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8–13 September 2014, Kraków, Poland

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Oral presentations

11th Conference of the European Foundation for Plant Pathology

Session 1

New pathogens and shifts in pathogenicity

8–13 September 2014, Kraków, Poland

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Molecular tools allow the description of new taxons at species and infra-specific levels among plant pathogenic bacteria

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Diagnostic of plant diseases is evolving and improving day by day. Molecular-based tools such as real-time PCR, LAMP, microarrays, barcoding and Next Generation Sequencing are enhancing the accuracy of the detection and identification of plant pathogenic bacteria. These technologies are currently based on the use of available nucleic acid sequences and information about the molecular interactions bacterial pathogen-plant host. The target DNA sequences selected for these techniques must be distinctive and based on conserved regions, in order to identify only one species, subspecies, pathovar or a particular set of genes. However, the currently available databases contain only a small portion of the sequences of the microbes present in complex environments. The use of integrated approaches for detection and identification of plant pathogenic bacteria has allowed the discovery of differences among strains classified to date in the same species but showing distinct characteristics in molecular identification tests. Three examples are presented: 1) *Erwinia piriflorinigrans*, a new pathogenic species described in Spain as causal agent of necrosis of pear blossoms that is closely related to *E. amylovora*, 2) a new *Xanthomonas* sp., also isolated in Spain and responsible of symptoms similar to those of *Xanthomonas arboricola* pv. *pruni,* but classified as different to this species according to multilocus sequencing analysis as well as to serological and PCR identification, 3) the new group of *Pseudomonas syringae* pv. *actinidiae* strains described from New Zealand, France and Spain, that show low virulence and different molecular characteristics. The description of new species or infra-specific groups leads to a more comprehensive knowledge of the complex pathosystems in agricultural industry and nature. Moreover, the partial or complete genomes of new taxons that are being determined will offer new omics-based tools for improving the detection, identification and taxonomy of bacterial plant pathogens.

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The genus Torradovirus, an overview

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In 2007, *Tomato torrado virus* (ToTV) was described as the causal agent of the devastating "torrado" disease, which was causing high economic losses in the tomato growing area of South-East Spain. It was a new virus, not belonging to any known plant virus genus at that time. Now, 7 years later, ToTV has been taxonomically designated as the type member of a new virus genus, the genus *Torradovirus*, which was created within the family of *Secoviridae*. Members of the genus are ToTV and *Tomato marchitez virus* (ToMarV), while Tomato chololate spot virus (ToChSV), Tomato chocolàte virus (ToChV) and Lettuce necrotic leaf curl virus (LNLCV) are still tentative members.

Torradoviruses possess spherical particles, approximately 30 nm in diameter, which harbour a bi-partite ssRNA genome. In the first, the largest, RNA a single polyprotein is encoded by ORF1, which is later processed into several functional replication proteins (e.g. RdRP, helicase). In the smaller RNA2, the putative movement protein and the three coat proteins are encoded by ORF2. The small ORF1 at the 5′ end of the RNA2 is a unique feature for torradoviruses, but it's function is still unknown.

Torradoviruses are transmitted by whitefly vectors. So far, three whitefly species were found capable to transmit the tomato-infecting torradoviruses; *Bemisia tabaci*, *Trialeurodes vaporariorum* and *T. abutilonea*. Recent work revealed the mode of transmission of torradoviruses, which are transmitted in a semi-persistent stylet-borne manner.

This paper will give an overview on the research done on members of the genus *Torradovirus*, including some new insights from recent work.

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Umbel browning and stem necrosis on some cultivated apiaceous hosts in European domain

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Several *Phomopsis* species were associated with destruction of reproductive parts of apiaceous host plants. The main symptoms consisted in umbel browning and stem necrosis. Recently, similar symptoms have been found on dill and coriander in Bulgaria and Lithuania. Pycnidia developed on the host tissue and nutrient medium. Because of the presence of α- and β-conidia, the causal agents were identified as *Phomopsis* spp. The isolates from dill and coriander shared many phenotypic characteristics with *P. diachenii* and *P. foeniculi* previously reported as disease inciters on caraway and fennel, respectively. Therefore, the accurate identification at species level required the use of genotypic techniques. Phylogenetic analysis was performed on *Phomopsis* isolates obtained from cultivated and wild growing apiaceous hosts using sequences from the Internal Transcribed Spacers 1 and 2, including 5.8S region of the nuclear ribosomal DNA. NCBI database, CLUSTALW and DNASTAR Lasergene 8.1.4 softwares were used in the study. Phylogenetic analysis revealed close relationship of teleomorph species *Diaporthe angelicae* and asexually reproducing fungi like *Phomopsis diachenii*, *P. foeniculi* and the newly obtained isolates from dill and coriander in Bulgaria and Lithuania. The isolates from some wild growing apiaceous species as *Heracleum sibiricum, H. sphondylium* and *H. sosnowskyi* showed high genetic similarity with them suggesting that they could serve as a source of inoculum for the cultivated ones.

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New data for the taxonomy and variability of the *Enterobacteriaceae* family

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The *Enterobacteriaceae* family contains approximately 150 bacteria species in 30 genuses. The fire blight – caused by the *Erwinia amylovora* – is one of the most devastating plant disease on pome fruits. In Hungary the first appearance was observed in 1995. During 2012–2014 the pathogen was isolated from plum, apricot and cherry plum. A wilting disease, caused by *Brenneria salicis*, inflicts serious damages on *Salix* sp. in several countries. The causative agent is present in Hungary, and makes mass tree decay in the country. The bacterium was isolated in Hungary in 2013 from the bark of an elm tree (*Ulmus* sp.). The shallow blight (vertical oozing cankers on the bark) caused by *Brenneria nigrifluens* was first reported from Hungary in 2013 from an old walnut tree from a home garden at Zánka, and from a sycamore also from this year. In our work we aimed the exploration of the kinship relations between the bacteria species of *Enterobacteriaceae* family based on sequence analysis, according to 16S rRNA gene and two housekeeping genes (*infB and atpD*).

Bacteria isolated from diseased leaf and stem tissues was macerated and streaked on King's medium B. Isolated bacteria grew at 26°C, were Gram negative. *Erwinia amylovora* isolates induced, *Brenneria nigrifluens*, *Brenneria salicis*, *Brenneria goodwinii* not induced a hypersensitive reaction in tobacco leaves. Biochemical test was also used for identification. Pathogenicity were tested by injecting five young healthy shoots, branches, fruits. After artificial infection the pathogen specific symptoms evolved on the plants. The pathogens were re-isolated from the infected plants and identified with the above method, thus Koch's postulates were fulfilled. For molecular identification and analysis of the variability of the pathogens, the 16S rRNA and two housekeeping genes (*infB and atpD*) were amplified and were sequenced from different isolates. The sequences were compared with the NCBI GenBank data.

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Biodiversity and phylogenetic position of bacteria causing bacterial canker of stone fruits trees in Poland

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According to pathogenic capability, within the bacterial species *Pseudomonas syringae* over 60 pathovars were distinguished. On the basis of DNA:DNA hybridization these pathovars were grouped into 9 genomospecies. Among them, the following pathovars infect plants of *Prunus* spp.: *syringae* (*Pss*), *morsprunorum* race 1 (*Psm*1) and race 2 (*Psm*2), *avii* (*Psa*) and *persicae* (*Psp*). The aim of our study was by using repeated sequences (rep-PCRs) and melting profiles (PCR MP) methods to characterize 168 *P. syringae* isolates obtained from different species of stone fruit trees with bacterial canker symptoms in various regions of Poland. Moreover, 65 strains selected on RFLP of 4 genes fragment analyses were also studied by Multilocus Sequence Typing (MLST). The tested bacteria were classified into pathovars *morsprunoum* (races 1 and 2) which was genetically very homogenous, and *syringae* – highly heterogeneous. Besides those pathovars a new highly homogenic group of isolates from cherry, marked *Pss*(A) was distinguished. Regarding phenotypic characters they were similar to pathovar *syringae* although in pathogenicity assay on cherry fruitlets they caused superficial lesions similar to those caused by pathovar *morsprunorum* strains. Sequence analysis of *gyr*B gene fragment of selected *Pss*, *Pss* (A), *Psm*1 and *Psm*2 strains and sequences of the same gene fragment of all available *Pseudomonas* type strains from GeneBank showed that they do not form a monophyletic group which is the basic criterion for classification into one species. Strains of *Pss* formed at least 9 phylogenetic lineages but only one of them contained the type strain of the species *Pseudomonas syringae* PDDCC 3023^T. Strains of *Psm*1 formed one cluster with type strains of species *P. amygdali* NCPPB 2607^T, *P. tremae* LMG 22121^T and *P. meliae* CCUG 51503^T whereas *Psm*2 strains were grouped with *P. avellanae* CIP 105176^T. The addition of the *gyr*B sequences of the type strains of *P. syringae* pathovars to the phylogenetic analysis showed that the distribution of studied strains was even more complex.

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Genetic diversity of *Colletotrichum acutatum* causing bitter rot on sour and sweet cherries in Poland

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In Summer 2012–2013, 29 various cherry and sweet cherry orchards, located in central, north and south west regions of Poland were examined for the presence of fungi causing bitter rot on cherries. In total, 111 of fungal isolates: 93 from sour and 18 from sweet cherries from visible rot symptoms were obtained. On the basis of morphological traits, examined during the fungal growth on PDA medium in pure cultures, all isolates were classified to the genus *Colletotrichum* and subsequently were identified as *C. acutatum* by PCR assay with species specific primers. In order to determine genetic diversity of all isolates, the analysis of amplification patterns obtained with 4 different ISSR and with 3 different RAPD primers as well as in PCR MP was conducted. For morphological and genomic diversity characterization, 10 *C. acutatum* isolates from other host plants as well as 2 other *Colletotrichum* species were included to analyses. Although low variability was observed within morphological traits of those isolates, genetic analysis revealed high level of diversity among isolates from cherries – the highest diversity was observed in amplification with ISSR primers. However, isolates from cherries were intermingled with the isolates from apple, pear and quince, showing very similar, grayish culture color, but they were clearly separated from grayish isolates from strawberry and pink-colored cultures from pear and cranberry. No correlation has been found between obtained amplification patterns and geographical origin of isolates, as well as host – sour or sweet cherry plant. Other two analyzed *Colletotrichum* species were clearly separated only in PCR MP assay.

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Session 2

Toxic metabolites of pathogens

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Mycotoxins in pathogen – patogen interactions: adaptation and response within *Fusarium* genus

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Usually mycotoxins are presented as harmful metabolites, acting as a pathogenicity or virulence factors or protecting the fungus from predators and competitors present in its environmental niche. The ability of fungal phytopathogens to exude toxic compounds is an important element of competition in a changing environment. The balance between producers and divergent non-producers plays an important role in the infection process.

For that reason, pathogenic fungi usually retain a number of adaptations related not only to the production of toxic compounds by themselves but also to the exogenous toxins present in the environment. We examine distinct effects of mycotoxins on morphology, growth patterns and gene expression after stimulation in both mycotoxin producing and non-producer isolates of divergent *Fusarium* species.

We demonstrate emergence of a distinct dosage-dependent "producer effect" (producing species being more resistant to their own toxin, relic resistance in species that lost the biosynthetic capacity) and possible trichothecene autotoxicity towards *F. graminearum* strains. By comparing the gene expression profiles and growth patterns of plant pathogenic *Fusarium* isolates, we obtained evidence that some trichothecene compounds can act as potential signalling molecules. We found that *Fusarium verticilioides*/*Fusarium proliferatum* isolates show a direct reaction to deoxynivalenol. The toxin present in the medium has strong effects on the mycelial growth rate, number of viable fungal cells in medium (luminogenic ATP assay) and early gene expression, in particular genes related to biosynthesis and transport of fumonisins (*fum1*, *fum19*, *fum21* genes).

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Current distribution of *Fusarium* fungi on small grain cereals in Russia: species complex and related mycotoxins

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Fusarium is the large genus that included pathogens, saprotrophs or endophytes. The *Fusarium* genus contains a number of heterogeneous species, some of which are more broadly adapted to environmental variability (*Fusarium poae*, *F. sporotrichioides*) than the other (*F. graminearum*, *F. culmorum*, *F. cerealis*). The adaptive ability of these fungi in different environments reflects their genetic plasticity and metabolic diversity. High levels of genome diversity may be instrumental in enabling the emergence of a new species. Some new phylogenetic species are associated with a geographical region, particularly in Russian Far East (*F. ussurianum*) and Siberia (*F. sibiricum*), but this does not preclude their detection in other regions of the world.

Fusarium head blight is the disease caused by a complex of several *Fusarium* species and resulting in lower grain yield and reduced quality due to mycotoxins. Mycogeographic survey of *Fusarium* species associated with grain of cereals has been done over the agricultural areas of Russia. The presence of toxigenic and non-toxigenic species within the disease complex, along with isolates of differing toxin-producing ability greatly complicate attempts to understand the factors that influence disease development and toxin accumulation.

The levels of T-2/HT-2 toxins are related to occurrence of *F. sporotrichioides* and *F. langsethiae*. *F. sporotrichioides* has detected on all cereal production territory. *F. langsethiae* has been found on the territory of European part of Russia. The levels of DON are positively related to infected kernels by *F. graminearum*. This pathogen belongs to geographically restricted fungi and detected largely in Far East and South-European area of Russia. Knowledge of distribution of toxigenic *Fusarium* species allows predicting and preventing mycotoxins contamination of grain yield and its products and, thus, reducing the risk of human and animal diseases.

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Molecular quantification and genetic diversity of toxigenic *Fusarium* species in northern Europe and Asia

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Fusarium graminearum is the most important DON producer in northern Europe and Asia and it has been replacing the closely related *F. culmorum* in northern Europe. The 3ADON chemotype of *F. graminearum* dominates in most northern areas, while the 15ADON chemotype of *F. graminearum* is dominating in Central and southern Europe. 15ADON chemotype is spreading to the north in western Europe, while 3ADON chemotype has spread to north-western Russia. The reasons for these changes in European *F. graminearum* populations will be discussed. We have also compared the correlation between *F. graminearum* DNA levels and DON levels in oats in Finland. DNA levels of *F. graminearum* were in all cases in agreement with DON levels in 2011 and 2012, when DON was measured by GC-MS. When the *RIDA®QUICK* SCAN kit results (DON) were compared to DNA levels of *F. graminearum*, the variation was much higher. The homogenization of the oats flour by sieving seems to be connected to this variation.

It is difficult to find morphological characters to separate all isolates of the European species *F. langsethiae* from the northern Asian species *F. sibiricum*, while the cosmopolitan species *F. sporotrichioides* can be easily identified based on morphological characters. The only species-specific primer pair for *F. sibiricum* is based on the long TG repeat in the ribosomal IGS region. Another way to identify *F. sibiricum* is to use a combination of four primer pairs, which are specific to different species of the species complex. *F. langsethiae* is a European species, while the main distribution of *F. sibiricum* is in northern Asia. One *F. langsethiae* isolate from Norway and another one from Iran were reidentified as *F. sibiricum*. Thus, the actual distribution of *F. sibiricum* may be larger than what is presently known.

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Gene expression of ABC-transporters in *Clonostachys rosea* IK726 in response to anti-fungal metabolites from *Pseudomonas chlororaphis* ToZa7 and *Serratia rubidaea* S55

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This study was undertaken in order to assess the possibility of combining *Clonostachys rosea* IK726 with the phenazine-producing bacterium *Pseudomonas chlororaphis* ToZa7 or *Serratia rubidaea* S55 in consortia of biocontrol agents (BCAs) for sustainable agriculture. The fungus *C. rosea* IK726, isolated from barley roots infected with *Fusarim culmorum* in Denmark, has proven to be highly efficient as a BCA against fungal pathogens of agricultural and horticultural crops. *P. chlororaphis* ToZa7 was recently isolated from the rhizosphere of tomato on Zakynthos Island, Greece and *S. rubidaea* S55 was isolated from the rhizosphere of wheat in Thermi, Northern Greece. In our previous study, we have shown the antagonistic activity of *P. chlororaphis* ToZa7 and *S. rubidaea* S55 against the tomato crown and root rot pathogen *Fusarium oxysporum* f.sp. *radicis – lycopersici* (Forl). *In vitro* results showed that *C. rosea* displayed a high level of tolerance to *S. rubidaea* S55 but not to *P. chlororaphis* ToZa7. We hypothesised that the tolerance of *C. rosea* could be attributed to the ATP – binding cassette (ABC) proteins, which are considered to be important for drug tolerance in microorganisms. Therefore, 14 *C. rosea* candidate ABC transporter genes representing the different ABC subfamilies were selected, and the gene expression patterns were monitored using quantitative reverse transcription PCR in mycelium by growing *C. rosea* in *P. chlororaphis* ToZa7 or *S. rubidaea* S55 culture filtrates for 1 and 4 hrs. Bacterial culture filtrates were obtained post 24 hrs and 72 hrs of incubation. Gene expression analysis showed a significant induction in expression of ABC transporter genes *ABC2419*, *ABC4987* (subfamily B) and *ABC3523*, *ABC3189* (subfamily G) in mycelium grown in filtrates of *S. rubidaea*'s S55 24 hrs culture. However, no significant induction was found in similar experiment using *P. chlororaphis* culture filtrates. Furthermore, when the fungus was grown in filtrates of *S. rubidaea*'s 72 hrs cultures, 6 genes (*ABC592*, *ABC3918*, *ABC3260*, *ABC2419*, *ABC4987*, and *ABC3433*) belonging to the subfamilies B and C, were significantly induced, whereas the *P. chlororaphis* 72 hrs culture filtrate caused an induction of only 3 genes (subfamilies B and C). In conclusion, this study could explain the higher tolerance of *C. rosea* IK726 to *S. rubidaea* S55 by underlining the importance of the ABC transporter genes in the fungus, since the majority of these induced genes are involved in secondary metabolite efflux, and induced after *S. rubidaea* S55 culture filtrate treatment.

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Segregation of *Alternaria* strains isolated from cereals in Germany and Russia according to their morphological, molecular and toxigenic features

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The genus *Alternaria* consists of saprophytic and parasitic fungi with a worldwide distribution. Parasitic *Alternaria* species are often plurivore and may be isolated from different cereals causing harvest losses and decreased grain quality. In the present study a polyphasic approach for the characterization of 95 small spored *Alternaria* strains from northeast Germany and Russia was made. Morphological examination of conidial appearance and arrangement sorted the strains into the morphological species groups of the *A. alternata*-, *A. arborescens*-, *A. infectoria*- and *A. tenuissima*-species group. Comparison of growth on potato dextrose agar showed no significant differences between the morphological species groups but a significant correlation of color and species group where most bright samples belong to the *A. infectoria*-species group. The examination of mycotoxin profiles resulted in two clusters one containing 92% of the strains of the *A. infectoria*-species groups with a low mycotoxin production and the other cluster containing 77% of the strains of the *A. alternata*-, *A. arborescens*- and *A. tenuissima*-species group with a high mycotoxin production. The molecular analysis via TEF-1α gene also resulted in two clusters one containing 79% of the strains belonging to the *A. alternata*-, *A. arborescens*- and *A. tenuissima*-species group and the other cluster containing 96% of the strains of the *A. infectoria*-species group. Furthermore no regional differences were found between the different strains, neither between growth, color or mycotoxin production nor in the TEF-1α gene sequence. The findings of the present approach suggest that the *A. infectoria*-species group isolated from cereal grains may be differentiated from the *A. alternata*-, *A. arborescens*- and *A. tenuissima*- by a very bright color, low production of mycotoxins and genetically by the TEF-1α gene.

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The ABC transporter *AbreAtr1* affects phytotoxin secretion and the pathogenicity of *Alternaria brassicae* isolates

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Alternaria brassicae is a necrotrophic pathogen causing black spot disease in cruciferous plants. *A. brassicae* is known to produce four cyclic depsipeptide phytotoxins belonging to destruxins. The role of destruxins in infection of the pathogen was investigated, but very little knowledge is currently available about molecular factors involved in pathogenicity in this fungus. In this study, the function of non-ribosomal peptide synthetase gene (named *AbrePsy1*) and ATP-binding cassette transporter gene (named *AbreAtr1*) examined by Real time PCR and the role these genes, during the synthesis and secretion of destruxins was considered. We also purified destruxin B from *A. brassicae* and the phytotoxicity effects of this toxin on detached leaves of Canola plants was confirmed. The toxicity and pathogenicity tests of culture filtrates (CFs) and six isolates of *A. brassicae* were also performed to investigate the effect of phytotoxins on infection behavior of different isolates of *A. brassicae*. Comparison of the results from transcription pattern of the *AbrePsy1* and *AbreAtr1* genes with phytotoxicity and pathogenicity tests on canola plants revealed the positive correlation between the pathogenicity, phytotoxicity and the *AbreAtr1* genes transcription. From these results, we can conclude that in pathogenicity mechanism of *A. brassicae*, the role of all two *AbrePsy1* and *AbreAtr1* genes is critical, but we also considered that ABC transporter *AbreAtr1* gene is a limiting factor in phytotoxicity pathway, since toxin secretion is indirectly influenced by the action of the ABC transporter.

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Fusarium Head Blight of spring wheat: mycotoxins, causal agents and their quantitative analysis with Real Time PCR

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Experiment conducted in 2011–2013 was located in Osiny near Puławy (south-eastern Poland). The objects were four cultivars of spring wheat grown in organic, integrated and conventional cropping systems. The highest average symptom intensity of *Fusarium* head blight (percent of infested ears) was observed in integrated system (13.6%) followed by conventional (11.7%) and organic one (8.3%). Average percent of ears with disease symptoms was as follows: cv. Trappe – 8.1%, cv. Brawura – 8.2%, cv. Katoda – 12.1%, cv. Hewilla – 12.3%. Mycological analyses revealed that highest percent of kernel colonization by *Fusarium* spp was observed in integrated system (30.7%) followed by conventional (26.0%) and organic (20.9%). Kernel infestation varied in assessed cultivars: Katoda – 38.3%, Hewilla – 31.3%, Trappe – 24.8%, and Brawura – 19.8%. Most commonly isolated species were *Fusarium graminearum*, *Fusarium poae*, *Fusarium culmorum Fusarium sporotrichioides* and *Fusarium tricinctum*. There was also conducted molecular quantitative analysis of *F. poae* and *F. graminearum* with Real Time PCR using SYBR Green I. There were confirmed differences in DNA amount of these species in analysed samples. It was found high diversity of DON content in grain of cv. Katoda harvested from researched cropping systems. In 2012, DON concentration exceeded accepted limit in all systems: organic – 1888, conventional – 2690, integrated – 5438 mg · kg^{-1}.

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A survey of genetic diversity of *Rhizoctonia solani* anastomosis groups associated with potatoes in South Africa

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Potato diseases caused by *Rhizoctonia solani* are a serious threat to potato commercial production globally, causing yield depression and reduced tuber quality. The aim of this study was to identify isolates of *Rhizoctonia solani* associated with potato diseases in South Africa. A total of 134 isolates were recovered from diseased potato plants, characterized for genetic, phylogenetic and pathogenicity diversity. The identity of AGs was confirmed using nucleotide sequences of ITS-5.8S regions of rDNA. Based on the ITS sequence analysis, *Rhizoctonia solani* isolates belonged to AG 3, AG 2.2 111B, AG 4 HG 1, AG 4 HG111, AG 5 and binucleate Rhizoctonia AGA with a frequency of 79.8, 6, 2.2, 2.2 and 9% respectively. *Rhizoctonia* AG 3 was the predominant AG and occurred in all the provinces whereas the other isolates occur in distinct locations. Phylogenetic analysis showed a greater interspecific and intraspecific genetic variation within and among AG groups. Sequence analysis of the internal transcribed spacers of the ribosomal DNA separated AG 3 group into two distinct homogenous clades. The 5.8S rDNA gene sequence (155 bp) was highly conserved among all isolates but differences in length and nucleotide sequence of the ITS1 and ITS2 regions were observed between AGs. There was no association between the geographical origins of the strains or the variety from which they were isolated and their genetic diversity. This study demonstrates the wide genetic diversity of *Rhizoctonia solani* in South Africa and highlights new genetic groups, AG 2.2 111B and AG 4 HG 111 not previously found to be associated with potato diseases in South Africa. During the pathogenicity experiments differences in virulence level between *R. solani* and binucleate *Rhizoctonia* spp. isolates were observed. Isolates of *R. solani* AG 2.2111B and AG 4HG1 and HG111 were the most virulent on stem cankers while AG3 caused black scurf on progeny tubers and its effects on stem and stolon cankers were variable. Binucleate *Rhizoctonia* spp. isolates were non pathogenic. This report is the first comprehensive survey of *Rhizoctonia solani* on potato fields in South Africa using molecular based approach.

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Modification of vegetative compatibility test

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Study on vegetative compatibility groups in *Fusarium oxysporum* Schlecht. emend. Snyder et Hansen based on *nit* – mutants started in 1985 in work of Puhalla who placed different formae speciales in different VCG (vegetative ompatibility groups. In the following 25 years VCGs were characterized in dozens formae speciales. However no heterokaryons were discovered between different forms.

After about ten years of using this approach we elaborated a modification of vegetative compatibility analyse. We realised that a proper criterion is necessary to elaborate the VC tester. We used this modification and characterized 30 isolates of the group under study and next the testers of the distinct group coud be assumed.

First on 6% calcium chlorate 30–60 *nit* mutants were recovered and phenotyped. All the *nit* M mutants, usually 5–9 and 2–3 *nit* 1 mutants from each isolate were paired with themselves. As the result one or two pairs of mutants forming heterokaryons most frequently were selected. These were testers of isolates. Using these testers selected on the objective criterion, it was possible to form heterokaryons between different formae speciales of. *F. oxysporum*

Additionally the short way of elaborating testers is presented.The perspective of applying this modified procedure is discussed.

