NC, Apr.
86. Quesada-Ocampo LM. 2016. Downy mildew updates for cucurbits. Southeast Regional Fruit and Vegetable Conference. Savannah, GA, Jan.
87. Quesada-Ocampo LM. 2016. Disease identification on vegetables. Certified Crop Advisor Training. Smithfield, NC, December 2016.
88. Quesada-Ocampo LM. 2016. Fungicides and host resistance for cucurbit downy mildew management. 31st Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2016.
89. Quesada-Ocampo LM. 2016. Management of cucumber downy mildew using fungicides and host resistance. Pickle Packers International Annual Meeting. Charleston, SC, October 2016.
90. Quesada-Ocampo LM. 2017. Cucurbit disease management. Commercial vegetable grower symposium. Henderson, NC, February 2017.
91. Quesada-Ocampo LM. 2019. Biology and control of Cucurbit Downy Mildew and Phytophthora capsici in Pickling Cucumbers. Saginaw Valley Research and Extension Center in Frankenmuth, MI. Feb. 14
92. Quesada-Ocampo LM, Adams M, Collins H, Salcedo A, Purayannur S, Standish J, D'Arcangelo K, Stahr M, Parada C, Wong S. 2019. Agent training on disease diagnostics and management in vegetable crops. Clayton and Raleigh, NC, July 2019.
93. Quesada-Ocampo LM, Adams M, Collins H, Salcedo A, Purayannur S, Standish J, D'Arcangelo K, Stahr M, Parada C, Wong S. 2019. Small Farms Tour: disease diagnostics and management in vegetable crops. Clayton, NC, June 2019.
94. Quesada-Ocampo LM, D'Arcangelo K, Adams M. 2018. Management of cucurbit downy mildew in pickling cucumber and other cucurbit crops. 33rd Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, December 2018
95. Quesada-Ocampo LM, Meadows I, Shew B, Eure E, Mauney C. 2018. Agent training on disease diagnostics and management in vegetable crops. Extension Conference. Raleigh, NC, November 2018.
96. Quesada-Ocampo LM. 2019. Biology and control of Cucurbit Downy Mildew and *Phytophthora capsici* in Pickling Cucumbers. Saginaw Valley Research and Extension Center in Frankenmuth, MI. Feb. 14
97. *Reyes, J.A., Villari, C., Brewer, M.T., Dutta, B., and McGregor, C.E. (2020) Detection of the Gummy Stem Blight-Causing Pathogens in Watermelon Using Field-Adapted Quick and Simple Technologies. Southeast Regional Fruit & Vegetable Conference, Savannah, GA. Poster*
98. Ribera LA. Trade impact talks:
    a. APHIS Project Kick-Off, Raleigh, North Carolina, January 31, 2017.
    b. C-FARE, Washington, DC, April 6, 2017.
    c. Viva Fresh 2017, Austin, Texas, April 21, 2017.
    d. Texas A&M AgriLife Program Planning Meeting, Rosenberg, Texas, May 9, 2017.
    e. Texas International Produce Association, Mission, Texas, May 30, 2017.
    f. Moosejaw, Canada, June 27, 2017.
    g. Imperial Valley EDC, Calexico, California, August 15, 2017
    h. Ag. Economics Extension Tailgate Workshop, College Station, Texas, September 30, 2017.
    i. Extension Outlook Conference, Stillwater, Oklahoma, October 20, 2017
    j. Del Rio Economic Development Council, Del Rio, Texas, November 2, 2017.
    k. Imperial Valley EDC Annual Banquet, Calexico, California, November 16, 2017.
    l. 29th Annual Texas Plant Protection Conference, Bryan, Texas, December 5, 2017.