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Genetic diversity among *Rhizoctonia solani* AG-4 isolates recovered from different hosts based on ITS-rDNA markers in Isfahan, IRAN

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Ribosomal DNA sequences have been widely used to study the phylogenetic relationships in different fungi. Fungal nuclear rDNA genes are arranged as tandem repeats with several hundred copies per genome. These spacer regions are considerably more variable than the subunit sequences and have been widely used in studies on the relationships among species within a single genus or among intraspecific populations. To evaluate the polymorphism between 18S and 28S genes several isolates of *Rhizoctonia solani* anastomosis group4 isolated from different hosts in Isfahan province in IRAN were examined based on PCR analysis. Genomic DNA was extracted from the isolates and prepared for PCR reaction. The amplification was performed using internal transcribed spacer (ITS) ITS1 and ITS4 primers. A DNA fragment of 700 bp in size was detected in amplified DNA in all isolates tested. To assess existence of any further polymorphism in the ITS region, the PCR products were digested with restriction endonucleases. However there was restriction sites for *Xba*I, *Hae*III, *Bam*HI, *Hind*III, *Sac*I, *Pst*I,and Taq1, but there was no any restriction sites for *Nde*I, *Xho*I, *Hinc*II, *Hinf*I, *Eco*RI and *Xho*I endonucleases. The endonuclease *Hinc*II recognized a restriction site in PCR products and discriminate the isolates belonging to AG4-HGII form isolates of AG4-HGI. Based on the results it has been concluded that AG-4 isolates of *Rhizoctonia solani* are heterogenic.

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How precisely diagnose whether the *Trifolium ambiguum* is infested by *Sclerotinia trifoliorum* or *Sclerotinia sclerotiorum*?

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Differentiation of *Sclerotinia trifoliorum* Eriks. and *Sclerotinia sclerotiorum* (Lib.) de Bary, with molecular methods is problematic. It was shown when identifying the cause of serious damage of Kura clover (*Trifolium ambiguum* M. Bieb.) which is reported to be resistant to most pathogens affecting other clovers. Species-specific primers developed based on genes *Aspr* and *Cad* that enable the identification of *S. trifoliorum* and *S. sclerotiorum* originating in the USA, in the case of Polish isolates proved to be non-specific. Also analysis of the ITS regions that, according to the literature, allows to distinguish the two species on the basis of two SNPs and based on the presence or absence of introns proved to be completely ineffective tool.

The study found that the IGS region sequences can be a reliable basis for distinguishing isolates. These regions, as shown in the literature and was confirmed in the case of Polish isolates, posses transitions and transversions within the amplified fragment differing both species. Therefore we tried to identify these species using real-time PCR using molecular probes.

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Identification of fungi associated with apple fruit rot before harvest and during storage in integrated agricultural system

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Apple rot can be caused by several filamentous fungi and the spectrum of pathogens can differ in the field in comparison to storage. In addition, the incidence depends on cultivar and climate conditions during growing season. The most common storage rot is caused by *Neofabraea* spp. (Bull's eye rot), bitter rot by *Colletotrichum acutatum* or *C. gloeosporioides,* brown rot by *Monilinia fructigena*. Grey mould caused by *Botrytis cinerea*, *Fusarium* rot and blue mold decay caused by *Penicillium expansum* are found less often. The main objective of the study was to obtain baseline information about apple rot-causing fungi in the integrated apple orchards in Latvia during the 2013 vegetation season and the rot incidence in the following storage period. Fungal species were identified according to morphological characteristics as well as using molecular methods. For most often isolated species, the Koch's test was carried out and the resistance against several fungicides was assessed. In the autumn, before storage, the main apple rot causal agents in six monitored cultivars were *C. gloeosporioides, C. acutatum, Neofabraea alba, M. fructigena*, *Panicillium* spp., *Fusarium avenaceum* and other *Fusarium* species (*F. acuminatum*, *F. equiseti*, *F. lateritium*, *F. sporotrichioides*). During storage the total percentage of rotted apples in various cultivars was as follows: 'Ligol' 4%, 'Tellissaare' 5%, 'Antej' 6%, 'Sinap Orlovskij' 6–9%, 'Belorusskoje Malinovoje' 10–13%, 'Auksis' 13%, 'Lobo' 23% and 'Forele' 59%. From infected apples Bull's eye rot caused by *N. alba* and *Neofabraea malicorticis* ranged from 0.33 to 9.26%, *Fusarium* spp. – from 0 to 2.22%, *Penicillium* spp. (*P. expansum*, *P. commune* and *P. echinulatum*) – from 0.29 to 5.34%, *Colletotrichum* spp. – from 0.12 to 3.13%, *Botrytis* spp. – from 0 to 3.37%, *M. fructigena* – from 0 to 21.08%. In part of the storehouses apple rot caused by *Cadophora luteo-olivacea* was detected. *Alternaria* and *Cladosporium* spp. were isolated only from a few apples as secondary infection agents.

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High Resolution Melting Analysis for identification of *Colletotrichum* species causing pepper anthracnose

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Colletotrichum coccodes, *C. acutatum* and *C. gloeosporioides* have been found to cause anthracnose of pepper fruits in Bulgaria. Recently, a new unusual species belonging to *C. gloeosporioides* group has been reported, which provoked similar symptoms and shared many phenotypic characteristics with the other *Colletotrichum* species. Enlarged number of *Colletotrichum* species occurring on *Capsicum annuum* cultivars made the accurate identification at species level crucial for resolving the complex and determination of an appropriate disease management. The application of real-time polymerase chain reaction (PCR) technology represents the most recent advance in the diagnosis of fungal pathogens. High resolution melting analysis (HRMA) is a post-PCR analysis method, which is a relatively new technique to detect nucleotide sequence variations. HRMA was applied to the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) of representative *Colletotrichum* isolates. The HRMA allowed clear discrimination of the new *Colletotrichum* species from closely related ones based on the normalized and derivative melting curve shapes. The results were confirmed by direct DNA sequencing. The HRMA is a rapid, sensitive and low cost procedure that appeared to be highly effective in differentiation of *Colletotrichum* species.

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The main causal agents of winter wheat crown rot in Latvia

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Wheat crown rot is a potentially harmful disease that can be caused by different pathogens. The aim of the present investigation was to indentify the causal agents of this disease depending on soil tillage and crop rotation. The incidence of crown rot fluctuated around 30% in our investigations. Reduced soil tillage and continuous wheat sowings increased the development of this disease. The symptoms of the disease were unspecific, and isolation was necessary to identify the pathogens. More than 2500 isolates were obtained from symptomatic wheat straw in 2012 and 2013. Genera of the pathogens were determined by morphological features of pure cultures, but species were confirmed by molecular analyses. Fungi from the genera *Fusarium* and *Oculimacula* dominated as causal agents; all other pathogens (*Gaeumannomyces graminis*, *Rhizoctonia* spp., and *Cochliobolus sativus*) were determined only in certain cases. The spectrum of pathogens was not influenced by soil tillage method. *Fusarium* spp. dominated in continuous wheat sowings – 66% of isolated pathogens were identified as *Fusarium* spp. The investigation showed that *F. avenaceum* and *F. culmorum* were the most frequent causal agents of crown rot, and only a few isolates of *F. graminearum*, *F. acuminatum*, *F. oxysporum* and *Microdochium nivale* were obtained.

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Virulence variation in cucurbit powdery mildew populations in the Czech Republic

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Pathogenicity structure (pathotypes, races) of cucurbit powdery mildew (CPM) species, *Golovinomyces orontii s.l.* (*Go*) and *Podosphaera xanthii* (*Px*) was studied on a set of 115 CPM isolates (71 *Px*, 44 *Go*) originated from different host species of family Cucurbitaceae from various locations of the Czech Republic from the years 2010–2012. For the screening, there was used a set of 6 CPM pathotype differentials from family Cucurbitaceae and 21 race CPM differentials of a single species, *Cucumis melo* L. proposed by Lebeda et al. (2008). In total, 6 different pathotypes (25, 27, 31, 47, 59 and 63) among Czech CPM isolates were detected. Differences in frequency of individual pathotypes were noted between both CPM species and also among surveyed years. Altogether, 106 different races (40 *Go,* 66 *Px*) in Czech CPM populations were determined. Differences in response to differential genotypes *C. melo* were found within individual CPM species, between both CPM pathogens and as well as among the studied years. Isolates avirulent to *C. melo* (Iran H) were found in both CPM species. *C. melo* differentials MR-1 and PI 124111 that are close relative were distinctly different from each other in response to both pathogens. Only five *Px* and four *Go* races were detected repeatedly (2 ×) in screened Czech CPM population. During the three-years of study, highly virulent pathotypes and races of both pathogens prevailed. Our results also revealed our previous investigations that Czech CPM populations are very heterogenous on their pathogenicity and differ from other countries. This research was supported by the following grants: MSM 6198959215, QH 71229 and Internal Grant Agency (PrF_2013_003, IGA_Prf_2014001).

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Crown gall and the diversity and detection of its causal agent – screening of Polish stone fruits nurseries

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Crown gall caused by tumorigenic bacteria of *Agrobacterium*/*Rhizobium* complex is one of the most dangerous diseases for nursery production of stone fruits and many other plants. Seventy nine stone fruits nurseries located in different regions of Poland were examined for the presence of crown gall. In half of them, the disease was observed and galls were sampled for agrobacteria isolation. Out of over 1200 isolates, about 418 were pre-identified as *Agrobacterium* (*Rhizobium*) spp. and out of them, 309 were pathogenic in test on sunflowers. Generally positive results obtained in pathogenicity tests were in agreement with positive results obtained in PCR with primers complementary to *tms*2 gene except 9 strains which were positive in *tms*2 PCR but negative in test on sunflower and 2 strains pathogenic on sunflower but negative in *tms*2 PCR. These 2 strains belong to new, hitherto unrecognized taxon and possess new type of pTi plasmid, which cannot be detected with any described PCR-based system. Other pathogenic isolates possessed nopaline type Ti plasmid and based on RFLP analysis of T-DNA fragment they were divided into 12 groups. Based on biochemical tests and MLST, majority of isolated agrobacteria belonged to biovar 2, only 18 to biovar 1 and 5 to newly described species *Rhizobium skierniewicense* and *R. nepotum*. Bacteria causing tumors were found to be very heterogeneous group of strains. Differences in agrobacterial populations were observed not only among nurseries but even in the single tumor.

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Nectrotrophic behaviour of *Erwinia amylovora* in apple and tobacco leaf tissues

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Erwinia amylovora, the causal agent of fire blight, very devastating disease of apple, pear and many other rosaceous plant species, has been detected in almost all world fruit production regions. One of the most important concerns related to fire blight epidemiology is how the pathogen survives and disseminates in the environment. Its parasitic relationship with host plants is best recognized. It has been also proved that *E. amylovora* can survive as epiphyte and endophyte. However, there is almost no information on necrotrophic behaviour of this bacterium.

We have used a model system with apple and tobacco plants to monitor during 3 seasons the survival of *E. amylovora* in dead leaf tissues. The system is based on inoculation of tip of actively growing terminal shoots on apple trees and spot inoculations of attached apple and tobacco leaves. Within 4–5 weeks after apple shoot tip inoculation fire blight has spread to the entire shoot and in some cases even to the woody shoot. Samples of necrotized leaf tissue were collected 5, 6, 7 and 8 months after inoculation. By using conventional methods, living *E. amylovora* cells were detected in the leaf midrib at all collecting dates on more than 50% of the samples, while in the lateral veins only in about 10% of samples; living bacteria were never found in the parenchyma. However, the pathogen DNA was detected in more than 50% of the samples of these tissues by applying nested- or Bio-PCR methods, which indicates the presence of both dead and possibly living bacterial cells. Analysis of the life status of necrotized leaves using Evans blue staining and observations with light microscope, performed five months after inoculation, showed that some of the plant tissue cells were still alive. Nevertheless, a month later, no living leaf cells were observed. In the dead tissues, a significant amount of glucose, fructose and starch, but not sucrose, was detected. We also found that *E. amylovora* survives and multiplies in apple and tobacco necrotic lesions, comprising only dead cells, that developed after leaf spot inoculation with the bacterium.

Based on these data, we conclude that *E. amylovora* can survive as necrotroph or seminecrotroph in plant tissues.

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Detection, identification and monitoring of *Dickeya* spp. in potato, water and weed samples with molecular methods

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In the past, bacteria from *Pectobacterium atrospeticum* (Pba) genus were thought to be the main cause of soft rot and blackleg in potato. In the last decades, the emergence of a new species *Dickeya solani* (Dsol) was observed (Toth et al., 2011; Wolf et al., 2014). Simultaneously, the *Dickeya* spp. isolates were found in water samples in different European countries (Great Britain, Finland, Israel).

We monitored the presence of *Dickeya* spp. in potato seed plantations in the growing season of 2005, 2009, 2010, 2011, 2013; in water in 2010, 2011, 2012, 2013 and in weeds growing near potato plantations in 2013. Monitoring of the bacteria present on potato seed plantations in Poland indicated that the number of samples infected with Pba is systematically decreasing, while simultaneously the number of incidences caused by Dsol is growing. Respectively, in 2005 we detected Pba in 53% samples and Dsol in 0.7% samples but in 2011 Pba was found in 41% samples and Dsol in 15% potato samples. Molecular characteristics of the whole genomes (e.g. ERIC, BOX, REP, PFGE patterns) and analysis of *dnaX , gyrA, rpoS* gene sequences of the isolates indicated that strains share high similarity and can be assigned to *D. solani* (Wolf et al., 2014).

In 2010, we isolated for the first time *Dickeya* spp. strains from water samples in Poland. Unlike the strains isolated from potato, these isolates of *Dickeya* spp. show high level of diversity regarding REP PCR and PFGE profiles. The incidence of *Dickeya* spp. in water is dependent on weather conditions. Our recent findings suggest that *D. solani* strains isolated from potato plants and many among those found in water samples can effectively infect potato tubers in laboratory tests and may cause severe losses in potato tubers under temperate climate conditions.

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Viruses infecting horseradish (*Armoracia rusticana* P.Gaertn., B.Mey. et Scherb) plants in Poland

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Fifty horseradish plants collected in few locations in Poland were tested in 2013 by ELISA for the presence of the following viruses: *Turnip mosaic virus* (TuMV), *Arabis mosaic virus* (ArMV), *Cauliflower mosaic virus* (CaMV), *Tobacco ring spot virus* (TRSV), *Cucumber mosaic virus* (CMV), *Cherry leaf roll virus* (CLRV) and *Tomato black ring virus* (TBRV). No plant was found to be infected with TRSV, CMV, CLRV or CMV. Three symptomless plants tested positive in ELISA for ArMV. One plant showing dark green spots was infected with CaMV. As far as we know this is the first report on CaMV and ArMV infecting horseradish in Poland.

Several horseradish plants showing distinct symptoms of chlorotic diffused rings on the leaves proved to be co-infected with TBRV and TuMV. We observed, however, low reliability of TuMV detection by ELISA. This problem has been overcome by using silicacapture-RT-PCR (SC-RT-PCR) assay, which was very sensitive and reliable, although not as simple as ELISA. One symptomatic plant was analysed using NGS (next generation sequencing) of two libraries prepared from polyadenylated RNA and small/short RNA fractions of total RNA. The complete genome sequences of two viruses: TuMV and TBRV (RNA1, RNA2 and satRNA) were recovered in this analysis. The sequenced isolate of TuMV showed very high similarity to the isolate CAR51 reported from Poland several years ago.

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Monitoring and characterisation of *Pectobacterium wasabiae* isolated from potato fields in Poland

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Pectobacterium wasabiae (Pwa) is a pectinolytic bacterium, responsible for soft rot and blackleg disease on economically important plants, including potato (*Solanum tuberosum* L). Pwa strains used to be wrongly classified as *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), because of significant genetical and phenotypical similarities between these species (Nykyri et al., 2012; Waleron et al., 2013). One crucial difference is that Pwa is more virulent and invasive than Pcc (Slawiak et al., 2013). Taking into account the fact that economic losses in potato production due to bacterial and fungal diseases reach even 22% annually (Czajkowski et al, 2012) in addition to Poland resting among the top 10 producers of this crop worldwide (Fao, 2012) our research group monitors potato fields and surface waters looking for pectinolytic bacteria. In 2013, we examined 248 potato stems, tubers or weeds and 1866 water samples, obtained from the The State Plant Health and Seed Inspection Service. We identified *P. wasabiae* in 30 samples of plant material, but it was completely absent in the water. Our first goal was to assess the virulence level of Pwa isolates by performing the infection test on potato slices. Later on, we examined their phenotypical traits connected with pathogenicity such as pectinase, cellulase or protease activity, motility and siderophore production (Toth et al., 2003). Interestingly, Polish Pwa strains did not vary much in pectinase and cellulase activity. Meanwhile, we observed differences in protease activity, motility and virulence. Furthermore, the genomic profiles of collected isolates were determined on the basis of repetitive sequence genotyping – BOX, REP and ERIC. Performed analysis revealed the presence of strains with at least 3 different REP, 2 ERIC and 2 BOX profiles. The genomic characterisation enabled us to track the spread of Pwa and hence the future disease control of blackleg and soft rot in Poland.

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Potato virus Y – important pathogen of tobacco

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Potato virus Y (PVY) is a pathogen of crops from *Solanaceae* family such as potato, pepper and tobacco. In a recent survey of a journal Molecular Plant Pathology, this virus was nominated the fifth out of top 10 plant viruses in plant pathology based on its scientific and economic importance (Scholthof et al. 2011, Mol. Plant Pathol. 12: 938–954). In tobacco, PVY is causing leaf vein necrosis thereby leading to significant losses of the crop. Current systematics of PVY is based on a combination of observation of symptoms after inoculation, serological tests and virus genome sequencing. There are seven strains of PVY, of which five are relatively common and capable of infecting tobacco. None of the currently available sources of PVY resistance in tobacco provides a universal resistance against all strains of the virus. Literature data suggest that selective pressure created by growing resistant cultivars was responsible for this. We will summarize efforts of acquiring new resistant tobacco cultivars done in our Institute by transferring resistance from a wild species *Nicotiana africana,* genetic transformation and gene pyramiding.

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Special session

Special session in Wieliczka Salt Mine

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EFPP Conferences

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European Foundation for Plant Pathology was founded in Wageningen, The Netherlands, in 1990. Its conferences are organized every two years, except for the years of International Society for Plant Patholgy congresses.

1990 – 1st EFPP Conference 26.02–02.03.1990, Wageningen, The Netherlands

1991 – EFPP Workshop: New approaches in biological control of soil-borne diseases Copenhagen, Denmark 30.06–04.07.1991

1992 – 2nd EFPP Conference Strasburg, France Council of Europe, 24–27.08.1992 Mechanisms of Plant Defence Responses

1994 – 3rd EFPP Conference Poznań, Poland 05–09.09.1994 Environmental biotic factors in integrated plant disease control

1996 – 4th EFPP Symposium Bonn, Germany 09–12.09.1996 Diagnosis and Identification of Plant Pathogens

2000 – 5th Congress of the EFPP Taormina-Giardini Naxos, Italy 18–22.09.2000 Biodiversity in plant pathology

2002 – 6th Conference of the EFPP, Prague, Czech Republic 09–13.09.2002 Disease resistance in plant pathology

2004 – 7th EFPP Conference – Aberdeen, Scotland

2006 – 8th EFPP Conference Frederiksberg, Denmark 13–17.08.2006 Sustainable disease management: the European perspective

2010 – 9th EFPP Conference Evora, Portugal 15–18.11.2010 Integrated Plant Disease Management

2012 – 10th Wageningen, The Netherlands 01–05.10.2012 IPM2.0 Towards future-proof crop protection in Europe

2014 – 11th Cracow, Poland 08–13.09.2014 Healthy plants – healthy people

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Biological monitoring in the treatment salt chambers of the 'Wieliczka' Salt Mine Health Resort

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In the treatment salt chambers of the 'Wieliczka' Salt Mine Health Resort, the pulmonary and allergy-related diseases are treated, because of specific stable microclimatic conditions, like low temperature, high relative humidity, salt aerosol, and lack of allergens. This innovative treatment, named "subterraneotherapy" needs to be performed under the permanent microbiological control. For this reason, the occurrence of biological particles in the air of two treatment salt chambers has been measured quarterly since 2012. The volumetric and impact methods are used to measure the fungal spore and bacteria concentrations in the air samples. The results are given in FS/m^3 and fungal spores/m^3 (CFU/m^3) of air, respectively. In the air samples, only 10 genera of fungal spores were found, out of which four genera were indicated in both methods (*Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*). *Alternaria* spores prevailed in impact method, while *Cladosporium* spores constituted the majority of total spores content in volumetric method. The highest spore concentrations were obtained in summer. The mean daily spore concentrations up to 300 CFU/m^3 and 100 FS/m^3 were achieved. In the same period the daily spore concentrations outdoor achieved 7000–10 000 FS/m^3 (volumetric method only). Among 24 recognized bacteria taxa (genera, species), only three dominated in all samples: *Micrococcus*, *Bacillus* and *Coagulae negative Staphylococcus*. No significant differences were found between fungal spore and bacteria concentrations in different study sites (two different chambers), under different study conditions (before and during patients stay), while the clear differences between the particle concentrations in different seasons were observed. The qualitative content of microorganisms in the air of salt chambers seems to be related to the biological material carrying in by patients and staff. The low concentration of particles favours the treatment of patients with allergy diseases.

11th Conference of the European Foundation for Plant Pathology

Session 4

Genomics, proteomics and bioinformatics

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Role of different molecular mechanisms in resistance to synthetic fungicide substances

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The resistance of fungal pathogens to fungicides limits their effectiveness and usefulness in agricultural applications. Selection pressure resulting from regular, long-term fungicide application, leads to the emergence and spread of new fungal strains with increased resistance to new groups of compounds. At the moment there are several well-known molecular mechanisms directly reducing the efficacy of fungicides. Among the best known is the spread of mutations in the sequences encoding target proteins, overexpression of genes encoding the target proteins and the adaptation of transport proteins to increase efflux of substances with antifungal activity.

With the available information on the mechanisms of fungal resistance to fungicide substances, now we are able to design molecular diagnostics procedures allowing rapid analysis of multiple, environmental samples. As part of the batch in vitro testing, we conducted bioassays, PCR and gene expression experiments on fungicide sensitive and resistant fungal strains. Our experiments aim to correlate morphological changes in mycelia with polymorphism of candidate resistance genes and their expression in stress conditions (fungicide treatment). We demonstrate polymorphisms in sterol demethylase, β-tubulin and in two MFS transporter genes from *Fusarium graminearum* which appear to provide one of the best candidate genes for wide specificity MDR pumps capable of exporting fungicides. One of them (FGSG_02865) is closely related to established resistance factors (FLR1 – fluconasol resistance in *Saccharomyces cerevisiae*) and its orthologs are present in a number of cereal pathogens.

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Towards probing the toxigenic potential of fungal communities in environmental samples

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The advances in next-generation sequencing presently allow for efficient sampling of fungal taxa present in the environment, via high throughput sequencing of taxonomic marker sequences. However, the same cannot be said for directly sampling the core genes determining different fungal chemotypes. The diverse and ancient biosynthetic gene families are of both evolutionary (horizontal transfer, extensive duplications and losses) and economic (biosynthesis and accumulation of major mycotoxins, such as aflatoxin or zearalenone). At the same time, the increasing coverage of model genomes finally provides the necessary resolution to describe and analyse the diversity of extant genes in multiple, divergent fungal taxa.

On basis of our phylogenomic analysis of core secondary metabolism genes (including synteny and exon-intron structure) of over 150 model fungal genomes, we develop a novel sample enrichment approach allowing for direct assay of the diversity of fungal non-reducing polyketide synthases present in environmental and/or cultured samples. Our qualitative test is based on serial preamplification and analysis of the conserved ketoacyl synthase domain. Preliminary assays were conducted on samples directly obtained from the environment, as well as on the pooled samples of cultured fungi. We discuss the phylogenetic background of chemotype diversity and its influence on the sensitivity and specificity of our protocol in regards to detection and characterisation of chemotypes, as well as possibilities of future extensions towards different gene families of interest.

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Cell wall-degrading effectors are crucial for the fungal plant pathogen *V. longisporum* to infect *Arabidopsis*

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The ascomycete *Verticillium longisporum* is a soil-borne fungal pathogen causing disease on a number of plant species worldwide. We have studied defense responses to *V. longisporum* in the model plant Arabidopsis and sequenced the *V. longisporum* genome to enrich the understanding of this particular plant-pathogen interaction. Upon fungal inoculation, plants mutated in the nitrate/peptide transporter gene *NPF5.12* display a wild-type phenotype, and a significantly increased fungal colonization. Pull-down experiments revealed interaction between NPF5.12 and a major latex protein family member, MLPL6. Fluorescent imaging demonstrated interaction of the two proteins in the plasma membrane and in the endoplasmic reticulum. These experiments also revealed the *V. longisporum* β-glucosidase VlBGL1 to be an interactor of NPF5.12. β-glucosidases are capable of degrading plant and fungal cell walls, and the VlBGL1 effector may act to assist *V. longisporum* colonization in the plant. Illumina sequencing with a base-coverage of 64x showed the *V. longisporum* genome to be ~70 Mbp in size, approximately twice the size of *V. dahliae* and *V. albo-atrum*, and comprising 21,000 gene models. An estimated 86% of the genome is shared with *V. dahliae* and *V. albo-atrum* genomes, with a high extent of gene duplication. 122 *V. longisporum*-specific secreted effectors were identified, including cellulose-binding proteins, pectate lyases and glycosyl hydrolases.

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Role of a pleiotropic drug transporter protein abcG5 in xenobiotic tolerance and antagonism in fungal biocontrol agent *Clonostachys rosea*

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ATP-binding cassette (ABC) transporters mediate active efflux of natural and synthetic toxicants, and are considered to be important for drug tolerance in microorganisms. In biological control agents (BCAs), ABC transporters can play important roles in antagonism by providing protection against toxins derived from the fungal prey, and by mediating the secretion of endogenous toxins. The fungus *Clonostachys rosea* is a ubiquitous soil borne ascomycete known for its antagonistic abilities against a wide range of plant pathogens, and also has entomopathogenic and nematophagous behaviour. In addition, *C. rosea* can tolerate diverse groups of fungicides when exposed to doses similar to those recommended for controlling plant pathogenic fungi. Recently, we identified a putative pleiotropic drug resistance (PDR)-type ABC transporter gene *abcG5* in the fungal BCA *C. rosea*, that was induced by the *Fusarium* spp. mycotoxin zearalenone (ZEA), through a suppression subtractive hybridization based transcriptome approach. In the present study, by generating *abcG5* deletion and complementation strains, we characterize the function of abcG5 in *C. rosea* aiming to understand its role in xenobiotic tolerance and antagonism. Gene expression analysis shows induced expression of *abcG5* in presence of ZEA, secreted metabolites of *F. graminearum* and different classes of fungicides like Amistar (active ingredient azoxystrobin), Apron (active ingredient mefenoxam), Cantus (active ingredient boscalid), and Chipco Green (active ingredient iprodione). Phenotypic analysis of the *abcG5* deletion and complementation strains showed that the deletion strains were more sensitive towards *F. graminearum* culture filtrates, ZEA, and Apron and Chipco Green, thus suggesting the involvement of abcG5 in cell protection. The Δ*abcG5* strains displayed reduced antagonism towards *F. graminearum* in a plate confrontation assay. Furthermore, the Δ*abcG5* strains failed to protect barley seedlings from *F. graminearium* foot rot disease. These data shows that abcG5 is a PDR transporter and important for xenobiotic tolerance and biocontrol traits in *C. rosea*.

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The effect of reversible point mutations in the *Pepino mosaic virus* coat protein gene on viral aggressiveness

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Pepino mosaic virus (PepMV) is one of the most widespread viruses infecting tomatoes. The virus population is highly diverse and four different genotypes have been distinguished so far: European (EU), Chilean 2 (Ch2), Chilean 1 (Ch1) and Peruvian (LP). Recently, new virus isolates representing the CH2 genotype, which is currently dominant, have appeared in greenhouses in Poland, France, The Netherlands and Belgium. Newly discovered variants display severe yellowing symptoms on the leaves of tomato plants (interveinal yellowing). The full length genome sequences of these isolates were determined and compared with other PepMV sequences deposited in the GenBank database. Two separate nucleotide substitutions in the coat protein (CP) gene (E155K and D166G in the protein) were identified which differentiated yellowing isolates from others described to date. A collection of viral mutants were created and biologically active RNA transcripts were produced to infect tomato plants. The analysis revealed that both mutations individually cause the development of yellowing symptoms. Analysis of tertiary models of the CP revealed that the amino acids in positions 155 and 166 of the CP are located on the surface of the protein. After one month the yellowing symptoms started to disappear gradually and later on the head of the plants became green again. Analysis of CP sequences derived from apical plant parts revealed that the yellowing mutations are unstable and tend to be back-mutated. Accumulation studies using different PepMV variants with and without the identified mutations clearly showed that reverse mutation towards the wild-type sequence, rather than a difference in accumulation speed or efficiency, is responsible for the disappearance of the yellowing symptoms.

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Fungal cellulases on both sides of the fence: cellulolytic enzymes as an infectious agent of *Fusarium* pathogens and as an inducer of plant resistance by the *Trichoderma* fungi

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Complex of cellulolytic enzymes is present both in the fungi of the *Fusarium* genus, which are frequent pathogens of many plants, including important crop species, as well as in the fungi of the *Trichoderma* genus which, in turn, show a potential as Biological Control Agents in biological plant protection. The activity of the cellulases from *Fusarium* fungi slightly etch plant tissues, thereby allowing the pathogen to penetrate the interior of the plant and promoting the infection process. The role of cellulose action is different in fungi of the *Trichoderma* genus, in which the cellulolytic enzymes catalyze the hydrolysis of cellulose e.g. in the root zone of plants, allowing the fungi to penetrate the plant tissue. *Trichoderma* presence increases the systemic immune response of the plant. Activities of cellulolytic enzyme complexes from fungal isolates belonging both to *Fusarium* and *Trichoderma* genera were measured in a standard laboratory test. It was spectrophotometric method – Filter Paper Assay (FPA) with used 3,5-dinitrosalicylic acid (DNS). The absorbance was measured at wavelength $\lambda = 530$ nm. Dedicated primer sets were designed to amplify partial sequences of cellulase-coding genes from strains studied. PCR-amplified marker fragments were sequenced. A phylogenetic tree was calculated based on the sequence of the gene fragment encoding the endoglucanase V (*eg5*) from *T. reesei* and with the use of the sequences its homologue obtained for 14 strains of *Fusarium* and 15 *Trichoderma* genera. An evolutionary comparison has been made between the fungi from genus *Fusarium* and *Trichoderma*. Considerable level of DNA sequence divergence was found between both genera. Moreover, numerous inter-specitic polymorphisms were detected allowing to distinguish individual species, which makes the studied fragment another good candidate for phylogenetic marker to be used in the evolutionary studies of diverse *Hypocreales* fungi.

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Molecular detection and identification of begomoviruses and its associated satellite molecules affecting some important plants in India

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India is rich in plant diversity and has good climate conditions for agricultural and other economically important plant species which makes the ideal conditions for insect vectors and begomoviruses to perpetuate. Begomoviruses have bipartite (DNA-A and DNA-B genome) or monopartite having only DNA-A genome. The satellite molecules referred to as DNA-β and DNA-α have also been found to be associated with begomoviruses. The existence of begomovirus and the associated satellite on various plant species grown in India are known to cause serious economically losses, therefore, study on detection and identification of begomoviruses and its associated satellite has been carried out. During the survey in 2008 to 2013 some important plants species viz. *Capsicum annum, Gossypium hirsutum, Hibiscuss rosa-sinensis,*, *Alcea rosea*, *Aster alpinus*, *Nicotina tabaccum*, *Parthenium hysterophorus* and Ageratum species growing at various locations in Lucknow, India were found to be exhibiting begomovirus like symptoms. To identify the begomovirus the complete DNA-A genome and their satellite molecules was amplified, sequenced and analyzed. The sequence analyses suggested occurrence of diverse begomovirus species and its satellite molecules on these plant species viz. *Tomato leaf curl New Delhi virus* (EU309045) & Chilli leaf curl betasatellite (DQ343289) on *C. annum; Cotton leaf curl Burewala virusand* (HM461866), Cotton leaf curlMultan betasatellite (HM140826) & Cotton leaf curl Shahdadpur alphasatellite (HQ343234) on *G. hirsutum*, *Cotton leaf curl Multan virus* (JN807763) & Ludwigia leaf curl distortion betasatellite (JQ408216) on *H. rosa-sinensis; Papaya leaf curl virus* (JQ954859) & Ageratum leaf curl virus betasatellite (JQ408217) on *A. alpinus*; *Tomato leaf curl Patna virus* (GU253915) on *N. tabacum; Tomato leaf curl Karnataka virus* (JX524172), & Nanovirus (JX570736) on *P. hysterophorus;* and *Ageratum enation virus* (JQ911767) & Ageratum yellow leaf curl betasatellite (JQ408218) on Ornamental Ageratum species. The results obtained during the study of these begomoviruses will be presented in conference.

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Leaf symptoms image analysis by using biology inspired algorithms

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Most of the pathogens colonizing plant tissues induce hypersensitive reaction accompanied by tissue necrosis. The hypersensitivity reaction is triggered by plant-pathogen interaction and in cases of necrotrophic (*Botrytis cinerea*) and hemibiotrophic parasites (*Phytophthora parasitica*) development of disease symptoms directly correlates with the development of the pathogen. In these cases proliferation of the disease symptoms could be directly correlated with susceptibility and resistance of the host plant.

In order to standardize and speed-up the analysis of visible symptoms, usually exhibiting as a necrosis of tissue, we designed and implemented standalone software solution based on combination of certain types of artificial neural networks and common image processing algorithms. We tested our tool on leaves infected with different common studied plant pathogens and compared determined results with other analysis methods. Presented software proved much higher reproducibility with significant speed up in data processing compared to results of manual evaluation. After successful training on the trial datasets, classification accuracy was usually up to 0.01 false positive rate.

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Quantifying the resistance risks associated with systemic seed treatments: a modelling analysis

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Systemic seed treatments are being developed for use on cereals for targeting foliar and seed-borne diseases. These treatments have the potential to improve disease control and raise yields. However, fungicidal application causes selection for fungicide resistance, and the strength of selection determines how rapidly resistant strains dominate pathogen populations and erode control. There are therefore potential risks associated with the same mode of action being used for both seed and foliar treatments, yet there has been little research to assess these risks. The FRAG-UK statement on SDHI fungicides and resistance risk in cereals, May 2013, states that SDHI seed treatments with foliar pathogen efficacy should count as one of two maximum SDHI applications, unless risk mitigation measures are in place. However, without empirical evidence it is unknown whether this measure is justified or is too restrictive. We have therefore developed a mathematical model, parameterised with field data, to simulate an epidemic outbreak and resistance evolution to test different application strategies. The model is similar to a SIR system, contains 11 explicit simulated leaves that emerge throughout each season, and models eradicant and protectant fungicide activity through changing parameters as a function of dose. We have generated disease severity profiles at different seed treatment doses, with and without foliar sprays, and have inferred the resulting selection coefficients. The model will be used to (i) explore the resistance effects of a wider range of seed treatment foliar fungicide combinations than can be tested experimentally, (ii) quantify the rate of selection for fungicide insensitivity and the resulting effect on effective lives for modes of action, and (iii) interpret the findings for a range of pathogens.

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Session 5

Diseases of trees in forests and recreation sites

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Healthy forest – healthy society

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A concept of recreational bioclimate and factors that positively influence the health of people resting in forests is presented. Different features of forest ecosystems affect the filtration, detoxification, biotherapeutic, psychoregulatory and aesthetic characteristics of recreation sites.

The focus is also on the biotic, abiotic and anthropogenic factors, reducing the healing abilities of forests. The results of forest health condition monitoring in Poland are discussed. In Poland 9,3 mln ha of forest and forest grounds (with 9,1 mln ha stands) contribute to 29,1% afforestation of the country, with 0,24 ha of forest per inhabitant. However, the forest area not available to the public (forest plantations below 4 m, experimental forests, seed stands, animal reserves, strictly protected areas, areas damaged by pests or diseases, etc.) makes 1,5–2,5 mln ha of forest permanently not available for recreation. This results in 25% reduction of average area of forest per capita accessible for people, so that only 0.18 hectare forest per inhabitant serves the tourism, recreation and health-improving functions, with a considerable share of stands suffering from tree diseases. This approach to forest value and function makes the job of forest pathologists particularly important from the point of view of social health and well-being. That requires, last but not least, proper promotion of forest pathology role and value for the society.

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Alien invasive threats to UK forests: a reassessment in the wake of ash dieback

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UK forests and woodlands, and trees in other situations are facing unprecedented challenges from the influx of alien invasive pests and pathogens resulting from increased global trade. Confirmation of the presence of the ash dieback, caused by *Hymenoscyphus fraxineus*, in UK woodlands in late 2012 provided a wake-up call to the authorities, leading to a flurry of activity from the government, sometimes prompted by the noisy clamour raised by the media on the subject, aimed initially at containing the problem, but rapidly evolving into planning for a future without substantial numbers of *Fraxinus excelsior* in the environment. The arrival of this 'new' disease, however, should not have been a surprise. Ash dieback was well-known from a steady advance across Europe.

Ash dieback, however, is only one of many known pests and pathogens threatening trees in the UK. Add the potential numbers of unknown pathogens, and the number of threats could become very large indeed. Current threats, including the panoply of *Phytophthora* species already present in Europe, along with pathogens such as *Ceratocystis platani* and *Fusarium circinatum*, will be put in perspective against the potential hosts present and grown widely in the UK. In addition, the recommendations of the UK Government's Tree Health Task Force will be presented for discussion.

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An integrated approach to monitor and control the invasive fungal pathogen *Heterobasidion irregulare* in European forest stands

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The North American fungal pathogen *Heterobasidion irregulare* is currently distributed in pine and oak stands along 103 km of coastline of central Italy. This paper reviews the pathways of introduction and invasion, the factors driving the invasion, and the dispersal abilities of this pathogen in Italy. Furthermore, an integrated disease management program to minimize the risk of spread of the fungus in Europe is suggested, based both on published literature and on new unpublished data. Observational and genetic evidence support a single introduction through infected wood during WWII, and a subsequent invasion through spore dispersal. Experimental evidence suggests transmission potential of the pathogen rather than hyper-susceptibility of native hosts is the major determinant of invasion. The current range of *H. irregulare* is too vast to suggest eradication, however we recommend minimizing the risk of spread of *H. irregulare* outside the zone of infestation while reducing the magnitude of infestations within its current range. We provide evidence suggesting the most cost-effective management approach hinges on preventing the saprobic establishment of the fungus in stumps in a "buffer" area surrounding the current zone of infestation.

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Role of *Armillaria* species in tree dying in Turkey oak (*Quercus cerris* L.) and Hungarian oak (*Quercus frainetto* Ten.) forest

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Armillaria species are designated as one of the factors responsible for oak forest dying both in Europe and in North America (Oszako and Delatour, 2000). Previous research shows that five species in the genus *Armillaria* are present in Serbia (Keča et al., 2006). Decline of Turkey and Hungarian oak forests is, in recent years, present throughout the Serbia and different abiotic (desiccation, drought, low temperatures, etc.) and biotic (defoliators, *Phytophthora* spp., etc.) factors are included in the process. Of the mentioned species, *A. gallica* Marx. & Romagn. is often reported in references as a weak pathogen on broadleaved trees and oaks (Guillaumin et al., 2005). The study area covers 30 areas, with selected symptomatic, asymptomatic trees and declining trees. Presence of rhizomorphs, decay and mycelial mats of *Armillaria* were noticed on all studied areas. The species were identified by PCR-RFLP method (Keča et al., 2006). The clones were identified based on SIGs, by pairing two neighboring diploid isolates (diploid/diploid). The pairing was evaluated based on the following criteria: 1) there is no delineation and no differences in the isolate morphology (reaction sign +); 2) there is no clear delineation, but the isolates differ morphologically (|); 3) clear delineation (|). *Armillaria* species were isolated from eight trees. Dominant species was *A. gallica*, while only couple of isolates were identified as *A. mellea*. Only *A. mellea* was isolated from a living tree, and all isolates of *A. gallica* were obtained from the declining or dead trees. SIGs test shows that the controlled isolates can be classified in groups and from 2 to 6 individuals (clones) are present in the controlled areas.

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Modelling the effects of climate on the incidence of the nut rot of chestnuts caused by *Gnomoniopsis castanea*

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Gnomoniopsis castanea is an emerging fungal pathogen causing nut rot on chestnut trees. In order to model the incidence of *G. castanea* as a function of climate a Partial Least Squares Regression (PLSR) analysis was performed in four steps: I) assessment of the pathogen incidence, II) pre-selection of predictors, III) models fitting, IV) external validation.

I) 40 to 120 ripe nuts were sampled in each of 12 sites located in the north-west of Italy in 2011. The incidence of *G. castanea* in each site was assessed by determining the amount (in %) of infected nuts. The diagnosis of the pathogen was performed with both isolation trials and molecular analyses. The incidence ranged from 20% to 93% depending on site.

II) Geostatistical analyses involving the Ripley's function, the Nearest Neighbor Hierarchical Clustering (NNHC) and the spatial autocorrelation index of Moran revealed that, despite the geographical clustering of sites ($P < 0.05$), the incidence of *G. castanea* was not spatially autocorrelated ($P > 0.05$). This finding suggests an influence of site-dependent factors on the disease. A Principal Coordinates Analysis (PCoA) followed by a Hierarchical Cluster Analysis (HCA) on maximum, mean and minimum temperatures and on rainfalls showed that warmer temperatures were associated to a significant increase of the incidence (+10.4%; $P < 0.05$).

III) The temperatures of the months before nut harvesting were selected as predictors for the fit of PLSR models on the logit transformed values of *G. castanea* incidence. Cross-validation and bootstrap analyses were carried out to perform models selection.

IV) External validation performed on data collected from sites not used for models fitting showed the good predictive abilities of the models ($\rho > 0.70$; $P < 0.05$).

All the above findings demonstrate that there is a relation between the climate and the incidence of *G. castanea*, providing statistical tools to forecast the incidence of the disease at site level.

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Genetic differentiation within and between Swiss and Lithuanian populations of *Hymenoscyphus pseudoalbidus* using microsatellite analysis

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The ascomycetous fungus *Hymenoscyphus pseudoalbidus* (anamorph: *Chalara fraxinea*) is an invasive pathogen which causes a severe dieback of ash trees (*Fraxinus* spp.) in Europe. The knowledge on genetic structure and information on changes in the ash dieback pathogen populations over time has important practical relevance for the sustainable management of forest disease as high ecological plasticity of *H. pseudoalbidus* is observed. Thus, the main aim of this study was to characterize epidemic (Swiss) and post-epidemic (Lithuanian) populations of *H. pseudoalbidus* using eleven polymorphic microsatellite markers (SSRs).

In this study, DNA of 847 isolates originating from five Lithuanian and five Swiss subpopulations was successfully amplified using two newly developed multiplex PCR reactions. Genetic distance values (F_{ST}) between pairs of the subpopulations varied unequally – from moderate to very high genetic differentiation and showed very high genotypic diversity as 390 multilocus genotypes (MLGs) were detected. Whereas, genetic distance between isolates of *H. pseudoalbidus* originating from infected leaf petioles (saprophytic phase) and from necrotic lesions (pathogenic phase) within subpopulations varied from little to reasonably low genetic differences indicating that allelic composition was nearly the same in both sample categories. Allelic richness (A_R) and private allelic richness (A_{PR}) were insignificant both in epidemic and post-epidemic *H. pseudoalbidus* populations. However, STRUCTURE analysis confirmed the presence of a single genetic cluster (no clustering). The results of the present study indicated a founder effect in both investigated *H. pseudoalbidus* populations and absence of significant contemporary gene flow from the post-epidemic populations in Lithuania to the epidemic front in Switzerland. Moreover, this study provided new information on genetic population structure of *H. pseudoalbidus* at different geographical scales in Europe, and gave a better understanding of how genetic diversity may vary across *H. pseudoalbidus* populations and in different substrate types (lesions vs. petioles).

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Virulence of *Hymenoscyphus pseudoalbidus* isolates originating from Lithuanian (post-epidemic) and Swiss (epidemic) populations

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A severe dieback of common ash (*Fraxinus excelsior*) caused by the ascomycetous fungus *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*) is currently observed in most European countries, including Lithuania and Switzerland. The main aim of this study was to determine if there are significant differences in virulence among *H. pseudoalbidus* genotypes originating from two geographically distant populations: epidemic (Swiss; disease started in 2008) and post-epidemic (Lithuanian, 1996). Additionally, our interest was to check whether there are differences in virulence between pathogen genotypes isolated from necrotic lesions (pathogenic phase) and from fallen ash leaf petioles (saprophytic phase). In September 2013, a large-scale inoculation experiment was conducted on 3-year-old *F. excelsior* seedlings (N = 1,000) using 100 *H. pseudoalbidus* isolates from each population, of which 50% were "lesion" and 50% "petiole" isolates.

In May 2014, bark necroses were observed on 89.0% of the seedlings, and were caused by 97.5% of the isolates. Mean overall lesion length was 8.64±0.21 cm (ranged between 0.0 and 37.0 cm). Lesions induced by Lithuanian isolates were significantly (at $p < 0.05$) larger than those induced by the Swiss ones (9.08±0.30 cm vs. 8.20±0.29 cm), although this was due to a higher proportion (12.5% vs. 9.5%) of seedlings inoculated with the Swiss isolates that remained asymptomatic. Significant differences (at $p < 0.001$) were observed as lesions induced by "lesion" isolates from both countries were compared with the "petiole" isolates (7.68±0.29 cm vs. 9.59±0.30 cm). High variability in virulence was detected among different isolates; significant differences were also found among some of the investigated subpopulations of the fungus. In conclusion, isolates of *H. pseudoalbidus* in the post-epidemic population were in general more virulent than at the epidemic front suggesting the occurrence of trade-offs between pathogen's fitness components such as adaptability to a new environment (at the epidemic front) and virulence in cost of the latter. "Petiole" isolates showed significantly higher virulence than "lesion" isolates which supports a hypothesis that saprophytic and pathogenic phases of pathogen life cycle may select for its different traits including virulence.

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Fungal diseases in forest nurseries in Poland

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Scots pine (*Pinus sylvestris*), pedunculate oak (*Quercus robur*) and common beech (*Fagus sylvatica*) are the major tree species in Polish forest nurseries, where 90% of the seedlings is grown in an open field. Modern nurseries must optimize culture conditions to maximize seedling production and minimize the risk of a disease outbreak. Furthermore, abiotic stress caused by environmental conditions, such as: frost, sudden rainfall, or injury, can also expose rapidly growing seedlings to fungal attack.

During the last years oak powdery mildew (*Erysiphe alphitoides*), pine needle cast (*Lophodermium seditiosum)*, rusts on leaves and needles (*Melampsoridium betulinum*, *Melampsoridium hiratsukanum*, *Melampsora larici-populina*, *Melampsora larici-tremulae*) and grey mould (*Botrytis cinerea*) have been the major problems in Polish forest nurseries. However, damping-off of deciduous and conifer seedlings, caused by *Fusarium* spp., *Rhizoctonia* spp., *Cylindrocarpon* spp., *Cylindrocladium* spp. and *Oomycetes*, is still the main factor causing losses in nursery production. Damping-off is a fungal disease of young seedlings which causes high mortality during the first few weeks after germination. Moreover the last year's weather conditions are particularly conducive to the fungal diseases development, especially damping-off of seedlings, grey mould and oak powdery mildew.

Currently fungal diseases are still a serious problem in maintaining continuity of production in forest nurseries, despite using cultivation practices and applying the principles of Integrated Pest Management (IPM, according to Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides).

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Rhizoctonia spp. in North Wielkopolska forest nurseries

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Coniferous species cover 69,9% of the total forest area in Poland, of which Scots pine is growing on 59,5% of the forest area of all forms of ownership (Report on the state of forests in Poland, 2012). A large percentage of pine woods in Poland requires constant renewal of pine stands and, therefore, continuous production of large quantities of high-quality planting stock for reforestation.

The aim of this study was to learn the diversity of *Rhizoctonia* spp., damping-off pathogens, occurring in selected nurseries in Regional Directorate of State Forests in Poznań and Regional Directorate of State Forests in Piła (central-west Poland). The diversity of pathogens found and further used in the study was tested with conventional and molecular methods. With the help of molecular techniques isolates were assigned to a specific anastomosis group.

Rhizoctonia isolates were obtained from soil samples using two kinds of trapping methods. The first method based on the use of pine seeds which were sown (25 seeds per pot) to a volume of 700 ml of unified soil. The second method used in the study was a modified method described by Paulitz and Schroeder (2005) where wooden toothpicks were used.

The number of cell nuclei per cell was determined with the method described by Bandoni (1979) and the growth rate of individual isolates was measured every 24 hours. Individual pathogenicity of isolates to Scots pine (*Pinus sylvestris*) seedlings was defined.

Eight different anastomosis groups were isolated. The nurseries differed in diversity of anastomosis groups.

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Success and challenges of crop protection in the past, presence and future

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Since 10,000 years ago agriculture started with the domestication of plants, they were selected for better growth and on yields and quality of consumable products. Therefore they became vulnerable for diseases as well, and a form of crop protection was needed and invented. It took till the end of the 19th century that science based crop protection emerged first with an-organic compounds but after the 2nd World War with primarily organic compounds. Modern crop protection was a great success and responsible for the enormous increase in crop production to feed the rapid increasing human population. In the 1960th the first concerns on the unwanted side effects of organic compounds led to the present focus on IPM. The challenge of today's research is provide tools for prevention of diseases before culture, to monitor precisely disease occurrence during culture, to provide crop protection products , and tools for precision application. However with all this research the concept has not changed: the cultured plant is still very sensitive for diseases. The great challenge of the 21st century is to keep crops with the highest food value, but giving them back traits for disease-insensitivity, that have been lost during the domestication started 10,000 years ago. Some new features of resistance tools and mechanisms, endophytes in the plant organs, the adjusted microbiome in the rhizosphere, and advanced cropping systems will be reviewed, which all lead to innate or inducible resistance, or less sensitivity to diseases of the single plant or the crops as a whole. These exciting new science will contribute to the development of new generations of resilient crops and cropping systems

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Coping with plant protection crises: a case study for managing fire blight outbreaks in Israel

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For preventing disease outbreaks and the resultant yield losses, growers implement diverse management practices. In most cases the efforts are effective and, in general, the impact of plant diseases on a national scale is confined. Occasionally, it is possible to foresee processes or circumstances that may potentially lead to creation of plant protection problems. For example, changes in regulations that would eventually phase out a widely used, highly effective, pesticide (such as methyl bromide), or the migration of a novel virulent isolate of a destructive pathogen (such as Ug99 of *Puccinia graminis* f. sp. *tritici*). In such cases, it is possible to take the necessary precautions to tackle the problem and prevent its occurrence. However, sometimes plant protection crises occur unexpectedly. In such cases prevention of a long-term impact of the outbreak on the relevant industry is dependent on a rapid response and on investing the necessary efforts to cope with the problem. An exceptionally severe fire blight epidemic developed in 2010 in pear orchards in Israel and there was a fear that the industry would be devastated. A national effort including all the bodies related to the pear industry was promptly initiated. A team of experts was assembled with the responsibility of identifying the events leading to the outbreak and formulating the actions to be taken to overcome the problem. The same team was nominated to lead the control efforts. The management actions were effective and in the coming year, 2011, disease levels on a national scale were negligible. Although the specific details and the procedures to be employed for managing plant protection crises vary from case to case (and from country to country), some generalities may be established. In this presentation, the experience gained in Israel while coping with the 2010 fire blight outbreak will be discussed.

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Management of postharvest diseases of apples

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Blue mould caused by *Penicillium expansum* and gray mould caused by *Botrytis cinerea* are the two important postharvest diseases of apples in Canada in long term storages. Resistance to thiabendazole fungicides has been reported in storages where thiophanate methyl was used as a postharvest control. Over the past decade we have investigated the efficacy of biocontrol, *Pseudomonas syringae*, and reduced-risk fungicides, fludioxonil, pyrimethanil, difenoconazole, and pyraclostrobin + boscalid against postharvest blue mold and gray mold on fruit of eight apple cultivars, Ambrosia, Empire, Fuji, Gala, Honey Crisp, Jonagold, Red Delicious and Silken apples in postharvest storages. Inoculum only and thiophante methyl were used as positive controls. The results showed that the biocontrol and fungicides were effective against postharvest *P. expansum* and *B. cinerea* on all apple cultivars for up to 6 months in air (4°C) or in controlled atmosphere (CA) apple storages. In recent years, 1-methylcyclopropene (1-MCP) has shown tremendous potential in maintaining fruit quality in apples during storage and so we have investigated the effect of 1-MCP on the control of postharvest diseases of apples with fungicides. The results indicate that 1-MCP has neither positive nor negative effect on the control of blue mold with postharvest fungicides, fludioxonil (300 μg a.i./ml) and pyrimethanil (500 μg a.i./ml) in air and CA storages for up to 6 months. Quality of apples may be maintained for up to 6 months by using postharvest control with reduced risk fungicides and/or biocontrol on 1-MCP treated apples in air (4°C) and CA storages.