99. Schultheis, J.R. 2016. Grafted vs. nongrafted watermelon studies. NC Watermelon Convention. Wrightsville Beach, NC, Mar.
100. Schultheis JR. 2017. A perspective on melons; some North Carolina cultivar results and some "food" for thought. Eastern Cantaloupe Growers Association. Nashville, TN, 16 February 2017.
101. Schultheis JR. 2017. The North Carolina pickling industry and use of parthocarpic fruiting types. Pickling Cucumber Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids,MI, Dec.
102. Schultheis, J. 2017 Great Lakes Fruit, Vegetable & Farm Market EXPO, "Perspectives and opportunities for growing orange flesh and specialty melons" in Vine Crops Session Summaries, pages 2-7. http://glexpo.org/summaries/2017summaries/VineCrops.pdf
103. Schultheis, J. 2017 Great Lakes Fruit, Vegetable & Farm Market EXPO, "The North Carolina pickling industry and use of parthenocarpic fruiting types" in Pickling Cucumber Session Summaries, pages 12-14. http://glexpo.org/summaries/2017summaries/PicklingCucumber.pdf
104. Schultheis JR 2018. The North Carolina pickling industry and use of parthenocarpic fruiting types.. Regional Pickling Cucumber meeting, Wilson Co., NC, March
105. Schultheis, J. 2018. Melon varieties; orange flesh and various specialty melon opportunities. DelMar Vegetable meeting. City, DE, January 2018.
106. Schultheis, J. 2018. A melon mix of specialty and cantaloupe types. Ontario Fruit and Vegetable Convention. Niagara Falls, ON Canada. February 2018
107. Schultheis JR, Birdsell T. 2017. Butternut squash production. Winter Vegetable Conference and Trade Show. Asheville, NC, 8 February 2017.
108. Schultheis, J.R. and S. Johnson. 2015. Grafted versus nongrafted watermelon studies using bare ground or plasticulture production methods. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC. Dec
109. Schultheis J, Starke K. 2017. Standard size watermelon cultivar and quality results, North Carolina, 2017. Georgia Watermelon Association. Saint Simons Island, GA, January 2018.
110. Schultheis, J. and K. Starke. 2017. Specialty melon opportunities. Annual North Carolina Vegetable Growers Association, Myrtle Beach, SC, November.
111. Schultheis, J. and K. Starke. 2017. Watermelon production considerations; pollenizer and grafting. Annual North Carolina Vegetable Growers Association, Myrtle Beach, SC, November.
112. Schultheis J, Starke K. 2018. Standard size watermelon cultivar yield and quality results, North Carolina, 2017. North Carolina Watermelon Association, Wrightsville, NC, March.
113. *Schultheis, J. and K. Starke. 2019. Review of the newest summer squash cultivars. AgExpo. Wilmington, NC. Dec*.
114. Schultheis JR, Thompson WB. 2016. Watermelon cultivar yield and quality trial results, North Carolina, 2015. 2016. Watermelon Research Group, San Antonio, TX, Feb.
115. Smart C. 2015. Disease problems common during the 2015 growing season. Twilight meeting, Eden Valley, NY.
116. Smart C. 2016. Managing cucurbit diseases. Empire State Producers Expo. Jan
117. Smart C. 2016. Disease update. Western NY Vegetable Growers meeting. Lockport, NC, Mar.
118. Smart CD. 2016. Vegetable Diseases (for beginning growers), March 16, 2016. 1 hour webinar.
119. Smart CD. 2016. How the NY Farm Bureau helped established the *Phytophthora* blight farm. Midwest Farm Bureau visit to NYSAES, June 24, 2016.
120. Smart CD. 2016. Vegetable disease management (1.5 hour discussion with growers and educators). Canton, NY, Aug 3, 2016.
121. Smart CD. 2016. Vegetable disease management (1.5 hour discussion with growers and educators). Willsboro, NY, Aug 4, 2016