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EU project BIOCOMES develops new biological control products for IPM in agriculture and forestry

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The objective of the EU project BIOCOMES (www.biocomes.eu) is to develop 11 new biological control agents (BCAs) and 2 production technologies for key markets in European agriculture and forestry. BCAs were identified through market analysis by six manufactures of biological control products. BCAs will primarily be for use in open field crops of vegetables (3), of which 2 are also for use in protected crops, arable crops (3), fruit crops (3), and three different types of forests (2). Primary targeted pests are: gypsy moth (*Lymantria dispar*), pine weevil (*Hylobius abietis*), tomato leaf miner (*Tuta absoluta*), white flies, aphids of fruit tree crops and *Mamestra brassicae*. Primary targeted pathogens are: damping-off diseases in forest nurseries, soilborne pathogens in oilseed rape, toxigenic *Fusarium* spp. in maize and wheat, brown rot (*Monilinia* spp.) in stone fruit, and powdery mildew in cereals (*Blumeria graminis*). The economic sustainability during the entire development process will be assessed by the responsible industrial partners. The environmental sustainability will be quantified for each BCA by means of the Sustainable Process Index method. The entire developmental process for each of the 11 BCA products is guided by a consultancy partner specialized and leading in (bio) pesticide registration including risk assessments for European (bio) pesticide industries.

IPM is an important approach to reduce dependency on pesticides use. Before pesticides are used, biological control measures, together with physical and other non-chemical methods, should have first preference (Directive 2009/128/EC). The new BCAs developed in BIOCOMES will provide new opportunities in IPM. An early testing of the products under development within other IPM projects is envisaged as soon as appropriate prototype formulations will be available.

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Approaches on *Trichoderma* strains usefull for protection of horticultural crops in Romania

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The presentation summarizes the activity performed in Romania during the last years for: (*i*) selection and characterization of several *Trichoderma* strains biologically active against several diseases of horticultural crops (as development on different culture media, physical conditions – pH of substrate, temperatures, carbon and nitrogen nutrients – electrophoretic and fatty acids analysis of their protein profile, volatile and non-volatile compounds produced by *Trichoderma* isolates) and (*ii*) development of bioproducts based on selected strains for soil, seed and foliar treatments.

The strains were screened for their antagonistic activity by using dual culture technique and then tested for their ability to control vegetables (tomato, cucumber) and grapevine diseases. *Trichoderma* strains Td_{49} and Td_{50} present specific characteristics as plant growth promotor, activating the tomato plant defense genes and stimulating the growth of cucumber seedlings cultivated on sterile growth substrate. Isolates Td_{49} and Td_{50} have proved a high efficacy in protecting tomatoes and cucumber against white rot (*Sclerotinia sclerotiorum*) and damping off (*Pythium*, *Rhizoctonia*, *Fusarium*). The isolate Td_{50} had, also, proved a high efficacy in protecting grey mould (*Botrytis cinerea*) in grapevine. These Romanian isolates are stored at the National Collection of Agricultural and Industrial Microorganisms, H-1118 Budapest, Somlói út 14–16, Hungary. *Trichoderma asperellum* T57 and T83 from ICECHIM Collection have been analyzed concerning their volatile and non-volatile metabolites production and tested and screened in controlling *Fusarium graminearum*, *Rhizoctonia solani* and *Pythium umtimum*. Both antagonist strains produced non-volatile metabolites that inhibit the mycelial growth of plant phytopathogen tested. The tests showed that *T. aspellerum* T83 was more active in controlling all pathogens tested, among them *R. solani* being the most sensitive to the antagonistic activity of both *Trichoderma asperellum* isolates, T57 and T83, from ICECHIM Collection.

The bioproducts based on Romanian *Trichoderma* strains were developed using fungal biomass, dried after inclusion into beds obtained by ionotropic gelation, and ingredients for two mains form of application: as seed treatment (registered in Romania under the commercial name Trichosemin 25 TS) and spray treatment, for foliar application and soil drenching (registered in Romania under the commercial name Trichopulvin 25 PU) (Patents OSIM 113103/1998, 126125 and 126363/2012). On the end of presentation there are discussed the perspectives of research in biological control of plant diseases in the present conditions under the EU regulations.

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Sclerotinia Rot in Brassicas – effective management without chemicals is finally possible

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In Australia, current management options against *Sclerotinia sclerotiorum*, the cause of Sclerotinia rot, mainly rely on cultural and chemical control measures that are often unreliable and can be cost prohibitive. Recently, however, high-level resistance against Sclerotinia has been identified. These include stem resistance in *Brassica napus* and *B. juncea* genotypes from Australia and China, in *B. napus*, and *B. juncea* introgression lines developed in India following hybridization of wild crucifers, and across diverse *Brassica*, *Raphanus* and *Sinapis* species. Effective seedling resistance to Sclerotinia has been located in genotypes of *B. oleracea*; as has leaf resistance in *B. rapa* and *R. sativus*. Separate genetic control for adult stem, adult leaf and seedling resistances has been demonstrated, which is crucial to developing resistant cultivars across different cruciferous oilseed, forage and vegetable crop types. New understanding on the functioning of host resistance mechanisms offers avenues for further improving disease control. Recent ability to characterize physiological specialization in pathogen populations has provided the first means to identify and monitor current and new pathotype distributions, identify resistances against predominant pathotypes, and combine resistances to different pathotypes within future cultivars. Further, specific genotypes that are more isolate-independent in their resistance expression have been identified as important resistance sources to target and exploit in developing new commercial cultivars with more effective resistance to Sclerotinia across multiple pathotypes. Finally, breeding populations with similar levels of resistance, but narrow variation in their resistance range, have been highlighted. These should provide breeders with advanced populations that not only display the level of resistance expected consistently, but do so while reflecting the genetic diversity of resistance sources needed to develop new more-resistant cultivars successfully. The progress achieved from identifying appropriate host resistances against the prevailing pathotypes ensures that successful management based on host resistance is now possible for Australia.

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Integrated pest management to control *Claviceps purpurea* in cereals

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Ergot, caused by the fungus *Claviceps purpurea*, is a severe disease in rye (*Secale cereale* L.) leading to purplish-black sclerotia in the ear that contain > 30 mycotoxins, ergot alkaloids. Because of their toxicity, EU threshold levels for ergot exist on the basis of percentage of sclerotia in the grain.

Instead of grain, a dark ergot (sclerotia) formed in single floret of the infected ears. It differs in shape and color from the healthy grain. Ergots remaining after the harvest on the field, can germinate in spring and ascospores can infect early flowering grasses and cereals. Two weeks after the primary infection honey dew drops will produce in the host for secondary spread. The formed conidia can be transferred to other flowers by insects or rain with the honeydew and re-infect.

Mainly susceptible crops are rye and triticale, whose flowers remain open. In cool and wet springs wheat and barley can be infected. The infection causes contamination with ergot and an exposure with toxic ergot alkaloids. A control is currently possible through plant construction and the cultivation of resistant varieties. Approved chemical fungicides are not available, although effective active substances such as prothioconazole, tebuconazole and pyraclostrobin exist.

In the framework of integrated pest management strategies, the variety resistance represents an essential part to minimize the uptake of toxic ergot alkaloids by the consumer. In particular the decomposition of sclerotia in the soil can be promoted by crop rotation and the use of the applicable tillage. A homogeneous flowering of all tillers can be achieved by the seed density, nitrogen fertilization and the use of growth regulator. With reinforced field hygiene such as the elimination of other host plants, inoculum will be minimized. Infested arable land has to be separately harvested and bring it to another use.

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Strategies in using fungicides to successfully control *Cercospora Beticola* in sugar beet in the USA

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Cercospora leaf spot, caused by the fungus *Cercospora beticola* Sacc., is the most important foliar disease of sugar beet in Minnesota and North Dakota, USA. The pathogen destroys the leaves which results in significantly lower root yield, sucrose concentration and extractable sucrose. Cercospora leaf spot is managed by the integrated use of tolerant varieties, crop rotation and tillage, and fungicides. Inoculated field trials were conducted at Foxhome, MN in 2011 to evaluate the efficacy of individual fungicides, mixtures, and a fungicide rotation program at controlling the pathogen and managing fungicide resistance. Single site and broad spectrum fungicides were evaluated. Fungicides provided significantly greater disease control and recoverable sucrose compared to the inoculated check. Fungicide mixtures typically provided similar or greater disease control and recoverable sucrose compared to using individual fungicides. The fungicide rotation program resulted in effective control of *C. beticola* when using individual products and/or mixtures. Results demonstrated that rotation of fungicide chemistries effectively controlled Cercospora leaf spot and may be used to manage fungicide resistance.

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Effectiveness of actinomycetes as biocontrol agents against root diseases is enhanced by their ability to produce 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase

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Fungal pathogens not only inhibit plant growth directly, but also induce the plant to synthesize the stress hormone ethylene. Much of the damage sustained by plants infected with fungal phytopathogens occurs as a result of the response of the plant to the increased *in planta* levels of ethylene. To compare the effectiveness of antagonistic actinomycetes on the suppression and severity of three soil-borne diseases; tomato wilt (*Fusarium oxysporum f. sp. lycopersici*), cucumber damping-off (*Pythium aphanidermatum*) and lettuce basal drop (*Sclerotinia minor*), actinomycetes were isolated from the rhizosphere of tomato, cucumber and lettuce in the United Arab Emirates (UAE). They were evaluated for their ability to produce the immediate precursor of ethylene (1-aminocyclopropane-1-carboxylic acid (ACC) deaminase) in the presence of the pathogens, as well as their ability to produce antifungal metabolites and cell-wall degrading enzymes that can inhibit the pathogens growth *in vitro*. The most inhibitory isolates produced chitinase, β-1,3, β-1,4, β-1,6-glucanases, diffusible antifungal metabolites, volatile inhibitors, siderophores, and were able to lyse the hyphae of the three pathogens *in vitro*. Under greenhouse conditions, the ACC deaminase-producing isolates (ACC^+) were significantly more effective in reducing the incidence and severity of the three diseases compared to ACC deaminase-non-producing (ACC^-) isolates. The application of ACC^+ isolates also resulted in the reduction of the endogenous levels of ACC in both roots and shoots and increased plant growth compared to ACC^- isolates in the presence of the pathogens. This is the first study to demonstrate the superiority of antagonistic rhizosphere actinomycetes to enhance their effectiveness as biocontrol agents by their ability to produce ACC deaminase, antifungal metabolites and cell-wall degrading enzymes. The results clearly showed the potential to enhance most, if not all, of the biocontrol agent's performance by including the ACC deaminase ability into the biocontrol strains.

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Suppression of Alternaria black spot disease of persimmon by application of plant growth regulators in the orchard

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Black spot caused by *Alternaria alternata* is one of the most damaging diseases of persimmon (*Diospyros kaki*) fruits in Israel. The pathogen penetrates and infects the fruit through wounds and microscopic cracks created on the fruits peel. Infection usually results in the development of black rot of the flash that disqualifies the fruit for marketing. Crack development in fruit peel is thought to be correlated to rain occurrence during the last stages of ripening. There are currently no effective means to cope with the disease in the orchard. Persimmon fruit development consists of two major stages. In the first, the cells divide and differentiate; in the second, the cells expand and mature. Based on reports from other fruit crops (*i.e.* apples) we hypothesized that application of growth regulators during specific timing of fruit development would increase fruit peel flexibility, thus, reducing cracking of the peel. The outcome would be a reduction in fungal infection and black spot intensity in the orchard. Superlon (Fine Agrochemicals, UK), a mixture of gibberellin and benzyl adenine (GA4+7 and BA), was applied at different times during fruit development in 4 orchard experiments and disease incidence was evaluated at harvest. It was found that application of Superlon at 70 or 100 days post budding (the first and second stages of fruit development, respectively) reduced black spot incidence in the orchard by 18% and 30% as compared to the untreated control. Application of Superlon at both times had an additive effect resulting in a reduction of 42% in disease incidence compared with the untreated control.

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Prediction of *Zymoseptoria tritici* based historical weather data

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Risk models based on climatic data as well as disease monitoring and control thresholds for decision on fungicide use are traditional used as important IPM elements in a sustainable cropping situation. The need for control of *Zymoseptoria tritici* vary significantly between years and the severity of septoria leaf blotch is well known to be very driven by humidity events, typically days with precipitation, during the growing season. The aim of a new project has been to improve the prediction of the current risk models for septoria leaf blotch. It has particularly been a wish to include relative humidity and leaf wetness parameters in the new models as well as making better links between macro and micro climatic parameters. New climate based scenario models have been investigated based on historical data using weather data from ten years and 10 Danish sites. The prediction values of the different models have been linked to disease events and yield responses in specific years. The outcome of the analysis will be presented.

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Resistance of Italian *Monilinia laxa* and *Monilinia fructicola* strains to thiophanate methyl

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Brown rot caused by *Monilinia* spp. is responsible of considerable damages in stone fruits production in the temperate regions of the World with a significant economic impact. Fungicides are the main mean for the disease control, however their intense use has determined the appearance of resistant *Monilinia* isolates. In particular in Italy, it is important to monitor *Monilinia fructicola* and *Monilinia laxa* resistant strains to fungicides to control economic losses associated with the peach and nectarine market. To monitor the development of resistance three methods were compared to assess isolate resistance/sensitivity: the amended medium, the Spiral Gradient Endpoint Method (SGD) and the Alamar Blue assay (AB). Eighty-five single-spore isolates of *M. fructicola* and 84 isolates of *M. laxa* were collected during the summer from 2009 to 2012 and tested with thiophanate methyl. Results revealed that 29% of *M. fructicola* isolates were sensitive (S), while 67% was low-resistant (LR) and the remaining 4% was high-resistant (HR). The majority of *M. laxa* isolates were S (74%), while the remaining 26% were LR. The molecular analysis showed, as expected, a point mutation at codon 198 in *M. fructicola* R isolates that is not present in S isolates, with GCA instead of GAA. LR and HR isolates have a point mutation at codon 83 in the β-tubulin gene where the arginine is converted in glutamine with a punctual allelic change CAA instead CGA. Interestingly, the alignments of nucleotidic and aminacidic sequences of five *M. laxa* S and five *M. laxa* R isolates showed that all ten isolates contained the sequence CTC at the codon 240 in the β-tubulin gene and no mutations were present in the rest of the sequences. This suggested the effect of other factors, such as the temperature, on the resistance.

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Development of a device that uses steam condensation heat to disinfect rice seeds

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To increase efficiency and reduce costs associated with chemical-free, environment-friendly disinfection of rice seeds, we developed a device that uses steam condensation heat to disinfect rice seeds. Compared to the conventional aerated steam treatment, the high-temperature and short treatment administered using the device can be adjusted for rice seeds. To sterilize seed-borne pathogens that mainly exist around the husk layer and to not injure the rice kernel, which has organs necessary for seed germination, the device focused on surface heating by steam condensation. An optimum heating condition was designed by controlling the humidity of the heating media and regulating the heating time. Disinfection against rice seed-borne diseases caused by bacteria, fungi, and nematodes was evaluated under the optimal condition (maximum heating without affecting germination). Under the optimal condition, this treatment was almost as effective as the conventional hot water treatment (60°C, 10 min). We observed that the artificial inoculation method to prepare infected seeds was greatly influenced by the extent of disinfection achieved using the physical seed treatments. In addition, heat tolerance of naturally infected and stunted seeds was lower than that of healthy seeds. We are currently developing a disinfection device for practical use on the basis of these findings.

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The potential of biofumigation for control of root-knot nematode on tomato

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The Phase out of methyl bromide in 2005 and the chemical traditional soil fumigants are damaging to the environment, are toxic to humans, and have negative effects on beneficial soil organisms. The use of mustard as a seed meal provides a promising alternative to synthetic chemical fumigants in our study. Mustards possess glucosinolate compounds in their seeds and foliage that upon soil-incorporation act as "biofumigant", hydrolyzing to form isothiocyanates that are highly toxic to Root-knot nematode (RKN). This study was carried out to evaluate the mustard powder *in vitro* and *in vivo* at different application levels (3, 5 and 7%) and times (two and seven days before nematode inoculation) against root-knot nematode, *Meloidogyne javanica* on tomato plants. *In vitro* test confirmed that mixing mustard powder at 4% with sandy clay soil as a biofumigant significantly reduced the percentage of egg hatching at all different incubation periods 24, 48, 72, 96 and 168 hrs, compared to control (soil without mustard and eggs in free water). Results indicate that the percentage of egg hatching reduction was 88.5, 90, 81.4, 74 and 69.4%, respectively. Laboratory results observed that the mixed soil with mustard encourage the larval mortality and lead to high mortality percentage at the different intervals periods. The mortality percentage was 94, 100, 90, 90 and 79%, respectively compared to control. Generally, *in vitro* results confirmed that the highest reduction in egg hatching and larval mortality obtained after 48 hrs of incubation period. *In vivo* experiment revealed that incorporation the soil pots with mustard at three different levels 3, 5% (48 hrs and one week before nematode inoculation) and 7% of soil weight significantly reduced the nematode parameters compared to plants treated with nematode alone. Nematode parameters i.e. number of galls/root system, root galling indices, number of egg masses/root system, as well as number of juveniles/250 g soil showed high reduction with mixing the soil pots with mustard at 5% (one week before nematode inoculation), followed by the same treatment (48 hrs before nematode inoculation). Mustard application at 5% one week before nematode inoculation reduced the nematode parameters by 97, 64, 97, and 93%, respectively compared to control. It could be concluded that the application of biofumigation by mustard is very effective for RKN control especially after the phase out of methyl bromide. Biofumigation with high concentrations of mustard can control effectively Root-knot nematode, and demonstrates the potential for mustard biofumigation as an alternative soil fumigant to methyl bromide.

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Effects of micro nutrient application on the incidence of root rot in red clover (*Trifolium repens L.*)

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Red clover is an important component of mixed grass-legume leys; however, fungal root rot decreases red clover viability over time. Root rot is caused by the soil-borne pathogens *Fusarium avenaceum, Fusarium culmorum, Cylindrocarpon destructans* and *Phoma medicaginis*. The aim of this study was to investigate whether the addition of micronutrients could reduce the development of root rot. A pot experiment with red clover cv. SW Ares was conducted in a glasshouse, 2010. Seeds and field soil was treated with Mn, Zn, B or a combination of all elements (n = 4). Soil from a garden centre was also used in for comparison (i.e. 'commercial soil'). After 19 weeks, the intact red clover plants were removed from the pots, the root rot disease severity index (DSI) determined and macro- and micronutrients was analysed in the roots. The results showed that root rot DSI decreased with increasing root concentrations of Mn. There were no linear relationships between root rot DSI and other elements. Treatments with high translocation of micronutrients from the root to shoot (i.e. high shoot:root ration) resulted in increasing root rot DSI. Plants in the 'commercial soil' had the highest translocation and highest DSI. Application of Mn or Zn had low micro nutrient translocation and the higher Mn root concentration and the lowest DSI. Thus, application of Mn and Zn may reduce the development of root rot in red clover and may thereby increase sustainability in red clover. Further investigations about the effect of Mn and Zn on root rot in red clover will be performed in a field experiment in Sweden.

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Applications of *Trichoderma* in vegetable protection and cultivation – EU project

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The aim of EU project, conducted since 2010, is to develop commercial preparations containing composition of selected strains of fungi genera *Trichoderma* for use in integrated agriculture and organic farming. Organic wastes are planned as a medium to produce *Trichoderma* biomass. The technology, combining recycling of farm wastes with complex of jointly acted biocontrol agents, may increase crop quality and reduce food and environment pollution. The goal is to develop a cheap, easy and competitive method for agriculture. Several biopreparations containing selected, single strains or combinations of *Trichoderma* with different modes of action, produced on composed organic carriers, were examined in field and greenhouse experiments. *Trichoderma* preparations were used as soil application, seed coating or foliar spray in main vegetable crops. It was found that soil applications increased marketable yield of pepper, potatoes and lettuce. Drenching of soilless media with conidial suspensions in tomato cultivation increased yield and quality parameters of fruits. *Trichoderma* used as seed coating decreased *Pythium* damping off. In field experiments, reduction of plants infection was also observed. Different strains of *Trichoderma* were effective in different crops. The mode of application had also a significant influence on fungal activity. Therefore, for every crop proper preparation of *Trichoderma* and technology should be developed.

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Options for the use of the biological control agent *Cladosporium cladosporioides* H39 in IPM strategies for apple scab control

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Venturia inaequalis causes apple scab leading to high losses in apple production. The disease currently is controlled by multiple fungicide applications. Isolate *Cladosporium cladosporioides* H39, originating from a sporulating colony of *V. inaequalis*, has been selected for its potential to control apple scab epidemics. Eight trials were conducted during two years in orchards in Eperjeske (Hungary), Dabrowice (Poland) and Bavendorf (Germany). Treatments were conducted during the primary season or the summer season. The effect of timing of antagonist applications before or after infection periods was assessed in an additional trial in an orchard in Randwijk (The Netherlands). The overall results of the field trials consistently showed for the first time that stand alone applications of the antagonist can control apple scab in leaves and fruits. This was demonstrated in organic and integrated growing systems with control levels as for common fungicides schedules. Control efficacies were 42 to 98% on incidence of leaf scab and 41 to 94% on fruit scab. The antagonist also was effective if applied one or even several days after infection events. This has been found in several field trials and has been confirmed by a trial with single spray applications at different intervals before or after infection events. Based on the available data on timing, concentration and fungicide compatibility of *Cladosporium cladosporioides* H39, the antagonist now can be tested as component of the complex IPM schedules needed to control diseases and pests in apple production.

Parts of this study are results of the EU projects PURE 265865 and CO-FREE 289497 which were co-funded by the European Commission. We also acknowledge the financial support by the Dutch Ministry of Economic Affairs (BO-25.10-005-001-PRI) (JK), the Hungarian Scientific Research Fund (K108333) and the European Commission and the State of Hungary (European Social Fund, TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program', project number: A2-SZJTOK-13-0061) (IH).

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Advancement of education in plant pathology and crop protection at the University of Göttingen

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In view of the constantly decreasing land available for agricultural food production and the increasing world population yield stabilization and its increase have to be ensured, which will only be possible with an inventive plant health management while protecting the environment, reducing emissions and biodiversity loss. The development of safe crop protection methods requires the education of highly skilled scientists and executives in sustainable crop protection management, in the developed countries as well as in the developing world. Over the past years however, research has taken a sharp turn towards molecular and basic approaches in many agricultural faculties, which has led to significant advances in diagnostic techniques, but has not sufficiently improved our holistic understanding of the biology of a detrimental organism such as its life cycle and damaging potential within certain cropping systems and environments. Experts who understand plant pests and pathogens, the risks they pose and the ways how these risks can be managed are urgently needed, both on a practical and scientific level. As a consequence, the urgently needed strengthening of the technical and scientific basis in crop protection requires a broader and multi-disciplinary approach both in teaching and in applied crop protection research.

For these reasons, a master programme 'Crop Protection' has been newly established at the University of Göttingen starting in the winter semester 2010 The programme consists of four semesters of advanced study courses covering all aspects of crop protection in lectures, seminars, laboratory classes, field excursions and the master thesis. A special feature in 'Crop Protection' is an internship of 6 weeks, in which students work on a project in a company or external research institution in the field of crop protection. This provides a deep insight for students into the 'real world of crop health management' and the industrial sector devoted to it. The language of instruction is English.The crop protection study program cooperates with research institutions and the agrochemical industry at different levels (Internship, lectures and practical courses) supporting the generation of young academics which will take the responsibility in future research and innovation in crop health management.

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Biological soil mapping – infestation of *Plasmodiphora brassicae* and soil characteristics

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Club-root, caused by *Plasmodiophora brassicae*, is a soilborn pathogen that causes severe yield losses in Brassica crops. It is an increasing problem. The spores survive for 15–20 years and might cause significant yield losses (>10%), already if 20% of plant are infected. An infestation with a couple of thousands spores/g soil is considered to have the potential to give significant losses. Infestation levels of >>100,000 spores/g soil has been observed. This soil borne pathogens can be transported with soil on machinery and even airborne. DNA-based methods have been developed to determinate levels of infestation in soil and a protocol to check level of contamination of soils on farms is being developed in Sweden. Presently this mapping approach includes two steps. The first is to check the level of infestation on the field level is a sampling with approx. 40 subsamples along a W-line. This step might then be followed by second point-sampling method. Point samples are drawn on the field where you suspect in-field variation. The aim is to identify areas were Brassica can be grown with a low risk of club-root infection and loss! At a farm in the County Skaraborg, Sweden, fields were sampled using W-line and analysed. The levels of spores varied between field from <5 to >700 fg DNA equivalent to > 400,000 sporers/g soil. Two field where investigated using point sampling and analyzed of *P. brasssicae*, pH-value and amount of boron in the soil. The pH-value and boron are both reported to effect the development of club-root and the combination of soil analysis of DNA from *P. brassicae* and boron might open a possibility to use variable rate application of nutrients *ie* boron to improve control of club-root in an integrated pest management approach. The pH-value and boron content varied between the fields and with-in the tested fields.

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The main fungal pathogens which infect sugar beet roots in Europe

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Several fungal pathogens attack sugar beet roots and they can cause considerable yield losses. Among the most economically important fungal sugar beet root diseases in Europe are *Phoma betae, Pythium ultimum, Aphanomyces cochlioides, Rhizoctonia solani, Fusarium* spp and *Verticillium* spp. Of these fungi *P.betae* is mainly seed borne; the other pathogens are soil borne. *P. betae*, *P. ultimum* and *A. cochlioides* can cause damping off in seedlings in most sugar beet growing areas. Also *R. solani* infects young plants, but the fungus is still uncommon in colder regions like Scandinavia. Most of the root fungi also infect older beets and reduce the storability of the sugar beet roots considerably.

At Syngenta phenotypic tests are performed for all mentioned root fungi in controlled conditions and/or in field. The aim is to evaluate resistance/tolerance in breeding materials. This is an important step in development of new cultivars. For improved breeding, markers have been developed e.g. for resistance to *R. solani*. Deep sequencing is one method that is applied for further improvement of markers.

Syngenta is also developing integrated solutions, combining genetics with new seed treatment and foliar compounds. This integrated strategy will provide a reliable control against e.g. *R. solani* in a broad range of field conditions worldwide.

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Olpidium virulentus strongly enhances soil transmission of Olive mild mosaic virus but not of *Tobacco necrosis virus D* or *Olive latent virus 1*

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Transmission ability and efficiency by the soil fungus *Olpidium virulentus* of *Olive latent virus 1* (OLV-1), *Olive mild mosaic virus* (OMMV) and *Tobacco necrosis virus D* (TNV-D), commonly found in Portuguese olive orchards, were evaluated. A single-sporangial culture of *O. virulentus* was recovered from soil using Chinese cabbage as bait plant and species was PCR confirmed. Transmission assays involved a 10^5/mL zoospore suspension, incubation with virus for 15 minutes, inoculation onto cabbage roots and virus detection after 6 days by ELISA. Assays revealed that the three viruses are transmitted to cabbage in the absence of that fungus. An efficiency of 10% transmission was found when 0.5 μg of OMMV/mL, 0.26 μg of OLV-1/mL and 0.5 μg of TNV-D/mL were used whereas 100% efficiency was attained when concentrations of 50 μg/mL of OMMV, 65 μg/mL of OLV-1and 150 μg/mL of TNV-D were applied. When each virus was separately mixed with fungal zoospores prior to plant inoculation, an efficiency of 10% transmission of OMMV was reached using 0.26 μg/mL, and 100% transmission obtained with 2 μg/mL whereas transmission rates of OLV-1 and TNV-D were similar to those detected in the absence of the fungus.

Such distinct transmission properties of OMMV versus TNV-D was unexpected as they are serologically indistinguishable and the coat protein, known to play an important role in binding to vectors, shares *ca*. 85% amino acid sequence identity.

Our data showing that OMMV soil transmission is greatly enhanced by *O. virulentus* zoospores, being 25 times higher than that in absence of the vector, is epidemiologically relevant, as virus contamination of growth substrates, especially if in presence of *O. virulentus,* may lead to an extensive viral dissemination causing deleterious effects to plants.

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Effect of static magnetic field on growth and sporulation of plant pathogenic fungi *Colletotrichum acutatum*

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The interaction of static magnetic fields (SMFs) with living organisms is a rapidly growing field of investigation. However, despite the increasing number of studies on the effects of the interaction on of SMFs with living organisms, many gaps in our knowledge still remain. This paper represents the study of the biological effects of the SMFs on the plant polyphagous pathogen *Colletotrichum acutatum* (*C. acutatum*). The effects of the exposure to SMFs of 163.84 mT to 176.28 mT (size of magnetic flux density of components XYZ) on the *C. acutatum* were investigated in vitro. The value of magnetic flux density was measured using a Hall probe and subsequently was created a numerical model, which shown the course of magnetic flux density. After the incubation the cultures, uniform from the point of view of growing and morphology, were placed into the static magnetic fields. SMFs inhibition of mycelia growth and sporulation was accompanied by morphological and biological changes. Fungal conidia germination and cell viability were also reduced. We provide evidence of the influence of SMFs on sporulation of the *C. acutatum*. The degree of sporulation was examined by Burke chamber and statistically evaluated.

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Accurate genotyping of soil inhabiting *Fusarium oxysporum* formae speciales complex using high-resolution melting analysis

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The soil fungus *Fusarium oxysporum* is the causative agents for vascular diseases infecting a wide variety of hosts, ranging from agricultural to ornamental plant species. Individual *F. oxysporum* pathogenic strains infecting similar hosts are classified together into groups named formae speciales. The high diversity in *F. oxysporum* suggests that this fungus is encompassed by a number of distinct lineages representing a species complex, whereas it is quite difficult to discriminate this complex because of its small genetic variation. Additionally, accurate and early diagnosis of *F. oxysporum* formae speciales is a crucial issue towards employment of efficient quarantine and management strategies. To gain insight into this issue and in order to distinguish *F. oxysporum* formae speciales, implementation of precise and rapid molecular diagnostic tools is a prerequisite. A new technique called high-resolution melting analysis (HRM) has been developed and already utilized for DNA genotyping. HRM is an automated analytical molecular technique that measures the rate of double-stranded DNA dissociation to single-stranded DNA with increasing temperature. Here we developed a real-time PCR assay using universal internal transcribed spacer (ITS) primers coupled with a high-resolution melting (HRM) analysis in order to differentiate *F. oxysporum* formae speciales complex. The melting curve analysis of amplicons specifically classified all isolates into *F. oxysporum* formae speciales and generated respective unique HRM curve profiles. The smallest DNA sequence difference recognized in this study was just one nucleotide. The results presented show that HRM curve analysis of ITS sequences is a simple, rapid, and reproducible method that allows the identification of *F. oxysporum* formae speciales. Our genotyping assay used the combined information of simultaneously acquired HRM data from an unlabelled probe and the full-length amplicon. Finally, the completion of both reactions in a closed tube saves time by eliminating the separate steps and reduces the risk of contaminations.

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Climate change increases risk of fusarium ear blight on wheat in central China

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To estimate potential impact of climate change on wheat fusarium ear blight (FEB), simulated weather for the A1B climate change scenario was inputted into a model for estimating FEB in central China. A logistic weather-based regression model for estimating incidence of wheat FEB in central China was developed, using up to 10 years (2001–2010) of disease, anthesis date and weather data available for 10 locations in Anhui and Hubei provinces, central China. In the model, the weather variables were defined in relation to the anthesis date for each location in each year. The model suggested that incidence of FEB is related to number of days of rainfall in a 30-day period after anthesis and that high temperatures before anthesis increase the incidence of disease. Validation was done to test whether this relationship was satisfied for another five locations in Anhui province with FEB data for 4–5 years but no nearby weather data, using simulated weather data obtained using the regional climate modelling system PRECIS. How climate change may affect wheat anthesis date and FEB in central China was investigated for period 2020–2050 using the wheat growth model Sirius and climate data simulated using PRECIS. The projection suggested that wheat anthesis dates will generally be earlier and FEB incidence will increase in central China.

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Healthy air – healthy plants – healthy people: Methods for detection of air-borne pathogens and allergens

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Aeroplankton includes numerous airborne macro- and micro-organisms, such as pollen grains, fungal spores, bacteria and viruses, many of which contain allergenic proteins to which a number of humans are increasingly sensitive. The concentrations of these air-borne organisms can be determined with varying measures of precision using aerobiological methods. The detection of pollen grains and airborne spores is based on air sampling with suction traps. Use of volumetric air sampling methods permits the accurate determination of the concentrations of airborne organisms collected. Seven-day spore samplers are routinely used to detect and quantify the number of pollen grains and spores of sporulating fungal taxa. The determination of spore concentrations may be based on light microscopic approaches that are increasingly aided by molecular biological techniques. The latter deliver a means of precise quantification of DNA concentration that can be re-calculated and related to the number of spores or gene copies from the target species. Molecular biological tools permit the detection of fungal variants in spite of close morphological similarities in spore shape or size. This precision, combined with high throughput, resolution and accuracy, renders PCR-based techniques at least comparable but often superior to microscopy. Abundant production of pollen grains by wind-pollinated plants or of wind-dispersed spores by sporulating fungi is necessary to fulfill their biological functions. However, high concentrations of airborne spores or pollen may lead to skin, eye or nasal irritation and diseases of human respiratory systems. Small particles are able to get inhaled even more deeply, to cause shortness of breath, alveolitis and asthma that sometimes have very serious consequences on human health. Modern traps enable the collection of aeroplankton with allergenic proteins that can be easily detected with ELISA tests. There is a whole selection of different apparatus, ranging from static samplers to minute, portable instruments. Similar technologies may be used to detect fungal spores of plant pathogens, thereby permitting the development of forecasting systems based on the biology of the plant pathogens. There are numerous examples of the successful airborne detection of pathogen mating types, Avr gene alleles, mutations for resistance to fungicides, effectors of pathogenicity and chemotypes from different plant pathogens. Aerobiological monitoring is increasingly widely used for the detection and quantification of pathogen inoculum and can be deployed as a crucial part of disease forecasting systems that, in turn, constitute a part of modern integrated pest management practices.

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Monitoring of plant and airborne inoculums of *Sclerotinia sclerotiorum* in spring oilseed rape using real-time PCR

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Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is a major disease of spring oilseed rape in Sweden. The pathogen survives in the soil for long periods as sclerotia. A real-time PCR assay was developed and used to determine the incidence of *S. sclerotiorum* DNA on petals and leaves of spring oilseed rape as well as in air samples. Five field experiments were conducted from 2008 to 2010 to detect and study pathogen development. The presence of *Sclerotinia* DNA on petals and leaves at different leaf levels of the plant of two different cultivars was determined regularly during the flowering period. Air samples were collected using a Burkad 7-day continuously recording spore sampler starting in late May 2010. There were significant differences in *S.sclerotiorum* incidence at different stages of flowering. The incidecnce of *S.sclerotioum* DNA on the leaves varied (0–100%), with significantly higher incidence on leaves at lower levels. In one field experiment, *S sclerotiorum* DNA was not detected on petals during flowering, whereas the pathogen was detected on leaves, with that the spore release did not coincide with flowering on that experimental site. Using a real-time PCR assay to assess the incidence of *S.sclerotiorum* on oilseed rape leaves could potentially improve disease risk assessment in a disease support system based on predictive tests, field data and local climate.

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Molecular detection of *Alternaria* spores from air

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The presence of fungal spores in the air is one of the most important issues studied by aerobiology. Fungi grow in all climatic zones and their vast majority reproduces by spores, thus providing a potential source of allergens. In addition to the outcome of their presence in our surroundings, we also encounter their negative effect both on plants, animals and humans. Knowledge of plant pathogen spore fluctuations and their relationship with weather variables can be used for the early detection of plant infections, thus allowing a more efficient and reliable use of pesticides. Combined with clinical data the forecasts allow to prevent of respiratory allergic diseases, improving diagnosis and treatment. The species belonging to *Alternaria* can cause seedling blights and diseases of numerous crop plants, mostly called black or dark spots. They are also a well-known agent of asthma and/or rhinitis. The monitoring of *Alternaria* spores in this study was done in three geographic regions of Poland: Great Poland, Lublin Upland and Carpathian Foothills. These regions greatly differ in geobotanical properties, climate and the type of landscape. Experiment was carried out in 2010–2012. The aim of this research was to investigate seasonal fluctuations and compare the abundance of *Alternaria* spores sampled in different regions of Poland. We have used a combination of light microscopy and a quantitative PCR. Both methods were highly correlated, what suggests that microscopic observations can be replaced by or supplemented with molecular detection. The dynamics of the abundance of spores corresponded to fluctuations in the levels of DNA. In all cases, there were positive correlations between spore counts, obtained by light microscopy, and molecular detection based on yield of DNA obtained from the most prevailing species, belonging to this genus of fungi.

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Deciphering transcriptomics of *Pythium porphyrae* – the pathogen of red seaweed genus *Porphyra*

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Species of the red seaweed genus *Porphyra* sensu lato are of important value worldwide with a global crop industry of US $ 1.8 billion. The oomycete genus *Pythium* involves approximately a hundred species of which are either saprophytic or pathogen on a broad range of hosts. Here, we investigated the gene repertoire of *Pythium porphyrae,* the agent of red rot disease, and its transcriptional regulation using next generation sequencing of 454 EST libraries constructed during time course of infection in *Porphyra*. The final assembly contained 10819 unigenes, representing 11.1 Mb of non-redundant sequence data. In order to identify multigenic families, we further clustered the predicted proteome into tribes; the largest one was composed of 194 unigenes containing a kinase domain either alone or in combination with others domains. Additionally, we performed *ab initio* predictions of candidate secreted pathogenicity effectors and related virulence factors. The secretome was depleted of sequences associated to cytoplasmic and nuclear functions. *De novo* motif search was performed in order to identify potential RxLR-type host translocation motives. Using similarity and PFAM searches, we identified elicitins, cellulose binding elicitor lectin (CBEL) as well crinklers genes; expression levels of both elicitins and CBEL were generally reduced at zoospore and zoosporangium stage. In order to get insight into gene families potentially involved in pathogenicity, we focused in manual curartion of genes such as secreted proteins, toxins, and homologues of known oomycete pathogenicity effectors. In agreement with the general view that *Pythium* pathogens are opportunistic, necrotrophic pathogens less specialised than other biotrophic oomycetes, *P. porphyrae* contains a gene repertoire very similar to this that is observed in other *Pythium* species. Finally, we also asked evidences in an effort to identify *de novo* pathogenicity-related genes that being belong to highly expanded and fast-evolving gene families.

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Twelve year results of the field trial of GM plum *Prunus domestica* L. cv. 'HoneySweet' resistant to the *Plum pox Virus*

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Sharka disease caused by *Plum pox virus* (PPV) is the most harmful disease of stone fruits in Europe. There is no highly PPV resistant cultivar of plum. Biotech approach has led to the development of resistance through genetic engineering. The result is plum cv. 'HoneySweet' and PPV protection is based on RNA interference (RNAi). GM plum *Prunus domestica* L, clone C5 (cv. 'HoneySweet') has been evaluated for twelve years (2002–2013) in a regulated field trial in the Czech Republic for resistance to PPV, *Prune dwarf virus* (PDV), and *Apple chlorotic leaf spot virus* (ACLSV), all ecomic important viruses of plum. Even under high and permanent infection pressure produced through grafting, PPV has only been detected in 'HoneySweet' trees in several leaves situated close to the point of inoculum grafting in the first nine years. Mild symptoms of PPV disappeared year by year. No PPV symptoms were observed in the last three years and results of ELISA detection were negative. The same tendency is in results of PPV detection by RT-PCR. Aphids were not able to transmit PPV in the natural conditions on 'HoneySweet'. Co-infections of PPV with PDV and/or ACLSV had practically no influence on 'HoneySweet' trees. Twelve years of field testing in the Czech Republic clearly demonstrated the high level of resistance of C5 to PPV infection through aphid vectors and by graft inoculation. Minimal PPV symptoms even disappeared during years, and no PPV leaf symptoms were found after the nine years duration of field trial, and in the following three years. Pomological evaluation of the quality of fruits harvested from non-graft-inoculated cvs. 'HoneySweet', 'Stanley', and 'Domácí švestka' trees, and from 'HoneySweet' trees growing twelve years under the high and permanent infection pressure of PPV-Rec, PPV-Rec + PDV, PPV-Rec + ACLSV, PPV-Rec + ACLSV + PDV proved the high quality of 'HoneySweet' fruits which are large, sweet, and of high eating quality. Four year results indicate that the characteristics of 'HoneySweet' fruits harvested from control virus non-inoculated trees are well within the range of the characteristics of control cultivars 'Stanley' and 'Domácí švestka', and are of higher quality in some characteristics. The resistance to PPV is highly effective, stable, durable, and heritable as a dominant trait.

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Decreasing pot plants' susceptibility to disease by application of biostimulants

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During the propagation and cultivation of pot plants, plant pathogenic bacteria and fungi can cause serious problems, including plant death. Well known examples from Dutch greenhouses are: *Phytophthora* in Kalanchoë during flower formation or *Fusarium foetens* in Begonia. It is desirable to lower plant's susceptibility to the infection by these pathogens. We hypothesize that is can be realised by stimulating the plant's own defense systems by using biostimulatory products.

We tested the influence of six different biostimulant additions on development of disease symptoms of *Fusarium foetens* in *Begonia* and *Phytophthora nicotianae* in Kalanchoe. Greenhouses trials were carried out in 144 m^2 greenhouses with 24 ebb and flow tables. Each treatment was done in triplicate. Parameters of plant growth and development were measured. Additionally, we sampled plant material for the measurement of plant metabolites by Nuclear Magnetic Resonance Spectroscopy (NMR).

Significant suppression of *Phytophthora* symptoms in Kalanchoë was observed in salicylic acid and silicium treatments. Decrease in disease occurrence was 60% and 87%, respectively when compared to untreated plants inoculated with *Phytophthora nicotianae*. In Begonia trial we were unable to obtain suppression against *Fusarium foetens* with tested biostimulants.

Influence of three biostimulants (silicium, salicylic acid and chitosan) and fungicide Ridomil on expression of 24 plant defence genes of *Arabidopsis thaliana* infected by *Botrytis cinerea* or *Phytophthora capsici* was also studied. Gene expression was modulated differently by each biostimulant. Addition of salicylic acid caused upregulation of genes important in jasmonic acid pathway, making plant less susceptible to *Botrytis cinerea* infection. Chitosan upregulated expression of genes from salicylic acid route. Interestingly, use of fungicide resulted in down regulation of plant defense genes.

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The mannose binding lectin gene *FaMBL1* is involved in the resistance of unripe strawberry fruits to *Colletotrichum acutatum*

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The fungal pathogen *Colletotrichum acutatum* is the causal agent of strawberry (*Fragaria* x *ananassa*) anthracnose. The fungus can infect strawberry fruits at both unripe and ripe stages, however the symptoms appear only on red ripe fruits. On immature fruits, the pathogen becomes quiescent as melanized appresso ria after 24 h of interaction. We found previously that a mannose-binding lectin (MBL) gene was the most up-regulated gene in 24 h-infected white strawberries, suggesting a role for this gene in the fruit response to pathogen.

Here, we present the time course analysis of the expression of this MBL gene, named *FaMBL1* (*Fragaria* × *ananassa* MBL 1), which was undertaken in order to monitor its expression profile in white and red fruits at early interaction times. *FaMBL1* was expressed exclusively in white fruit after 24 h, when the pathogen was quiescent. *Agrobacterium*-mediated transient transformation was then used to obtain white and red strawberry fruits with silenced and overexpressed *FaMBL1* gene, respectively. Upon *C. acutatum* 24 h infection *FaMBL1*-silenced unripe fruits showed an increase in susceptibility to *C. acutatum*. These 24-h-infected tissues contained subcuticular hyphae, indicating pathogen penetration and active growth. In contrast, overexpression of *FaMBL1* in ripe fruits decreased susceptibility; here, 24-h-infected tissues showed a high percentage of ungerminated appressoria, suggesting that the growth of the pathogen had slowed. These data suggest that *FaMBL1* plays a crucial role in the resistance of unripe strawberry fruits to *C. acutatum*.

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Influence of *Neotyphodium lolii* endophyte on defense reaction of perennial ryegrass (*Lolium perenne* L.) infected by pathogenic fungi

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Neotyphodium endophytes form very often symbiotic associations with grasses. Presence of the endophyte can increase resistance of the host to diseases. Such effect can be associated with presence of the toxic metabolites. Apart from antibiosis, the other mechanisms are involved in higher resistance of the grass/endophyte association to pathogenic fungi. There is still little known about these mechanisms. Thus, the detailed study was conducted to examine the effect of *Neotyphodium lolii* endophyte on perennial ryegrass, one of the most important grass species in Europe. Total production of phenolic compounds and Patogenesis Related Proteins (PR Proteins), emission of Volatile Organic Compounds (VOCs) by perennial ryegrass genotypes infected with pathogenic fungi were researched. The competitive activity of N. lolii towards pathogen inside the host plant was also determined. Endophyte infected (E+) and non-infected (E–) plants were used. The plants were artificially inoculated with *F. poae* and *R. solani* infection material by foliar spray. There was observed a highly significant effect of the perennial ryegrass genotype, endophyte status, time of the analysis after inoculation (DAI) and their interactions on phenolics content, PR proteins production and VOCs emission in the plants. Most of the phenolics and VOCs reached their highest levels of production/emission on the second to fourth DAI and on the sixth DAI respectively. Content of PR proteins varied depending on the enzyme and association. No antagonistic effect of the endophyte on the development of mycelium of the pathogen was observed. Our results suggest that phenolics, PR Proteins and VOCs can play an important role in defense mechanisms of E+ perennial ryegrass against pathogens.

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Resistance responses against *AvrLm1* and *AvrLm4* from *Leptosphaeria maculans*

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To better understand the interaction between the apoplastic fungal pathogen *Leptosphaeria maculans* and its host oilseed rape (*Brassica napus*), the molecular basis of effector-triggered defence (ETD), which is based on interactions between pathogen effectors and receptor-like proteins (RLPs) was explored. *B. napus* responded to the phoma stem canker pathogen *L. maculans* with an oxidative burst. Diaminobenzidine (DAB) staining showed that H_2O_2 accumulation increased significantly 7 days post-inoculation (dpi) of cultivar Excel, which carries the *R* gene *Rlm7*, with a *L. maculans* strain carrying the corresponding effector *AvrLm7*. Infiltration of *B. napus* leaves with conidia of *L. maculans* induced the expression of defence genes. Induction of *PR-1* and *WRKY70* occurred between 3 and 7 dpi. Our data suggest that *L. maculans* effectors vary in their influence on plant defence reactions. *B. napus* cv. Excel responded more strongly to a *L. maculans* strain that expresses *AvrLm4* than another strain that expresses *AvrLm1*. The interaction between *AvrLm4* and *Rlm4* is being studied at the level of RLP candidate genes and protein structure predictions.

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Transcriptional and phytohormone responses characterising susceptible and resistant genotypes in the *Arabidopsis* – *Verticillium longisporum* pathosystem

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Verticillium longisporum is a vascular pathogen infecting oilseed rape. Quantitative disease resistance (QDR) is the major control means against this disease but is poorly understood. To study QDR against *V. longisporum* QTL-mapping was performed using a new (BurxLer) RIL population of *Arabidopsis thaliana*. Near-isogenic lines (NILs) that differed only in QTL regions were used for fine-mapping and for attributing transcriptional and phytohormone reactions to these regions. Two NILs were selected for microarray analysis: NIL9 contained alleles of the susceptible parent Ler in the variable regions, whereas tm (tailor-made) NIL130 was selected to contain confined introgressions from the resistant parent (Bur).

QTL controlling colonisation resistance were mapped to chromosomes 2 and 4. tmNIL130 was more resistant to systemic colonisation than NIL9 and showed an increased transcriptional response to *V. longisporum*: At 13 dpi, only 18 genes were differentially expressed by *V. longisporum* infection in NIL9, but 295 in tmNIL130, among them PR-proteins, cell wall-modifying enzymes, receptor-like kinases and transcription factors. NILs were also used to measure contents of SA, ABA and JA in response to *V. longisporum*. Bur and L*er* differed in their phytohormone response to *V. longisporum*, and some of these responses could be attributed to the chromosomal regions of interest. Fine-mapping and a candidate gene approach are applied to clone gene(s) controlling resistance and recent results will be presented.

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Oligosaccharides of natural origin as a new group of plant resistance inducers to diseases

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Plants are static organisms and have developed high performed mechanisms to protect themselves against biotic and abiotic attacks. Plants cells are able to recognize some molecules called plant resistance inducers or elicitors. These molecules can be endogenous or external. Laminarin is an oligosaccharide extracted from the seaweed *Laminaria digitata*. This external elicitor triggers plant natural resistance *via* the induction of plant resistance mechanisms. In fact, it has been shown that laminarin is rapidly recognized by plant cells; this recognition is characterised by membrane ionic flux stimulation. It has also been demonstrated that phenylpropanoid and oxylipin pathways are stimulated by laminarin application. Laminarin contributes to different plant diseases resistance, as apple scab or botrytis on strawberry.

Field tests in Europe show that laminarine included in protection programs against scab at the orchard and for storage ensures similar protection to conventional programs, with no pre harvest interval constraint. The reduction of chemical fungicide treatments before harvesting allows reducing the quantity of residues and the number of detected fungicide molecules in apple.

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Intercropping of maize and faba bean influences the uptake of micronutrients in organic production

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Intercropping of various plant species may have many advantages such as increased uptake of mineral nutrients and reduced incidence of plant pathogens. The aim of this study was to investigate the effect of intercropping on uptake of micronutrients and sulphur (S) in organically produced faba bean and forage maize. Also, relationships between shoot concentrations of micronutrients and leaf spots in faba bean were investigated. Three field experiments were performed in the South East part of Sweden, 2010–2011. Maize (*Zea mays L.* cv. Isberi) and faba beans (*Vicia faba L.* cv. Aurora) were either cultivated as sole crops or intercropped. Intercropping significantly increased the concentrations of Cu and Zn in maize and B and S in faba bean. Leaf spot disease severity index (DSI) decreased in intercropped faba beans (mean DSI 50) compared with sole crop (mean DSI 64) ($p < 0.05$). A negative linear relationship between Cu concentrations in shoots and leaf spot DSI was found ($p < 0.006$). Intercropping of maize and faba bean improved mineral content of the forage and reduced the incidence of leaf spots in faba beans. Sufficient shoot Cu concentrations reduced the severity of leaf spot diseases in faba bean.

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Studying the epidemiology of *Ramularia collo-cygni* for the improvement of an Integrated Pest Management system in a changing climate

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Long term surveys show a shift in pathogen population in Bavaria. As a consequence the efficiency of established control strategies has been reduced. Unsatisfying barley yields concerning quantity and quality have been dominantly attributed to the occurrence of heavy leaf spotting caused by environmental factors and Ramularia leaf spotting (RLS).

In a joint project between the Technische Universität München and the Bavarian State Research Center (LfL) the causes of the leaf spotting are being investigated by monitoring different sites in Bavaria and conducting fungicide trials in spring barley and winter barley. Complementary intense studies of the pathogen biology are carried out. The aim of the project was to integrate the results into an established Integrated Pest Management tool (Gerstenmodell Bayern) for improved control. With the focus on the detection of *Ramularia collo-cygni* in the field and interaction of the epidemics with climatic factors, the project has the advantage of intense observations in a region with high incidence and high agricultural and climatic variability.

While the fungicide trials proved the high impact of the disease complex on yield quantity and quality, the monitoring showed a broad and regular occurrence on all sites. Results with an improved strategy, based on the experience from the specific fungicide trials, generally gave a positive yield benefit under different environmental conditions.

New insights into pathogen biology discovered the opportunity of seed transfer. Looking into barley seed from an archive discovered that *Ramularia collo-cygni* could constantly be detected with a strong increase from the 1980s on. A survey of samples from different regions of Bavaria in indicated a dominance of seasonal effects. A new seed treatment gave good control.

The impact of the pathogen biology, environmental factors and new treatments on control strategies will be discussed with current results.