122. Smart CD. 2016. Field walk and discussion of diseases of cucurbits and other crops. Western NY Field Days. Portland, NY, Aug 31, 2016.
123. Smart CD. 2016. Why is the *Phytopthora* blight from important? New York State Ag Experiment Station Task Force, October 10, 2016.
124. Smart CD. 2016. Managing cucurbit downy mildew in organic systems in the northeast. December 6, 2016. 1.5 hour webinar with 135 participants.
125. Smart CD. 2016. Understanding and controlling diseases of cucurbits. Crop Consultant Meeting, December 1, Syracuse NY.
126. Smart CD. 2017. Vegetable Diseases (for beginning growers), March 15. This was a 1.5 hour webinar.
127. Smart CD. 2017. Twilight discussion of cucurbit diseases. Western NY Field Days. Portland, NY, Aug Smart CD. 2017. On-farm discussion of methods to control Phytophthora blight in summer squash. Seneca Falls NY, July 2017
128. Smart CD. 2017. Role of cover crops in Phytophthora blight control. Northeast Cover Crops Council. November 2017
129. Smart CD. 2019. Real-world management strategies for Phytophthora-infested ground. Oceana County MSU Extension, Hart, MI. Jan 31.
130. Smart CD. 2019. Update on Phytophthora blight research. 30 minute talk to about 50 growers. New York State Producers Expo. Syracuse NY. January 2019
131. Smart, C.D. Eastern NY Fruit & Vegetable Conference. February 26, 2020. A year in review – Diseases from 2019 and what to expect in 2020. 30 minute talk to 100 growers. Contact hours = 50.
132. Smart, C.D. Empire State Producers Expo Syracuse, NY. Jan 15, 2020 Workshop on Phytophthora blight organized by Smart's labl 9:00 – 10:15 AM including presentation by Vogel Options and outlook for Phytophthora resistance in peppers and squash. 1.25 hour session to100 growers. Contact hours = 125
133. Smart C, Lange H. 2016. Vine Crop Update 2015. Proceedings of the 2016 Empire State Producers Expo, Syracuse, NY.
134. Smart CD, Lange H. 2018. Fungus, Water Mold or Bacteria: Which is Which in My Vine Crops? New York State Producers Expo. January
135. Smart, CD and Lange, H. 2019. Disease Update: What we saw in 2018 and what to expect in 2019. Proceedings of the Empire State Producers Expo. January 2019.
136. Starke KD, Schultheis JR. 2018. Mini-watermelon cultivar yield and quality evaluations in North Carolina, 2017. Watermelon Res Dev Group Ann Meet Jacksonville, FL, February.
137. VandenLangenberg K, Wehner T. 2015. High resistance over the production season to the new downy mildew in cucumber. PPI Annual Meeting (October 30, 2015, Fort Worth, TX)
138. Wallace EC, Quesada-Ocampo LM. 2015. Controlling downy mildew in cucumber. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, Dec.
139. Wang Y, Haider KR, Weng Y. 2016. Pyramiding Downy Mildew Resistance Genes into Elite US Processing Cucumber with Marker-assisted Selection. Pickling Cucumber Commodity Meeting, Grand Rapids
140. Weng Y. 2015. QTL Mapping for downy mildew resistance in WI7120 cucumber. PPI Annual Meeting (October 30, 2015, Fort Worth, TX)
141. Weng Y. 2017. "Genetic architecture of downy mildew resistances in cucumber". 2017 PPI Annual Meeting (Chicago, IL, Nov 1 2017)
142. Weng Y. 2017. Genetic resources for cucumber breeding - a molecular perspective. PPI 2017 Spring Meeting (4-19-2017, Milwaukee, WI).
143. Weng Y. 2019. Marker-assisted pyramiding of downy mildew resistance genes into US processing cucumber – a update. Cucumber reporting session, Great Lakes Expo.

144. Weng Y. 2019. Improve downy mildew resistance of pickling cucumber through marker-assisted breeding. Oral presentation in Pickle Packer International (PPI) Annual meeting (November 11, 2019, St Petersburg, FL)

145. Weng Y. 2019. QTL pyramiding to enhance downy mildew resistance in pickling cucumber. Oral presentation at Midwest Pickle Association annual meeting (Dec 5, 2019, Grand Rapids, MI)

146. Wessel-Beaver L, Linares Ramirez AM. 2017. Dos Virus Importantes en la Calabaza: Mosaico Amarillo del Calabacín (ZYMV) y Mancha Anular de la Papaya (PRSV). Expo Hort, Lajas, PR 4 April. 118 attendees

147. Wintermantel, W.M. 2020. Development of Rapid Detection Methods for Evaluation of Germplasm for Resistance and Determination of the California Host Range of Cucurbit Chlorotic Yellows Virus and Squash Vein Yellowing Virus. California Melon Research Board Annual Research Symposium. San Diego, CA. January 9, 2020.

148. Wintermantel, W.M. 2020. Virus Diseases of Melons. University of California Cooperative Extension Winter Vegetable Crop Research Meeting, University of California West Side Research and Education Center, Five Points, CA. January 21, 2020.

149. Wintermantel, W.M. 2020. Identifying threats from virus diseases in watermelon and other cucurbit crops. Webinar, Mississippi State University Extension Service. May 13, 2020.

150. Wong TW, Quesada-Ocampo LM. 2018. Evaluation of SDHIs for management of root knot nematode, gummy stem blight, and Fusarium wilt of watermelon. NC Watermelon Convention. Wrightsville Beach, NC, March 2018.

151. Wong S, Stahr M, Collins H, Quesada-Ocampo LM. 2018. Host resistance and fungicides for integrated management of watermelon Fusarium wilt. 33rd Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, December 2018.