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State of art of *Ramularia collo-cygni* (leaf spot of barley) in Argentina and detection and quantification of *R. collo-cygni* by Real Time PCR in barley plantlets and seeds treated with fungicide

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Ramularia leaf spotting caused by the fungus, *Ramularia collo-cygni,* affects barley crops worldwide. In the last two crop seasons (2012 and 2013) this disease has been present in Argentinean barley crops. Disease severity was especially high in 2012, when the fungus caused important premature reductions of leaf green area and related yield losses. This disease was first detected in Argentina in 2001 with 100% of foliar incidence and severities from 60 to 100%. At that point in time, the disease was only recorded in the typical barley growing area of the southwest of Buenos Aires province. One decade later however, *Ramularia* leaf spot was again detected in barley crops but at the north of Pampa's region, in Entre Ríos, south of Santa Fe and north of Buenos Aires. Twenty days later the disease was also diagnosed in the southeast of Buenos Aires province, where several barley cultivars with high levels of visual symptoms were reported. All the reports were based on the description of typical symptoms and leaf samples microscopically assessed for conidiophore presence. The most affected crops were those with low-intensity of disease monitoring or without complete treatments with fungicides (single fungicide application or lower than recommended doses). The fungus *R. collo-cygni* can overwinter through sclerotial type structures on crops residues (structures not yet identified and described in Argentina) and via infected seeds. *R. collo-cygni* has an endophytic growth stage and its detection in seedlings or seeds cannot be performed by conventional isolation techniques.. The levels of pathogen DNA detected in grain, determines the quality of the seed and helps to decide on the future use of fungicides. Moreover, the detection of pre symptomatic *R. collo-cygni* infection during vegetative crop stages, allows an early decision on the use of fungicides to protect the crop from later disease epidemics. In this presented work, Real-Time PCR protocols to detect *R. collo-cygni* were applied, using specific primers to amplify DNA of the pathogen. These tests were used to analyze DNA samples extracted from barley seedlings grown from seeds infected by *R. collo-cygni* An experiment was set up to measure the effect of a new BASF seed treatment (carboxamide + triticonazole 10%) on fungal DNA levels in seed. Seeds were either untreated or treated with 30 or 75 ml of the mixture. DNA extracted

from barley seeds or emerging plant leaves had enough quality to perform the *R. collo-cygni* detection assay. Leaf tissues from plants grown from untreated seeds had higher quantities of *R. collo-cygni* DNA than the samples from the two seed treatments. The Real Time PCR technique with specific primers detects *and* quantifies *R. collo-cygni* DNA in plantlets and seeds faster, with high specificity and sensitivity. This technique can be applied to analyze seeds and to evaluate the performance of seed fungicides to control *R. collo cygni*. Control of the disease currently relies on chemical methods. Technical reports from commercial trials indicated that the best disease control could be achieved by triple mixtures composed of strobilurins, triazoles and carboxamides applied as a foliar spray at early stem elongation. Future research is required to study the epidemiology of *Ramularia* leaf spot of barley in Argentina and identify suitable management practices to mitigate its effects.

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Ramularia collo-cygni – a growing problem for barley growers

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Ramularia collo-cygni is the main biotic agent involved in the late season disease of barley, Ramularia Leaf Spot (RLS). The disease has been shown to reduce grain yield and quality. The interaction between the fungus and the host plant has been investigated using a *gfp* transformed isolate in controlled environment conditions. The colonisation of leaves and seed was visualised using confocal microscopy. The movement and presence of the fungus has been monitored in field crops using plant samples and Burkard spore samplers. Fungal DNA in seed was also quantified using a qPCR assay developed at SRUC. The risk factors associated with RLS epidemics are being identified and information used in the design of fungicide programmes. Control of RLS is still possible with a number of active fungicides available to growers. However, the rapid appearance of resistance to strobilurin fungicides has highlighted the need for monitoring of efficacy. The sensitivity of *R. collo-cygni* isolates is now being monitored using a multiwell plate assay.

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Detection of genetic variability in responses of spring barley cultivars to Ramularia leaf spot infection based on fungal DNA content and toxin accumulation in leaves

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Ramularia collo-cygni (Rcc) is the causal agent of a novel and increasingly important leaf spot disease in barley, Ramularia leaf spot (RLS). Necrotic spots with a yellow halo are formed at post-anthesis stages of the crop. Rcc produces phytotoxins (rubellins) which may cause complete browning of the leaves leading to die off within few days. In a field screening conducted in 2010, ten spring barley cultivars were evaluated for their resistance to Rcc. The cultivars displayed significant differences in their symptomatic response to Rcc infection. Fungal DNA was detected in all barley genotypes by qPCR. At early growth stages (GS 61–65), few days before first symptoms appearance, the amount of fungal DNA in leaves of the most susceptible cultivar (Barke) was five times higher than in the most resistant cultivar (IPZ 24727). A strong correlation ($p = 0.001$, $rs = 0.851$) was observed between the visual disease symptoms and amounts of Rcc DNA in F-1 leaves at growth stage 73–75 (milk ripeness). A novel, specific and sensitive HPLC protocol with a fluorescence detector was developed for the detection of rubellins in infected plant tissue. Rubellin D accumulated in all samples in the early stages (GS 61–65) of disease development, few days before symptoms became visible. Levels of Rcc phytotoxin (rubellin D) in the infected leaf tissue strongly correlated with the Rcc DNA content ($p = 0.002$, $rs = 0.842$) as well as with disease severity at GS 73–75 ($p = 0.000$, $rs = 0.966$). Our results demonstrate that both qPCR measurement of fungal DNA and rubellin D measurement are reliable methods for the accurate determination of cultivar differences in response to RLS under field infection conditions.

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Investigating *Ramularia collo-cygni* genetic diversity

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Ramularia collo-cygni is now recognized as an important pathogen of barley in Northern and Central Europe, New Zealand and South America and has also been reported recently on oats and wheat. It is the cause of Ramularia leaf spot (RLS), a disease which occurs late in the season. It induces necrotic spotting and premature leaf senescence, leading to loss of green leaf area in crops, and can result in substantial yield losses. The fact that the fungus can remain latent in barley plants until flowering, coupled with its very slow growth *in vitro*, makes it difficult to detect it in crops. As a result, the epidemiology of this pathogen remains poorly understood. To know more about its epidemiology, having the knowledge of its genetic structure and diversity is important. In this study, we tried to have a first look at the population genetics of *Ramularia collo-cygni*. Since *Ramularia* genome sequences were not yet available, a gene fishing strategy was performed to select putative housekeeping genes. We used the sequences of several housekeeping genes in *Cercospora zeae-maydis* and *Mycosphaerella graminicola,* reported to be two related species to *Ramularia*. After testing primers for these genes on *Ramularia,* five putative housekeeping genes were selected. To carry out the study, genes fragments had to reach a minimum size of 500 bp. To reach this minimum size, we performed Thermal Asymmetric Interlaced (TAIL) PCRs on these genes. Once the right size was reached and after making sure that the amplified fragments are homologous to their corresponding genes on other fungi, we amplified and sequenced them on 20 *Ramularia collo-cygni* isolates. We performed classic population genetics analysis (Theta-W, Theta-Pi, Tajima's D) to uncover genetic variability and population structure.

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Disease resistance trade-off between the *mlo* locus and Ramularia leaf spot in barley

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Ramularia leaf spot (RLS) is an emerging threat to barley cultivation across Europe. To advance our understanding of the genetics of resistance to RLS a doubled haploid population was produced from the Ramularia resistant barley variety Power and the susceptible variety Braemar. Disease development was scored at both adult and seedling stages and the resulting data was used to identify a single QTL associated with reduced Ramularia leaf spot. This QTL, from the resistant parent Power, was located on the long arm of chromosome 4H. In the susceptible parent Braemar, this region contains *mlo11*, the recessive broad-spectrum powdery mildew resistance gene, suggesting that mutations associated with mildew resistance at the *Mlo* locus enhance susceptibility to RLS. To further characterise the role of *mlo* mutations in RLS susceptibility a set of near isogenic barley lines containing different *mlo* mutations were inoculated. In all cases mutation at the *Mlo* locus increased susceptibility to RLS. Two additional genes (*Ror1* and *Ror2*) are required for *mlo* resistance against powdery mildew to be effective. Mutations at either the *Ror1* or *Ror2* locus, which increase susceptibility to mildew, reduced RLS symptom development. These results indicate a trade-off between *mlo*-mediated resistance to powdery mildew and susceptibility to RLS. We speculate that the widespread use of *mlo11* in European spring barley to control powdery mildew may have been a contributing factor in the emergence of Ramularia leaf spot as a major disease of barley.

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A European overview of the occurrence of *Ramularia collo-cygni* and its sensitivity to fluxapyroxad

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Ramularia leaf spot (RLS), caused by *Ramularia collo-cygni*, has become increasingly important in European countries since the middle of the nineties. This is particularly the case in Austria, Southern and Middle Germany, Denmark and Scotland, where RLS is one of the most important diseases in barley with yield losses up to 20–30% possible. To understand the potential for further spread of the disease, European pre-disposition maps will be discussed which were achieved by computing climate information with the conditions most favourable for the development of RLS. With the introduction of SDHI fungicides, a new dimension of fungicidal control of RLS has been achieved. This makes SDHI fungicides the product of choice for RLS control. Fluxapyroxad, one important active ingredient out of this group, has given excellent efficacy in the field in numerous European countries. However, SDHI chemistry is at risk from the development of resistance by *R. collo-cygni* and so there is a need for a broad monitoring program to survey the sensitivity of this pathogen over time. In 2012 and 2013, intensive monitoring of field and commercial sites in different European countries was carried out. Microtiter assays of the isolates obtained showed a wide range of ED_{50} values towards fluxapyroxad, with an obvious difference between individual locations. Molecular-biological analyses were initiated to see whether mechanisms or mutations could be identified which explained the observed sensitivity differences of single isolates. Despite the observed sensitivity differences of isolates from several locations *in vitro*, fluxapyroxad in the field gave consistent and reliable performance against Ramularia leaf spot. As a high level of variability in sensitivities to fluxapyroxad was found even between samples from the same field without any measurable impact on SDHI performance, it is therefore concluded that the levels of ED_{50} observed at this time do not indicate resistance development but are a result of a high level of genetic variability of *R. collo-cygni* in the field.

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Ramularia collo-cygni: production of different types of spores *in vitro*

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Since 2000 the complex of necrotic leaf spots on barley leaves is increasing. Yield losses of the order of 25–30% are often observed. For a better control against this phenomenon a good knowledge of the actors including the fungus *Ramularia collo-cygi* is absolutely needed. Isolation and culture of this fungus is not easy and even very difficult to produce spores on artificial medium. In aim to optimize the production of spores a new technique was developed to cultivate this pathogen. It is to inoculate the fungus in the very high density on nutrient medium as PDA (potato-dextrose-agar) and SBA (straw-bran-agar) in order to initiate a overcrowding-effect. The cultures were incubated at 15°C under alternating dark light and obscurity (12h/12h). The fungus grew well and produced forms of spores that have never been observed in our laboratory. They were a form of spherical micro conidia on short branched conidiophores. Also a lot of clamidospores were formed in the mycelium. On the poor artificial medium (SBA) environment *Ramularia collo-cygi* produced a lot of small sclerotia. Despite these efforts little amount of "normal" conidia on swan-neck shaped conidiophores were observed. Now the question is how these different types of spores and sclerotia react with each other and whether this can lead to the already long-sought teleomorph of this fungus (*Mycosphaerella*).

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Ramularia leaf spot on barley in the Czech Republic

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Ramularia leaf spot (RLS) was in the Czech Republic first identified in 1998. In current study were tested 144 spring barley cultivars at 3 locations in the Czech Republic over 3 years (2009–2011). Only minor and statistically insignificant differences were observed among the individual cultivars in reaction to RLS. No cultivars were observed to have resistance to *Ramularia collo-cygni* (Rcc), but significant influence of location and year on the intensity of RLS infection in barley was observed. Isolates of Rcc mostly originated from the Czech Republic, but also from the Slovak Republic, Germany and Swiss were tested using amplified fragment length polymorphism (AFLP) analysis. The level of genotypic diversity was higher within populations than among them. No significant population differentiation was observed thus extensive gene flow is assumed among populations. The inferred clusters did not represent geographical populations. A real time PCR assay was designed to quantify the pathogen in barley tissues. PCR primers and a TaqMan probe were designed to target Rcc-specific DNA sequence. The method was optimized using pure fungal DNA and plasmid standard dilutions. Barley kernels were dissected into lemma, pericarp, testa, endosperm and embryo which were individually tested by real time PCR for quantifying Rcc. *Ramularia* DNA was highest in the lemma, and occurred in lower amounts in the pericarp and embryo.

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Pathogenic *Ramularia* spp. in Poland

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The fungi described as *Ramularia* species are the anamorphic stages of the genus *Mycosphaerella* (Ascomycota). They are obligatory parasites of seed plants, but they also infect the spore-bearing (cryptogamous) plants, such as ferns. Some species are hyperparasites which infect other parasitic microfungi. There are 325 known species of *Ramularia*, worldwide. In Poland, 120 taxa have been found, including 116 taxa on 308 species of angiosperms, 1 species on fern (*Asplenium trichomanes*) and 3 hyperparasites on 5 rust species (Pucciniales). There are still new species being collected in Poland, with some that are also new to science. Over 40% of these new *Ramularia* species were found at 1–5 sites only, and grow mainly on wild plants. The common species are often pathogens of crop and ornamental plants, e.g. *Ramularia grevilleana* (on *Fragaria* and *Potentilla*), *R. geranii* (*Geranium* spp.), *R. inaequalis* (Asteraceae), *R. urticae* (*Urtica* spp.). Among *Ramularia* species there are also pathogens of important crops such as *R. collo-cygni,* infectious to barley (*Hordeum vulgare*) and *R. beticola*, which attacks beetroot and sugarbeet (*Beta vulgaris*). *R. grevilleana* has been shown to damage cultivated and wild strawberry crops since the end of the 19th century. The outbreaks of *R. beticola* on beetroot were first reported in 1932 and *R. collo-cygni* was identified for the first time in Poland in 2003. However *R. collo-cygni* has subsequently been widely studied in respect to basic genetic characteristics, as well as rubellin production. The first detection of didehydrorubelin E, using High Performance Liquid Chromatography – Mass Spectrometry, was reported in Poland. A total of 23 species have now been collected from cultivated plants. *Ramularia* species exhibit a biotrophic stage in their life cycle and some of them grow on rare plants, so it is sometimes extremely difficult to check whether the collected fungal species meets the demands of Koch's postulates. Genetic analysis and GenBank data are also very scarce thus the need for further studies is highlighted.

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Biosynthesis and mode of action of the rubellin toxins produced by the phytopathogenic fungus *Ramularia collo-cygni*

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The filamentous fungus *Ramularia collo-cygni* causes a late season disease on spring and winter barley known as Ramularia Leaf Spot (RLS). It has become an important disease in the past couple of decades in northern Europe and has recently been reclassified as a major disease of barley in the UK. If left unchecked, RLS can rapidly cause substantial yield losses that are estimated to be worth over £10 million each year in the UK. The lack of apparent varietal host resistance combined with the appearance of fungicide resistance has given this disease a higher profile.

The increased profile of this plant disease has greatly enhanced the fundamental understanding of the causal pathogen in recent years. Particularly analysis of the fungal life history has provided important insights into the cell biology of the anthraquinone-derived toxin, rubellin, produced by the fungus during its necrotrophic phase. In this study we focused on the analysis of the putative molecular pathways involved in rubellin biosynthesis and its *in planta* mode of action. We show that the recently sequenced *R. collo-cygni* genome revealed the presence of an aflatoxin-like gene cluster that resembles the biosynthesis gene cluster of the toxin dothistromin in the pine pathogen, *Dothistroma septosporum*. Moreover, we used the model plant *Arabidopsis thaliana* to investigate the non-host specific mode of action of rubellin. We show that the rubellin-induced cell death may involve proteasome and caspase-like activities, providing some of the first insights into the molecular pathway by which this toxin induces cell death.

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Phoma stem canker on oilseed rape cultivars with the resistance gene *Rlm7* in the UK

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Phoma stem canker, caused by the related pathogens *Leptosphaeria maculans* and *L. biglobosa*, is an economically important disease of oilseed rape (*Brassica napus*) worldwide. There is a 'gene-for-gene' interaction between *L. maculans* and *B. napus* at the phoma leaf spot stage. The resistance gene *Rlm7* has been widely deployed in new oilseed rape cultivars across Europe and it has been suggested that it is more durable than other *R* genes commercially available. Monitoring the frequency of avirulent and virulent isolates in pathogen populations can be important for predicting increases in virulence and managing the risk of severe disease epidemics.

Field experiments were done with cultivars carrying the *Rlm7* gene and a cultivar with no known *R* genes (Drakkar) at eight sites in the UK in the 2011/2012 and 2012/2013 and three sites in the 2013/2014 cropping seasons. Severity of phoma leaf spotting caused by *L. maculans* or *L. biglobosa* was assessed in autumn/winter and severity of phoma stem canker was assessed in summer before harvest. There were differences in severity of phoma leaf spotting on *Rlm7* cultivars between seasons. Single pycnidial isolates were obtained from leaf lesions and pathogen identification was done by visual observation on PDA. *L. maculans* isolates from Drakkar and *Rlm7* cultivars were used to investigate the frequencies of the *AvrLm2*, *AvrLm3*, *AvrLm4* and *AvrLm7* alleles at different sites in the UK by inoculation on cotyledons of cultivars with the corresponding *Rlm* genes. Only a small proportion of isolates obtained in 2012/2013 were virulent against *Rlm7* and they were all found to be avirulent against *Rlm3*.

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Evolution of the frequency of the *AvrLm7* allele of *Leptosphaeria maculans* in France under selection pressure: a 15-years survey

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Leptosphaeria maculans is responsible for the stem canker, a major disease of oilseed rape (*Brassica napus*). Specific resistance genes are used in commercial varieties to control the disease and the efficiency of a given resistance gene is a function of the frequency of the corresponding avirulence allele in field populations of the pathogen. After the release of the resistance gene *Rlm1* in the 90's, a very rapid shift in the frequency of virulent isolates toward this gene was observed. More recently in France, a new resistance gene, *Rlm7*, was introduced in commercial varieties, at a time when most (>99.5%) of the isolates possessed the avirulent allele *AvrLm7*. Since 2000, the frequency of virulent isolates was monitored in populations of *L. maculans* in either experimental fields with increased selection pressure, or at a national scale in more standard agronomic situations. While a rapid increase of virulent isolates was observed in an experimental field located at Grignon combining superficial tillage and lack of crop sequence (36% of virulent isolates after 3 years), the overwhelming of the *Rlm7* gene appeared much less rapid than that found previously for *Rlm1* in the surrounding fields, or at the national level (less than 20% of virulent isolates after 10 years of *Rlm7* cultivation). The possible reasons for this differential behaviour will be discussed.

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Towards unraveling the function of *Leptosphaeria maculans* avirulence effector *AvrLm4-7*

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Plant pathogens secret effector proteins to manipulate host defence system and facilitate colonization. Comparing to bacteria, the functions of fungal effectors are less explored. At least eleven effectors of *Leptosphaeria maculans* that are recognized by *Brassica napus* resistance genes have been genetically mapped and three of them cloned. One of the cloned genes is *AvrLm4-7*, which is recognized by Rlm4 and Rlm7 counterparts in plant. It is evident that AvrLm4-7 is closely linked with fungus virulence since an absence of the functional *AvrLm4-7* allele results in decreased aggressiveness on its host.

We focused on the possible biological function of *AvrLm4-7* in the interaction with *B. napus*, namely signalling events in infected tissues, manipulation with reactive oxygen species (ROS) and senescence establishment. Using RT-qPCR we examined the level of transcription of previously characterized marker genes of salicylic acid (SA), jasmonic acid, abscisic acid and ethylene signalling pathways in a susceptible cultivar of *B. napus* inoculated with *L. maculans* isolates carrying or lacking *AvrLm4-7*. Our results indicate that the effector *AvrLm4-7* suppresses SA and ethylene signalling and accumulation of ROS in infected tissues. In addition the establishment of senescence seems to be postponed in comparison with the isolate lacking *AvrLm4-7* allele.

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A Host-Pathogen Interaction Paradigm: can a grower change the pattern of rapid adaptation of new races of *Leptosphaeria maculans* to Canadian canola in western Canada?

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Blackleg caused by *Leptosphaeria maculans* is the most destructive disease affecting Canola (*Brassica napus* L.). Genetic resistance has proven to be an effective means of disease control in western Canada. However, this pathogen is now a growing concern affecting the Canadian Canola industry and has appeared on cultivars rated as resistant. Tighter rotations, low cultivar diversity, and an excess of 20 million acres has led to emergence of new virulent races. No single resistance gene can remain effective against dispensable avirulence genes and a changing pathogen population. Information on genetic variability in the population is essential in developing an effective control strategy. We sampled isolates of *L. maculans* in 2010 and 2011 across Alberta, Saskatchewan, and Manitoba. Race structure was assessed by differentials and/or PCR on avirulence alleles. Certain alleles were more prevalent in the pathogen population with *AvrLm6* and *Avrlm7* present in >90% of isolates and *AvrLm3, Avrlm9*, and *AvrLepR2* present in <10% of isolates. The latter 3 alleles have changed to the virulent forms affecting the majority of the Canadian canola cultivars which carry the corresponding Rlm genes (i.e. *Rlm3, Rlm2*). However, some loci differed greatly across geographic locations. For example, *AvrLm2* ranged from 86% in Vegreville, Alberta to 38% in Plum Coulee, Manitoba. Selection pressure from different race-specific resistance genes in commercial canola cultivars (i.e. *Rlm3*) is postulated as the most significant factor influencing the variation in the emergence of 'newer' virulence forms observed. The presentation will address some of the innovative measures undertaken by researchers, growers and the canola industry under the Growing Forward 2 Funding Program to address this change in race structure and mitigate blackleg spread across the Prairie Provinces.

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Managing blackleg of canola in western Canada – "new" strategies against an old disease

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Blackleg disease of canola (*Brassica napus* L.), caused by the fungus *Leptosphaeria maculans* (Desmaz.) Ces. & de Not, was controlled successfully for years in western Canada due largely to use of resistant cultivars and 3- to 4-year crop rotations. This disease has been on the rise in recent years with severe cases reported on some of the R- or MR-rated canola cultivars. To better understand the disease dynamics and minimize the risk associated with potential breakdown of host resistance, studies were conducted to identify specific resistance (*R*) genes in Canadian canola cultivars or germplasm, determine the frequency and distribution of *L. maculans* avirulence (*Avr*) alleles in the pathogen population, and assess the efficacy of fungicides and their application timing for blackleg management. Only a limited number of *R* genes were identified in Canadian canola cultivars/germplasm, with *Rlm3* (65%) and *Rlm1* (10%) being most common. Other *R* genes were rarely identified. Based on the analysis of *L. maculans* isolates collected from trap plots across the Canadian prairies in 2012 on a differential set of host genotypes carrying known *R* genes, very few isolates (<5%) carried the *AvrLm3* or *AvrLm1* allele in most regions. This indicates that *Rlm3* and *Rlm1* are ineffective. The *Avr* profile was also analyzed for pathogen population isolated from R-rated cultivars in commercial fields that showed severe blackleg infection in 2012; *AvrLm1*, *AvrLm3* and *AvrLm9* alleles were generally missing in these fields. Early application of a strobilurin fungicide at the 2–4 leaf stage reduced blackleg severity on cultivars with and without resistance to *L. maculans*, but the yield benefit would only be realized on a susceptible cultivar under high disease potential conditions.

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Leptosphaeria maculans in winter oilseed rape: distribution of different races in Germany and efficacy of monogenic resistance genes

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The occurrence of virulent races of *Leptosphaeria maculans* (LM), the causal agent of blackleg disease is a major threat to oilseed rape production world-wide. To identify races of LM in Germany, we collected leaves with typical Phoma lesions from a cultivar harboring no known major gene (NK-Bravour) and from a cultivar harboring the major gene *Rlm7* (Exocet) on a west to east and a north to south transect from 2011 to 2014. Single spore isolates from NK-Bravour were tested on a differential set consisting of 10 OSR genotypes with known major resistance genes for their virulence on *Rlm1, 2, 3, 4, 7, 9* and *LepR1, 2 and 3* in a cotyledon inoculation test. Exocet was cultivated to screen for *Rlm7* virulent isolates in local populations, which was proofed in the same cotyledon inoculation test. The frequency of virulent isolates on *Rlm1, 2, 3, 4* and *9* was very high with over 80%, whereas frequency of virulent isolates on *Rlm7* was very low (<5%). We assume that choice of cultivars with different compliment of resistance genes leads to a different spectrum of virulent isolates per region. Furthermore we tested the efficacy of major resistance genes against LM under varying temperatures for cotyledons and stems. Therefore, the resistant cultivars Caiman with *Rlm7* resistance and Uluru with *LepR3* resistance as well as Lirabon as susceptible control were used. Major gene resistance in cotyledons remained stable under rising temperature; only an increase of LM DNA was found for cotyledons of Caiman at higher temperatures (≥27°C). Major gene resistance could actively reduce disease severity in stem tissue. Especially Caiman was strongly dependent on its *Rlm7* resistance gene, whereas resistance of Uluru relied more on quantitative resistance. High temperature treatment did not change incompatibility into compatibility at stem bases.

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Ten years of system for forecasting disease epidemics (SPEC) in Poland

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According to European Union Directive (Directive 2009/128/EC), Member States were obliged to introduce from 1 January 2014 the principles of integrated pest management, which emphasized a healthy crop with the least possible disruption to agro-ecosystems and encouraged natural ways to fight diseases and pests. If the natural methods do not sufficiently protect crops, the basis for decisions on the execution of chemical plant protection treatments should be based on monitoring of harmful organisms using decision support systems. In Poland a network of 9–10 volumetric spore samplers located in different geographical locations has been constantly operating since autumn 2004. The monitoring is known as the System for Forecasting Disease Epidemics (*in Polish:* System Prognozowania Epidemii Chorob, SPEC). From the very start, the monitoring of airborne ascospores has been focused on *Leptosphaeria maculans* and *L. biglobosa*, two pathogens of oilseed rape, responsible for economic losses due to blackleg or stem canker. The communications about the concentration of the inoculum in air samples are immediately passed to farmers, using the website and SMS text messages sent to registered users. In Poland the communications of the SPEC decision support system are being sent using mobile nets to 3 thousand registered users, four times per season. The educational website (www.spec.edu.pl) offering scientific descriptions is visited by *ca*. 4 thousand website users per year and the commercial website (www.dupont.pl, SPEC sub-site) offering a complex information service as well as advice is visited by nearly 10 thousand users each year. The numbers show high interest in the use of aerobiological data in helping to undertake decisions in plant protection against the most serious diseases of agricultural crops. The methods can be easily implemented to other pathogens and geographical regions, as already demonstrated for the inoculum of *Pyrenopeziza brassicae, Alternaria* spp. and *Fusarium* spp.

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Potential spread of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape crops in China

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In China, the incidence of phoma stem canker observed in pre-harvest surveys from 2005 to 2012 was greater on winter oilseed rape in provinces in central China (in May) than on spring oilseed rape in north China (in August). In all cases when the causal pathogen was isolated from stem cankers, it was identified as *Leptosphaeria biglobosa* by morphology in culture and/or by species-specific polymerase chain reaction. Both *L. biglobosa* and *L. maculans* were detected on crop debris and seed in shipments of oilseed rape seed imported into China through Shanghai or Wuhan ports in 2009–2011. Descriptions of the observed spread of *L. maculans* into areas previously colonised by *L. biglobosa* across a spring oilseed rape growing region (Alberta, Canada, westwards, 1984–1998) and across a winter oilseed rape growing region (Poland, eastwards, 1984–2004) were used to estimate the potential westward spread of *L. maculans* in China across spring oilseed rape growing regions (north China) and winter oilseed rape growing regions (central China, generally provinces along the Yangtze River), respectively. The rates of spread were estimated as 47 km per year across spring oilseed rape in north China and 70 km per year across winter oilseed rape in central China. Dispersal modelling suggested that the rate of spread of *L. maculans* across Alberta, Canada (c. 17 km per year) could be explained by wind-borne dispersal of ascospores.

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Understanding the importance of *Leptosphaeria biglobosa* as cause of phoma stem canker epidemics on winter oilseed rape in the United Kingdom

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Phoma stem canker is a major disease problem on oilseed rape (*Brassica napus*) in the UK, causing losses worth £100M p.a., despite use of fungicides. The disease is caused by two closely related species, *Leptosphaeria maculans* and *L. biglobosa,* which co-exist on their host oilseed rape. *L. maculans* is generally considered more damaging, causing stem base canker; *L. biglobosa* is less damaging, causing upper stem lesions. Therefore, previous work has mainly focused on *L. maculans* and there has been little work on *L. biglobosa*. This work investigated the contribution of *L. biglobosa* to stem canker epidemics by comparing the amounts of DNA of *L. maculans* and *L. biglobosa* in diseased stems. Diseased upper stem and stem base samples were collected from nine oilseed rape cultivars in 2010/2011 and 2011/2012 field experiments in Hertfordshire, UK. The abundance of *L. maculans* and *L. biglobosa* in each stem sample was assessed by quantitative PCR. In 2010/2011, the amounts of *L. biglobosa* DNA were greater than those of *L. maculans* DNA in upper stem samples but were similar to those of *L. maculans* DNA in stem base samples. While in 2011/2012, the amounts of *L. biglobosa* DNA were greater than those of *L. maculans* DNA in both upper stem and stem base samples. These results suggest that the severe upper stem lesions and stem base cankers in the 2011/2012 season were mainly caused by *L. biglobosa*, suggesting that *L. biglobosa* can sometimes cause considerable yield loss in the UK. The frequency and quantity of *L. biglobosa* detected in 2011/2012 were greater than those in the 2010/2011 growing season. The differences between these two seasons may have been caused by differences in composition of the ascospore inoculum. For effective control of phoma stem canker, there is a need to understand epidemics caused by both *L. maculans* and *L. biglobosa*.

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Loop-mediated isothermal amplification (LAMP) is a speedy molecular tool to study *Leptosphaeria* spp. populations in air and plant samples

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LAMP technique is a useful tool for the detection and identification of harmful microorganisms, including plant pathogens. In this work the LAMP technique was used to study the population of the genus *Leptosphaeria*, the cause of phoma leaf spotting and stem canker of oilseed rape. The technique allowed to detect pycnidiospores and ascospores of *L. maculans* and *L. biglobosa* from samples prepared in the laboratory, as well as samples obtained using volumetric traps designed by Hirst. The results obtained with the LAMP technique were very similar to those obtained using the high-resolution but considerably more expensive method of Real-time PCR. Currently, we are using the LAMP technique for routine studies of the population of *Leptosphaeria* spp. in oilseed rape plants. Recent studies of the composition of *Leptosphaeria* spp. on leaves of oilseed rape showed a higher proportion of *L. maculans*, than *L. biglobosa*, as compared to the situation observed ten years ago. Recently we also use LAMP technique to search for the isolates of *L. maculans* that break the resistance of oilseed rape varieties with *Rlm7* – the gene controlling the resistance of oilseed rape to the current population of *L. maculans* in Poland and Germany.

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Occurrence, spread and management of clubroot on canola (*Brassica napus*) in Canada

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The obligate parasite *Plasmodiophora brassicae* causes clubroot of crucifers. Over the past decade, clubroot has emerged as a major constraint to canola (*Brassica napus*) production in some regions of central Alberta, Canada. The number of fields with confirmed *P. brassicae* infestations in Alberta has increased steadily from 12 in 2003 to nearly 1,500 in 2013. A few cases of clubroot on canola also have been reported recently in other western Canadian provinces, including Saskatchewan and Manitoba. The development of PCR-based technologies to detect and quantify *P. brassicae* has facilitated studies of pathogen biology and spread. While the movement of *P. brassicae* infested soil on farm and other machinery appears to be the main mechanism of dissemination, these PCR-based studies have revealed that wind erosion of infested soil also may represent a secondary mechanism of spread. Nonetheless, the management of clubroot on canola had been primarily focused on sanitization of field equipment and long rotations out of susceptible crops. The release of *P. brassicae*-resistant canola hybrids by several seed companies beginning in 2009–10 enabled improved management of clubroot through the cropping of resistant cultivars. These cultivars have strong resistance to the predominant pathotypes of *P. brassicae* found in Canada, while exhibiting good yields and other desirable agronomic characteristics. However, the recent identification of isolated *P. brassicae* populations with novel virulence phenotypes capable of overcoming at least some sources of resistance highlights the variable nature and adaptability of the pathogen, and the resulting need for good resistance stewardship and integration of multiple products and practices for successful management of clubroot on canola.

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The *Plasmodiophora brassicae* genome and transcriptome

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Plasmodiophora brassicae is the casual agent of the club root disease of the *Brassicaceae*, one of the most damaging diseases within this plant family. It is belongs to the Plasmodiophorids, obligate plant pathogens in the eukaryotic protist group of Rhizaria. This group contains important agricultural pathogens such as *Polymyxa betae* and *Spongospora subterranea*. Despite their agricultural importance, the biology of Plasmodiophorids is poorly understood. Due to their obligate biotrophic nature no genome sequence of Plasmodiophorids has been obtained so far. We succeeded obtaining the whole genome sequence from a *P. brassicae* single spore isolate. Transcriptome data from *P. brassicae* infected host plants and specific life stages, and from *S. subterranea* infected potatoes were obtained and analysed. The assembled genome has a length of 24 Mb, including 8601 gene models with transcriptional evidence. The genome is GC rich and low in repeats and transposons. The *P. brassicae* shows similarities to other eukaryotic biotrophic plant pathogens like the reduction of some metabolic pathways. Details of *P. brassicae*, effector candidates and life stage specific *P. brassicae* transcripts will be presented. The sequence of our *P. brassicae* single spore isolate will be substantial as a reference to determine differences to other *P. brassicae* races and for improving the understanding of Plasmodiophorid plant pathogens.

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Plant hormone metabolism by the clubroot pathogen *Plasmodiophora brassicae*

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The clubroot disease of Brassica species, among them *Arabidopsis thaliana*, is caused by the obligate biotrophic protist *Plasmodiophora brassicae*. The disease causes huge losses among brassica crops such as oilseed rape and cabbages, so to understand how the disease is regulated is of enormous interest. After colonization of the host, the roots are transformed into large gall like structures which gave the disease its name – clubroot. Plant hormones are important signals during the infection and colonization of plants by *P. brassicae*. Auxins and cytokinins act in the regulation of cell elongation and cell division to create the space in the host tissue that is needed for the propagation of the pathogen. Moreover cytokinins are involved in the generation of a metabolic sink to ensure the nutrition of the pathogen. Salicylic acid ant its methyl ester play a very different role and act in plant defense. The finely tuned regulation of these hormone levels seems to be the key for the efficient colonization of the host plant. Until now only one protein from *P. brassicae* is functionally characterized. Surprisingly, *P. brassicae* possesses genes with homology to plant hormone metabolism, but the ability of the pathogen itself to modulate plant hormone levels is not not understood so far. The corresponding proteins show homologies to auxin conjugate synthetases, tRNA isopentenyltransferases and methyltransferases. Specifically, we have cloned a GH3-like and a methyltransferase gene from the protist and characterized the corresponding proteins on the biochemical level. A possible in planta role for these enzymes will be discussed.

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The role of cytokinins in clubroot disease

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Plasmodiophora brassicae, the causative agent of clubroot disease, causes large galls to form on the roots and hypocotyls of its Brassica hosts. We have used the model system *Arabidopsis thaliana* to explore the mechanisms that drive gall formation in clubroot-infected plants. Recently we have demonstrated that in *A. thaliana,* gall formation occurs as a consequence of pathogen-induced reprogramming of host vascular cambium (VC) activity, the meristematic region that causes secondary thickening in roots and hypocotyls. Infection stimulates VC activity and alters the fate of cells derived from it. The development and activity of the VC is strongly influenced by the plant growth regulator cytokinin (CK). In mutants of *A. thaliana* where host CK biosynthesis is largely abolished, the VC fails to form. These mutants can still be infected by *P. brassicae* but large galls fail to develop and pathogen development is slowed. Measurements of host cytokinin-responsive gene expression in these mutants supports the view that *P. brassicae* can synthesise some CKs but in insufficient quantities to restore VC development. An analysis of the recently sequenced *P. brassicae* genome and RNAseq of infected tissue has identified candidate genes for *P. brassicae* CK biosynthesis that we are currently characterising. Our transcriptomic analysis of wild type plants shows that the expression of host CK biosynthesis and response genes declines at the onset of gall formation, coupled with a marked reduction in the CK content of infected tissue. We are exploring how CK homeostasis is regulated during infection and the interplay between host and pathogen-derived CKs using plants with mutations in CK receptors, biosynthesis and signalling pathways. As CKs can also act as triggers of plant defence, we are examining the interplay between susceptibility and defence and how this can be modified to restrict the damaging impact of gall formation on the host plant.

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Deciphering the mechanism leading to shifts in cell proliferation/differentiation balance accompanying clubroot infection

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Due to the fact that plants are sessile organisms, mechanisms coordinating balance between cell divisions and differentiation are crucial for adaptation to changing environments. The mechanisms responsible for the developmental plasticity of plants are frequently exploited by pathogens in order to modify the host and create a suitable environment for the pathogen needs. The subject of our study is reprograming of the host plant by *Plasmodiophora brassicae* – a soil-borne obligate pathogen infecting members of the *brassicaceae* family. Infection by this pathogen leads to increased cell proliferation within roots and hypocotyls causing development of galls (hence its common name, "clubroot"). The aim of our studies was a detailed understanding of the impact of *P. brassicae* on cell cycle progression in the hypocotyl of Arabidopsis plants. Microarray analysis and qRTPCR shows that transcripts of numerous core cell-cycle genes responsible for the G1-S and G2-M progression are up-regulated by the pathogen. At the same time transcripts of factors negatively regulating the cell cycle progression are decreased. Some factors in this group are also involved in a regulation of the shift between cell proliferation and cell differentiation thus influencing cell fate. Observed changes within the cell cycle have further implications on the activity of genes involved in direct regulation of phloem formation. Based on our data we suggest that the pathogen-driven reprogramming of the cell cycle progression may lead to anatomical changes influencing the overall distribution of soluble sugars.

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Mapping clubroot resistance genes in various sources of *Brassica rapa* and introducing these mapped genes into canola

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We used various *B. rapa* clubroot resistance sources including five F1 hybrids of Chinese cabbage and four turnip accessions of the European clubroot differential set to perform mapping and functional analysis of clubroot resistance genes. We mapped all clubroot resistance loci from all nine different *B. rapa* materials and all these clubroot resistance loci can be assigned precisely into three chromosome regions. All clubroot resistance loci were individually introduced into the same genetic background of a totally susceptible *B. rapa* to produce near-iso genic lines. Based on the indoor testing using Canadian clubroot isolates, all mapped clubroot resistance loci are effective to Canadian clubroot isolates, showing high levels of clubroot resistance. However, some clubroot resistance loci showed susceptible to partially resistant in China when a set of near iso-genic lines were tested under field conditions, suggesting that the Chinese clubroot isolates were more virulent, suggesting that some mapped clubroot resistance loci might be different while others might be the same loci and contain the same or similar clubroot resistance genes. Some of these mapped clubroot genes have been introduced into canola after crossing and backcrossing three times and eventually all these mapped clubroot resistance genes will be transferred into canola. Using known gene locations and closely linked molecular markers, multiple clubroot resistance loci will be pyramided into the same canola lines through molecular marker assisted selection. Meanwhile, candidate clubroot resistance genes are being tested through plant transformation.

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Understanding the mechanism of clubroot resistance gene *Rpb1* based on transcriptome, metabolome and fourier transform infrared (FT-IR) analyses

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Clubroot, caused by *Plasmodiophora brassicae* Woronin, is a serious threat to canola production in western Canada. Host resistance is a cornerstone to clubroot management and understanding resistance mechanisms is the key to optimal use of clubroot resistance (CR) genes for durable resistance. *Rpb1* is a CR gene identified recently from *Brassica rapa*. Transcriptomic analysis based on RNA sequencing identified several genes related to host defense responses, especially for signaling and metabolism of jasmonate/ethylene and pathogen-induced callose deposition, were up-regulated substantially in resistant (R) canola plants carrying *Rpb1* following *P. brassicae* infection. The possible role of jasmonate in *Rpb1*-mediated resistance was further supported by its increase in R plants upon infection, as identified in global metabolite profiling using direct-infusion Orbitrap mass spectrometry (DIMS). DIMS also identified flavonoid- and tryptophan-derived metabolites related to CR. Many of these metabolic alterations can possibly be induced via the jasmonate/ethylene pathways. In a further study, synchrotron FT-IR was used to assess differential biochemical markers in plants with *Rpb1* using ground bulk root samples. FT-IR data were analyzed using the principal components analysis (PCA) focusing on two spectral regions: 3,100–2,700 cm^{-1} (for lipids) and 1800–800 cm^{-1} (a fingerprint region for proteins, polysaccharides, and carbohydrates), respectively. Roots of R and susceptible (S) plants responded differently to infection; there was an increase in lipids, but decrease in polysaccharides and carbohydrates in inoculated R samples relative to inoculated S samples. This result echoes the RNA-sequencing data which identified several lipid compounds playing a role in clubroot resistance. In contrast, a decrease in lipids but increase in polysaccharides, carbohydrates and proteins were observed in inoculated S samples relative to non-inoculated control. Changes in proteins between inoculated R and S samples were insignificant.

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Transcriptome analyses of *Brassica napus* roots after infection with *Plasmodiophora brassicae* Woronin indicate differential dynamics of gene expression in resistant and susceptible lines

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Plasmodiophora brassicae Woronin causes clubroot disease in rapeseed *Brassica napus* L. and other Brassica crops. Understanding of the dynamics of root transcriptome is an important step for developing genetic strategies for combating the disease in a sustainable manner. We have determined, by large-scale RNA-Seq analyses, the root transcriptome at the primary and secondary stage of pathogenesis and also near the end-stage in disease development in Pathotype 3- infected *B. napus*. Clubroot-resistant and susceptible parental lines and doubled-haploids (DH) from F1 of a genetic cross of the two parents were investigated in this study. The transcriptomes of the parents and DH pools representing resistant and susceptible types were analyzed. At the primary infection stage, the genes pertinent to secondary metabolism, PR proteins and hormone regulation were induced. Later in infection, significant changes in the transcript levels of pathways affecting carbohydrate metabolism, auxin metabolism, oxidative stress, PR proteins, jasmonic acid (JA) and salicylic acid (SA) metabolism, and signaling pathways were evident. The gene expression dynamics in the resistant parent and the DH pool of resistant lines were dramatically different when compared with the susceptible parent and DH pool. Much stronger induction of genes pertaining to PR proteins, JA, SA and ethylene (ET) hormones, ubiquitin-assisted proteolysis, signaling, secondary metabolism as well as cell wall lignification genes was the main feature in the resistant lines, suggesting connectivity among these pathways in terms of resistance response. In general, a substantially greater number of genes (5–6 times more) were differentially expressed in the susceptible lines than in the resistant lines during the infection processes. Upregulation of hexose synthesis and transport, indicating coopting of host metabolism by the pathogen, and downregulation of auxin biosynthesis and resistance signaling were found.

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DNA-based soil test reveal clubroot as an emerging threat in winter oilseed rape in south Sweden

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One of the greatest challenges in Swedish oil seed rape production is now to manage *Plasmodophora brassice*, the organism causing clubroot, developed in Brassica crops during centuries. Dissemination of clubroot is increasing in winter oilseed rape districts in south Sweden where the crop has been grown successfully since the introduction in the early 1940´s. The real-time, quantiative PCR method recently developed is now offered by commercial laboratories enabling growers to get soil samples quickly analysed for the amount of *P. brassicae* inoculum. In connection with sampling farm fields for chemical soil data a soil analysis for clubroot was performed in 45 samples from 18 different farms in south west Scania. The results reveal an alarming dissemination as *P. brassicae* was detected at 10 of the farms and in 60% of the fields. A high level (>325 000 gene copies per gram of soil) was found in 14% of the fields whereas a moderate level (50,000–325,000 gene copies per gram of soil) of *P. brassicae* DNA was found in 33% of the fields. Besides, several fields are reported with overwintering injuries. Resistant cultivars are now available on the market, and field tests are at present performed in fields with different inoculum levels. Data for interpreting soil tests are currently based on results from field tests of partly resistant cultivars of summer oilseed turnip rape. There is an urgent need to carefully follow the reactions of resistant cultivars of winter oilseed rape at farm level as resistant cultivars are infected to some extent, to enable a sustainable oilseed rape production.

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Plasmodiophora brassicae – status and control in oilseed rape in the UK

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A survey in the years 2007–2009 revealed that 52% of 96 oilseed rape fields sampled in the UK were infected with clubroot caused by *Plamodiophora brassicae*, when tested in bioassays of the soil. The survey work showed that the disease was present in all areas of the UK where oilseed rape was grown. These positive detections were often at sub clinical levels in the crop, implying that commercial yield losses will increase from current levels. Yield losses to clubroot in three seasons of trials were 0.3 t/ha for every 10% clubroot severity. Losses in affected crops equated to over 50% of potential yield in the most severely infected crops. Calcium carbonate (LimeX70), Calcium cyanamide (Perlka) and boron (Solubor) were evaluated for control. These soil amendments gave variable control but showed some potential as part of a clubroot management strategy. Varietal resistance remained the more effective management tool for growers, but varietal resistance was under pressure at sites where it had been heavily used in back rotations and varietal control was not effective at sites in Aberdeenshire. The varieties Mendel and Cracker, gave 50–95% disease control at three sites in the West Midlands. Control with soil amendments ranged from 0–90% but analysed over the trial series control meaned at 25% for the most effective treatment (Calcium carbonate at 8 t/ha). Soil testing for clubroot and soil pH, and lengthening rotations are important to the long term management of clubroot on UK farms, as varietal resistance and soil amendments give inconsistent results.

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New strategies for clubroot management in western Canada

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Plasmodiophora brassicae causes clubroot of crucifers and poses a threat to Canadian canola (*Brassica napus*) production. Experiments were conducted in the greenhouse to assess the effect of the soil fumigant sodium N-methyldithiocarbamate (Vapam) on primary and secondary infection, final clubroot severity, and on the growth parameters of a susceptible canola cultivar. Application of Vapam at rates of 0.4 to 1.6 mL L^{-1} soil resulted in 12–16 fold reductions in clubroot severity and primary and secondary infection. Vapam also was effective in reducing clubroot severity and improving canola seed yield under field conditions. Additional experiments were conducted to examine the effects of various cropping sequences, consisting of clubroot-susceptible *B. rapa* (C), the non-host perennial ryegrass (*Lolium perenne*) (R) and a fallow (F) treatment, on *P. brassicae* resting spore populations and clubroot severity on a subsequent susceptible *B. napus* canola crop. Both host and non-host crops reduced clubroot severity in the susceptible canola compared with the fallow treatment, but plant height and biomass were highest in the fallow. Resting spore concentrations decreased in all treatments, but were lowest for the R–C and C–R sequences compared with the fallow. Mini-plot studies also were conducted to assess the effect of crop rotation on clubroot severity and resting spore populations. In one study, three crops of susceptible canola were compared with 2-year breaks with oat–pea; barley–pea, wheat–wheat; or fallow–fallow. In another study, continuous cropping of resistant canola was compared with rotations that included 1-, 2- or 3-year breaks with barley. Rotations that included barley or oat reduced clubroot severity and increased yield compared with continuous cropping of resistant or susceptible canola. The deployment of genetically resistant cultivars combined with targetted application of Vapam may represent an effective strategy to help manage and contain clubroot of canola.

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Clubroot on oilseed rape in Poland

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In the recent few years, clubroot – caused by a protist *Plasmodiophora brassicae*, became a fast expanding pathogen of oilseed rape (*Brassica napus*) in Poland. The aim of our work was to recognise the area of its occurrence on plants of oilseed rape and in agricultural soils of Poland and to identify races of the pathogen. Samples of infected plants were collected in 2010–2013 from 190 fields of oilseed rape. The most of clubroot infections were found in Pomerania, Lower and Upper Silesia, Opole as well as Varmia and Mazuria regions, but there were no regions free from the pathogen. Agricultural soils were randomly collected from 1102 fields. Soil infestation by *P. brassicae* was studied using a soil test performed in glasshouse conditions at 20°C, using seedlings of two species of the genus *Brassica*, susceptible to pathogen infection. Eight weeks after sowing the plants were individually removed from the soil and they were inspected for the presence of clubs on roots. The sample was regarded as free from serious occurrence of the pathogen when plant roots had no clubs. This methodical survey of soil infestation revealed the severe occurrence of the patogen in 84 out of 294 monitored counties (28.6%). The pathogen was found in all main growing areas of oilseed rape, as well as in regions with less intensive cultivation of this crop, such as Carpathian Foothills or Podlasie province. Moreover, it was also found in Great Poland – the most dry region of the country, with an average of 450–500 mm rainfall per year. The most prevailing races of the pathogen were P1 and P3, but P2, P4 and P5 races were also detected in some locations. Testing performed with the use of these races on plantlets of the cultivar Mendel, with one dominant and two recessive resistance genes, allowed further splitting of these races to forms not infective (A) and infective (B) to *P. brassicae*. Until now we have detected nine ‚new' races: A and B forms of P1–P4, as well as P5A race.

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Seed health testing: TESTA project – aims and prospects

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The name "TESTA" refers to the EU 7th Framework Programme collaborative project "Seed health: development of seed treatment methods, evidence for seed transmission and assessment of seed health". A wide range of diseases and pests are carried by seed and as well as spreading and increasing old problems, new problems may be introduced into the European Community via this route. The TESTA project will develop a range of novel methods to underpin the control of these diseases, including faster, more accurate methods to assess the mode of seed transmission, economic and practical sampling approaches for the detection of pathogens present in low levels in large seed lots, novel and efficient generic detection methodologies, non-destructive testing methods and improved, effective and sustainable disinfection procedures. The TESTA consortium comprises experienced researchers, representatives of European and Mediterranean Plant Protection Organisation (EPPO), International Seed Testing Association (ISTA) Seed Health Committee and International Seed Federation International Seed Health Initiative (ISF-ISHI) working groups as well as seed testing laboratories and SMEs involved in seed production. The TESTA project consists of seven work packages namely, WP1 – Seed transmission of plant pathogens; WP2 – Sampling strategies; WP3 – Generic platform-improved detection/diagnostics; WP4 – Disinfection methods; WP5 – Validation of detection methods; WP6 – Dissemination; WP7 – Management. These work packages aim to 1) enhance knowledge of the biology of seed transmission, both by developing novel methods based on labelled microorganisms and through extensive field trials; 2) establish a comprehensive web-based database as a global resource, detailing all known pests and diseases of crop plants transmitted by seed; 3) develop novel methods for assessing levels of seed transmission and their relevance to disease levels in crops; 4) improve sampling strategies and methodologies for seed lots, in order to generate representative samples for laboratory testing; 5) establish generic platforms for seed testing methods, together with the assessment of innovative protocols, possibly using non-destructive methodologies; 6) develop novel disinfection/sanitation procedures for seed, and assessing their proficiency; and 7) disseminate results to National policy stakeholders, testing laboratories and inspection services. Progress made in the project to date will be presented.

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Can a low level of *Clavibacter michiganensis* subsp. *michiganensis* infestation in a tomato seed lot give rise to a large number of infected seedlings in the nursery?

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Infected seedlings are considered to be one of the most important primary sources of inoculum of *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), the causal agent of bacterial canker and wilt in commercial production plots. In the current study we tested the hypothesis that *Cmm* epidemics may develop in nurseries. We first examined the possibility that *Cmm* cells are dispersed spatially from source-point infected seedlings (seedlings that are systemically infected by the pathogen, such as those emerging from infested seed) and colonize the leaf surfaces of surrounding seedlings. It was found that the pathogen was dispersed to distances of 65–75 cm from the point-source seedlings. A sub-irrigation system reduced, but did not prevent, *Cmm* dispersal. Later, we examined the possibility that ambient conditions in tomato nurseries in Israel may facilitate infection of seedlings colonized by epiphytic *Cmm* populations. It was found that infections occurred under a wide range of conditions. These included a short (*ca.* 10 min) foliar-wetness period prior to inoculation. A model based on the experimental data was used to calculate the significance of measures aimed at reducing the incidence of seedling infection. Interpretation of the results of the model suggests that the benefits gained from minimizing the spatial dissemination of the pathogen from source-point seedlings, exceed the benefits gained from reducing the seed infestation level. Based on the experimental results we conclude that the working hypothesis cannot be rejected; thus low level of infested seeds may give rise to a large number of infected seedlings in the nursery.

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The effect of selected essential oils on germination, vigour and health of carrot (*Daucus carota* L.) seeds

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Essential oils have potential as natural treatments for the control of seed-borne fungi. The aim of the study was to investigate the effect of volatile compounds of fir (*Abies alba* Mill.), pine (*Pinus sylvestris* L.) and thyme (*Thymus vulgaris* L.) essential oils on germination, vigour and health of carrot seeds. For treatment 1 g of carrot seeds cv. Perfekcja was placed in 50 cm^3 glass container. Next the small pieces of filter paper (1 cm^2) imbibed with 10 or 20 μl of each oil were placed 2 cm above the seed surface. The sealed containers were kept at 20°C in darkness for 6, 12, 24, 48 and 72 h. Control were untreated seeds. Germination, vigour and health tests were performed for treated and untreated seeds. To determine seed germination the total percentage of germinating seeds, germination at I^{st} and II^{nd} count, the percentages of abnormal seedlings and dead seeds were evaluated. Parameters T_{10} and T_{50} describing the speed of seed germination were calculated to determine seed vigour. Moreover, the deep-freeze blotter test was applied for seed health evaluation and the percentage of seeds infested with individual fungi was determined. None of the treatments affected the total percentage of germinating seeds. Treating seeds with essential oils did not improve their germination at II^{nd} count. Fir oil applied at the amount of 10 and 20 μl for 24 h and thyme oil used at the amount of 10 μl for 24 and 48 h improved T_{10} parameter. Treating seeds with thyme oil at the amount of 10 μl for 12 h reduced the incidence of *Alternaria alternata, A. radicina* and *Fusarium* spp. and did not detoriorate seed germination at I^{st} and II^{nd} count. The application of fir oil at the amount of 10 μl for 72 h decreased seed infestation with *Alternaria dauci*.

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The effect of hydrogen peroxide and organic acids on germination, vigour and health of onion (*Allium cepa* L.) seeds

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Natural origin compounds are possible alternative for chemical control in organic farming. The aim of the study was to evaluate the effect of hydrogen peroxide and organic acids on the quality of onion seeds (cv. Octavia and cv. Sochaczewska). The seeds were treated with 3% solution of hydrogen peroxide and 0.25%, 0.5%, 1% and 2% solutions of acetic and lactic acids for 30 min. Controls were untreated seeds, seeds soaked in distilled water for 30 min and seeds treated with fungicide Penncozeb 80 WP. Germination, vigour and health tests were performed for treated and untreated seeds at 20°C. The total percentage of germinating seeds, germination at Ist and IInd count, the percentage of abnormal seedlings and dead seeds were evaluated. Seed vigour, expressed with T_1 and T_{50} parameters describing the speed of germination, was calculated on the base of the number of seeds germinated daily. Moreover, seed health test was carried out on potato-dextrose-agar and the percentages of seeds infested with individual fungi and seeds free from fungi were determined.

Hydrogen peroxide treatment generally had no effect on germination and vigour of onion seeds of both samples. Soaking seeds in 0.25% and 0.5% organic acids, especially lactic acid, significantly improved germination capacity at Ist and IInd count and accelerated germination of tested seeds. Hydrogen peroxide as well as organic acids, regardless of concentration, increased percentage of seeds free from fungi in both samples. Soaking seeds in 3% hydrogen peroxide and 1% and 2% acetic and lactic acids controlled growth of most of the fungi to the higher extent. However, treating seeds with acetic acid at lower concentrations and lactic acid, regardless of concentration, favoured growth of *Botrytis cinerea* on the 'Sochaczewska' seeds.

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Fusarium spp. seed-borne pathogens of horticultural crops

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Diseases caused by *Fusarium* spp. in agricultural production areas crops are common all over the world. New first reports are published every year about different *Fusarium* pathotypes (either species, *formae speciales* or races of *Fusarium*), causing disease on different horticultural plant hosts or even on new cultivars all over the world. These include edible, ornamental, trees or cereals plant species in all continents. Either in open field or under greenhouse production, disease incidence in many cases has caused serious economic losses and yield loss of 40 to 80–100%. Different control methods are used: health of the plant material, seed treatments (because it can be seed-borne), resistant cultivars or varieties, grafting, substrates (organic or inorganic, because is soil-borne), soil and substrate disinfection, etc. Despite, new *Fusarium* spp. are reported or detected. It is known that fruits and seeds can be a pathway of transmission, but it has not been proven with reported written references on seeds. To prove that seeds are the pathway is long and laborious: pathogenicity tests must be conducted on several stages with a quantity of isolates (in order to see if inoculated plants develop disease symptoms on them) and re-isolations from infected inoculated plants (roots, crown, stems, etc.), to confirm Koch's postulates and to obtain seeds with the pathogen (that has to be tested again). *Fusarium* spp. have been detected on seeds and proven to be a seed-borne in several horticultural crops: melon (*Cucumis melo*), basil (*Ocimum basilicum*). There are new reports of *Fusarium* spp. on many crops that are still not fully proven on seeds. Because it is important to prevent the primary inoculum sources of pathogenic *Fusarium* spp. and its spread, growers (for local markets and export), technical advisors, and seed producers have to know and understand the management of the crop. In many cases, depending on the region, it is important to obtain information about: pathogenic capacity (which hosts can be diseased), how the pathogen can be avoided, how to produce healthy seeds, etc. Health of the plant material for planting, seed health testing, together with solarization, biodisinfection, biofumigation, crop rotation are also discussed. Future wider efforts of research and management are needed to control the introduction of *Fusarium* through the seed and others pathways.

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Storage of cereal seeds may reduce *Fusarium/Microdochium* infection frequencies and increase germination

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Fusarium spp. and *Microdochium* spp. may cause seedling blight and poor germination of cereal seeds. Increased infection frequencies of these fungi on cereal seeds in Norway recent years have resulted in poor seed quality and shortage of certified seeds, especially of oats. However, indications of poor survival of *Fusarium* in seed and improved germination after some months of storage have been observed. A study was carried out to investigate if seed storage can contribute to improved seed quality and possibly "save" seed lots. A number of barley (N = 10), oats (N = 9) and spring wheat (N = 11) seed lots harvested September 2012 were tested for germination capacity and *Fusarium/Microdochium* infection frequencies (PDA and blotter method) a couple of weeks after harvest, and after five, 12 and 15 months of storage. In barley, the average germination percentage increased slightly, from 92% in the autumn 2012 to 95% after first five months of storage. In oats, the average germination percentage increased from 82% to 85% during the first five months, and a slight further increase (to 87%) until end of storage period (December 2013) was observed. In spring wheat, the average germination percentage was reduced during storage, from 81% in autumn 2012 to 67% after five months. In barley and oats, average *Fusarium/Microdochium* levels were reduced during storage, with the highest reduction during the first five months, from 50% in autumn 2012 to 37% after five months (barley), and from 60% in autumn 2012, to 46% after five months, and to 27% in December 2013 (oats). In spring wheat, no significant reduction in average infection level was recorded (58% in autumn 2012, 50% after 15 months of storage). It is concluded that storage of barley and oats seeds for 5 months after harvest may in some cases increase the seed quality and thereby meet the certification requirements of minimum 85% germination.

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'*Candidatus* Liberibacter solanacearum' is a carrot seedborne pathogen

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'*Candidatus* Liberibacter solanacearum' is an emerging bacterium phloem limited that cannot be cultured in vitro and associated with economically important diseases in potato, tomato, carrots, celery and other crops. The transmission of any '*Candidatus* Liberibacter' species through true seeds has not been demonstrated yet but the bacterium is transmitted in a persistent (transovarial) way by different psyllid species in several crops. The transmission of '*Ca*. L. solanacearum' by carrot seeds was suspected because yellows decline and vegetative disorders in carrot crops have been reported recently in geographically distant areas in Europe. Using validated CaLsol/100 Plant Print Diagnòstics real-time PCR kit for specific detection of '*Ca*. L. solanacearum' the bacterium was detected in 43% of the 54 seed lots analyzed. Average number of total '*Ca*. L. solanacearum' cells ranged from 4.8 to 210 cells/seed, but using propidium monoazide to target live cells, only 5% of then were viable. Liberibacter-like cells were observed in the phloem sieve tubes of the carrot seed coat and in the seedlings. The bacterium was detected after 90 days, in seedlings grown from PCR positive seed lots. After 150 days, typical symptoms were observed in 12% of seedlings of cv. Maestro. Consequently, to prevent the introduction of the bacterium and its potential spread, control of '*Ca*. L. solanacearum' in seed lots is required. The epidemiological risks of this finding are discussed.

11th Conference of the European Foundation for Plant Pathology

Poster presentations

8–13 September 2014, Kraków, Poland

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Session 1

New pathogens and shifts in pathogenicity

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Morphological and pathogenic characterization of several *Fusarium solani* f. sp. *cucurbitae* isolates obtained in Almería Province, Spain

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Phytopathological prospection was carried out to know the importance and distribution of soilborne diseases in commercial greenhouses of cucurbits in Almería province, Spain; zucchini (*Cucurbita pepo*), melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) plants showed necrosis at the base of the stem, leaf wilting and death. From a high number of plants *Fusarium* spp. was isolated and preliminary classified as *F. solani*. The objective of this study was the morphological characterization of several isolates obtained in this prospection, and the study of their pathogenicity to zucchini plants. The morphological characterization was carried out according to the methodology proposed by Leslie and Summerell (2006). Characters as the colony coloration and the size and shape of the structures formed (macroconidia, microconidia, chlamidospores and phialides) were described. The study of their pathogenicity was conducted in a polyethylene-covered plastic house located at IFAPA Centro La Mojonera (Almería). Two consecutive experiments were conducted during the spring of 2012 on 32-liter perlite bags, in a Randomized Complete Block design with four replicates. Plots consisted in three perlite bags with three plants per bag. The same number of plants served as uninoculated controls. Plants were inoculated at the two to three true-leaf stage with propagule suspensions of the isolates with an approximate concentration of $5 \cdot 10^6$ propagules/ml. Inocula (50 ml per plant) were poured around the stem of each plant. The pathogenicity was evaluated by weekly observations of disease symptoms. The morphology of the isolates was similar to the descriptions of other authors, excepting chlamidospores forming long chains and sometimes with thin walls. The isolates showed a high variability in their morphological characteristics. All isolates excepting one of them were pathogenic and very aggressive causing the death of 100% of the plants at the end of the experiment. This work has been financed with the project INIA RTA 2010-00044-00-00 co-financed with EFRD (EU).

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Molecular diversity of *Raspberry leaf blotch virus* – a new pathogen of *Rubus* sp. plants in Poland

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In 2012, in Scotland a new virus named *Raspberry leaf blotch virus* (RLBV) was detected in raspberry 'Glen Ample' cv. RLBV is transmitted by mite *Phyllocoptes gracilis*. The virus causes yellow blotching and distortion of the leaves as well as reduces quality of fruits. As the similar symptoms were observed on raspberry plantations in Poland, the study on detection of RLBV and evaluation of the Polish isolates of this virus was conducted. Total nucleic acids from the leaves from 45 raspberry and four blackberry plants and from *Phyllocoptes gracilis* individuals were isolated by absorption on silica gel (SC). Fragments of RNA1 encoding a putative RNA-dependent RNA polymerase and RNA3 encoding glycoprotein precursor and the nucleocapsid of the RLBV isolates were amplified by RT-PCR using two pairs of specific primers. RLBV was detected in 15 samples of raspberry plants and one sample collected from blackberry growing in a natural environment. Multiple alignments showed that the nucleotide sequences in the analyzed region of RNA1 of Polish RLBV isolates were 97.7–100%, similar to each other and shared 92.3–93.2% of identity with the sequence of the reference strain of this virus from Scotland (GenBank acc. no. FR823299). Comparative analysis of nucleotide sequence of RNA3 fragments showed 94.6–99.8% similarity between tested RLBV isolates and their 94.8–97.9% identity with the Scottish reference strain (GenBank acc. no. FR823301). *In silico* restriction analysis of the both amplified genome fragments confirmed variability of the Polish RLBV isolates.

The individuals of mites collected from the leaves of raspberry growing on eight plantations were tested for the presence of RLBV by RT-PCR. The virus was detected in *Phyllocoptes gracilis* samples from four locations.

This research was conducted in frame of Multiannual Program of the Research Institute of Horticulture (PW7.5, 2008–2104) financed by Ministry of Agriculture and Rural Development

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Hypertrophy of *Momordica charantia* caused by *Cuscuta japonica* parasitization

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The holostemparasitic plant *Cuscuta* parasitizes various plants and sucks nutrients from the host stem. We used *Cuscuta japonica* as the parasite and *Momordica charantia* as the host plant, and described their interaction. The parasitized *Momordica* stems started swelling as a hypertrophic response within three days after parasitization. Concurrently, the *Cuscuta* stem grew rapidly and developed bigger scale leaves than usual. Parasitized *Momordica* stems reduced photosynthetic activity. Histological observation revealed no programmed cell death but an increased number of vascular bundles in the *Momordica* stem, especially near the *Cuscuta* hyphae. The defensive response of *Momordica* mainly involved the SA pathway. Drastic increase of tZ- and DZ-type cytokinins in *Momordica* stems would play an important role for hypertrophy. Comprehensive plant hormone analysis provides new insights into plant interaction studies.

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Competition between different species of the fungal genus *Alternaria* during infection

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Early blight (alternariosis) induced by *Alternaria solani* and *A. tomatophila* is a widespread disease of potato and tomato leaves, respectively. Usually those species are accompanied by small-spored *Alternaria* species, which were described as saprotrophs or rarely as pathogens. To define the role of small-spored *Alternaria* species in infection we carried out a series of inoculations of potato leaves and tomato leaf discs. Inoculum was prepared from conidia of 12 presumably pathogenic isolates (*A. solani* and *A. tomatophila*) and 12 presumably non-pathogenic isolates (*A. tenuissima*, *A. arborescens*, and *A. alternata*). Leaves were treated by pure and mixed conidial suspensions of different concentration. The size of necrosis induced by *A. solani* and *A. tomatophila* depended on conidia concentration. Small-spored species showed no pathogenicity irrespectively of inoculum concentration. The use of mixture of conidia of small-spored and large-spored species resulted in synergetic effect. The necrotic spots were two times bigger if any amount of small-spored species conidia were added in the inoculum. Thus we show that small-spored *Alternaria* species may play a role of a secondary pathogen increasing disease severity.

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Cucurbitaria piceae Borthw. destroyed large areas of the *Picea pungens* substitute forest stands in the mountain region of northern Bohemia.

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The large areas of mountaineous Norway spruce forests were destroyed in Northern Bohemia (especially Krušné, Jizerské and Orlické hory Mts.) in the second half of 20th century by sulphur dioxide depositions. Substitute tree species stands were planted in order to maintain forests under heavily air-polluted conditions mostly in 1970s and 1980s and one of broadly used tree species planted in several thousands of hectares was exotic Colorado blue spruce (*Picea pungens* Engelm.). From the phytopathological point of view, this species was considered as a relatively unproblematic with limited number of pathogens. The situation has dramatically changed recently, when several hundreds of hectares of blue spruce stands have been heavily damaged or killed by sudden outbreak of cucurbitaria bud blight.

This disease is caused by well known and to this date relatively unimportant pathogen of spruce and fire species *Cucurbitaria piceae* Borthw. (Pleosporales: Dothideomycetes; anamorph *Megaloseptoria mirabilis* Naumov; syn. *Gemmamyces piceae* (Borthw.) Casagrande). Blackish ascomata are superficial on a basal stroma, gregarious, globose, sessile or stalked, ca 300–600 μm in diam., with a rounded ostiole ca 30–50 μm in diam. Asci are eightspored, cylindrical, 180–250 × 25–30 μm. Ascospores are pale to dark brown, spindle-like, muriform, measuring 35–50 × 12–15 μm. Aggregated pycnidia are of similar colours, shapes and dimensions as ascomata, sessile, glabrous and thick walled. Phialides on septate conidiophores are ampulliform or doliiform and 8–15 μm long. Hyaline conidia are scolecoid, septate and measuring 220–320 × 5–8 μm.

The pathogen has been detected in several European countries nearly throughout the whole continent yet, however its recent outbreak in Bohemian mountains has apparently invasive character. The transformation plans of blue spruce stands to more natural ones in the affected areas have to be dramatically changed and accelerated under the pathogen pressure.

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Rhexocercosporidium carotae as a new causal agent of carrot disease

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Carrots are one of the main vegetable species cultivated in Poland. Because of their taste and nutritional value they have a very wide culinary use. Carrots are consumed practically all year round. During the cropping season they may be infected by many fungal and bacterial pathogens. In 2012, a new disease of carrots in Poland was noted. Infection symptoms are visible as brown spots located only on the roots, gradually enlarging elliptically. Development of disease results in the infection of deeper layers of parenchyma. Oval, brown spots are a bit brighter in the middle part, which progressively deepens, creating a kind of crater as a result of the development of the pathogen in the deeper layers of the crumb. Infested carrots lose their commercial and processing value. Microscopic observations and PCR assays confirmed that disease is caused by *Rhexocercosporidium carotae*, fungus belonging to *Ascomycetes*, known also as causal agent of black spot of roots. It is a soil-borne fungus, which during the cropping season spreads by conidia. Pathogen was already reported in the 60s of 20th century in Norway, as well as in the Netherlands, Sweden and Denmark.

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Loop-mediated isothermal amplification (LAMP): a novel method of plant pathogen identification

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Loop-mediated isothermal amplification (LAMP) appears to be alternative for PCR based method in detection of pathogens. It allows to detect pathogenic microorganisms DNA in a short time and at low cost. The reaction is based on a *Bst* DNA polymerase (and their mutants like *Gsp*SSD polymerase) and can be performed at isothermal conditions. The high specificity of the LAMP reaction is reached due to primers (2 or 3 pairs) annealing in eight different regions of the target sequence. LAMP is a very efficient method of amplification, which allows synthesis of large quantity of DNA in a short time. LAMP products can be visualized under UV light, after adding to the reaction mixture fluorescent dyes, such as calcein or GelRed. Fluorometric analysis using calcein can be analyzed directly in the tubes, which leads to faster reaction result and prevents from contamination of laboratory caused by undesirable DNA spread. Visualization of DNA products on gel electrophoresis is not required for assessing successful DNA amplification. LAMP reaction also allows rapid detection of pathogens even in the field by use of portable devices.

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Initial studies on transfering a maize pathogenic bacteria with *Diabrotica virgifera*

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Our previous studies reveald that the plant pathogenic bacteria like *Pantoea ananatis* are present in the population of bacteria colonizing an alimentary canal of *Diabrotica virigifera*. Since vectoring of bacteria via insects is a well known phenomenon and *D. virgifera* is a quarantine pest in Poland, we investigated transferring the plant pathogenic bacteria on maize plants via *D. virgifera*. Performed experiment involved a greenhouse tests in which an imagos of *D. virgifera* collected in the surroundings of Rzeszów in South East Poland were used. A pathogen free maize plants grown from the seeds in the greenhouse were also used. Pure cultures of *Pantoea ananatis*, *P. agglomerans* and *Enterobacter cloaceae* subsp. *dissolvens* were used. The insects captured in natural field conditions were incubated in the isolators containing pathogen free maize plants untill they were pathogen free, which was confirmed on a random sample of the insects. In the folowing part of the experiment the insects were divided into four groups corresponding to the three pathogens tested and the negative control and incubated in the isolators containing maize plants inoculated with each pathogen in the laboratory. A sterile distilled water was used for inoculation in the negative control. After incubation the presence of the plant pathogenic bacteria was confirmed in the alimentary canals of the tested insects. In the next part of the experiment an insects carrying the plant pathogenic bacteria were transfered to the seperate isolators containing pathogens free maize plants. After incubaction the presence of the pathogen was confirmed in the tested maize plants exhibiting the symptoms that might suggest the bacterial infection.

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Phomopsis mali Roberts – a new pathogen of fruit trees in Poland

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Studies conducted in 2009–2013 in orchards of south-eastern Poland showed that shoots of apple, pear, cherry and plum trees were inhabited by *Phomopsis* spp. Cultures of the fungus constituted from 2,5% to 23% of all isolates. Studied *Phomopsis* cultures formed on PDA medium white-gray colonies with beige and brown reverse. Conidiomata producing spores were formed with difficulty after 14–21 days of incubation. *Phomopsis* strains showed similarity in appearance and growth rate regardless of the host plant. Suitable conditions for mycelial growth was observed in the temperature range from 16°C to 25° whereas optimum temperature for sporulation was 25°C. All the tested isolates formed alpha and beta conidia with dimensions 6.3–8.6 × 1,5–3.2 μm × 19.5–45 × 1.2–3.1 μm. Results of cross-inoculation tests proved the pathogenic abilities of *Phomopsis* strains originating from apple, pear, cherry and plum shoots towards tested plant species. The analysis of RAPD products indicated 84% similarity within the studied population of *Phomopsis spp*. Nucleotide sequences of the internal transcribed spacer region (ITS1 and ITS2) of the ribosomal DNA (rDNA) of Polish strains showed 97% identity with the reference strain of *Phomopsis mali* Roberts (NBRC accession no. 31031).

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Monilinia spp. causing brown rot of pome and stone fruits in Italy

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Italy is the second producer of stone fruits and the fifth producer of apple fruits in the World. Brown rot caused by *Monilinia* spp. is a well-known pathogen affecting pome and stone fruit production worldwide. In Europe, three *Monilinia* species are present, causing serious blossom and twig blight and fruit rot: *M. laxa*, *M. fructigena* and *M. fructicola*. The aim of this study was to monitor the *Monilinia* population using multiplex PCR. For this purpose, between 2012 and 2013, a total of 136 *Monilinia* spp. isolates were obtained from infected fruits grown in different commercial orchards of stone and pome fruits, located in Emilia Romagna, Lombardy and Sardinia. The identification of the isolates was at first obtained with multiplex PCR and the results were confirmed on the basis of the colony shape, conidial dimension, germ tube and the sporulation according to the EPPO standards and the sequence analysis of the internal transcribed spacer (ITS1/5.8S rDNA/ITS2) region of ribosomial DNA.

In the assayed sample of the Italian population, *M. fructicola* was the prevalent species (38%) while *M. laxa* and *M. polystroma* were both detected in 24% of samples. The remaining isolates were identified as *M. fructigena* (14%). In the artificial inoculation of fruits, all examined *Monilinia* spp. isolates were pathogenic. To our knowledge this is the first report of *M. polystroma*

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Genetic diversity of *Monilinia* spp. causing brown rot on sour and sweet cherries in Poland

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In 2012–2013, 26 commercial orchards of sour (*Prunus cerasus*) and sweet cherries (*Prunus avium*) located in the central and north of Poland were monitored for brown rot presence. The disease was observed in all of them. From the rotted fruits, 44 isolates of *M. laxa,* 32 of *M. fructigena,* 8 of *M. fructicola* and 7 isolates of *M. polystroma* were obtained. They were identified according to the morphology of fungal cultures growing on PDA medium and on the base of multiplex PCR (Cóte et al. 2004). All obtained isolates and 25 *Monilinia* isolates originated from other host plants were submitted to genotyping using methods: PCR MP (with three restriction endonucleases), ISSR (3 different ISSR primers) and RAPD (4 different RAPD primers). Dendrograms constructed on the resulting amplification patterns showed that *Monilinia* spp. isolates were grouped according to their species classification. The results showed that these three genotyping techniques had a good discriminatory power, but the highest number of different amplification patterns was observed in RAPD. In all analyses the highest genetic heterogeneity was found among *M. fructigena* and *M. laxa* isolates, while *M. polystroma* and *M. fructicola* created more homogeneous clusters. No correlation has been found between isolates originated from cherries and other host plants, as well as between an isolate and its geographical origin or a year of isolation.

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Detection and identification of *Leek yellow stripe virus* in garlic and onion in Poland

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Leek yellow stripe virus (LYSV), genus *Potyvirus*, family *Potyviridae*, have been the most common and important virus infects a wide range of *Allium* species worldwide. LYSV is one of the several viruses that chronically infect garlic (*Allium sativum* L.) which resulted in the reduce garlic bulb weight by 25%. The aim of the study was the detection and identification of LYSV in 218 samples collected in April 2014. Fifty eight leek plants (*Allium porrum* L.), 80 onion bulbs (*Allium cepa* L.) and 80 garlic bulbs from Poland, Belgium, Egypt and Spain were examined by ELISA test. This test was carried out with extracts from leek leaf samples and onion, and garlic bulb samples to detect LYSV using commercial antiserum (DSMZ, Braunschweig, Germany). LYSV was detected only in 31 garlic and 8 onion bulbs. All leek tested plants were virus-free. ELISA positive plants were further subjected to molecular studies. Total RNAs from the infected bulb samples were extracted using the silica capture (SC) method described originally by Boom et al. (1990) and adapted to the diagnosis of plant viruses by Malinowski et al. (1997) and assayed by reverse transcription (RT)-PCR using primer pair 1-LYSV (5'-ACAAGTAAGAAACAGAAGGACAGC-3'), 2-LYSV (5'-GAGGTTCCATTTTCAATGCACCAC-3') (Parrano et al. 2012) to amplify 409 bases of partial coat protein gene. The amplified products were purified and sequenced in both directions.

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Fusarium temperatum as a new main factor of ear rot of maize in Poland

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The molecular studies conducted in 2014 were aimed at estimation frequency of *Fusarium temperatum* among selected isolates of *Fusarium* spp., collected in Głuchów and Radzików. Previous mycological analyzes (2005–2012) showed that *Fusarium subglutinans* was predominant, but recent EF1& gene analyzes showed high frequency (55 isolates) of new species – *Fusarium temperatum*. Among all tested strains just only one was identified as *Fusarium subglutinans*. The preliminary results of breeding demonstrate new look at etiology of corn cob fusariosis. Both species from *Liseola* section are morphologically very similar, and polymorphism of EF1& gene lets us recognize difference.

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The occurrence and frequency of races of *Phytophthora sojae* in Ontario during 2010–2012

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Phytophthora sojae is the causal agent of Phytophthora root (PRR), an economically important disease of soybean worldwide. To determine the occurrence and frequency of races of *P. sojae* in Ontario, where most Canadian soybean is grown, a total of 359 single-zoospore *P. sojae* isolates were obtained from plant and soil samples collected from 203 soybean fields and two PRSR nurseries during 2010–2012. Twenty-four races and two intermediate reaction types (IRT) of *P. sojae* were identified from the 359 isolates on a set of eight soybean differentials, each containing a single resistance (*Rps*) gene. Race 25 was the predominant race, representing 16.4% of the pathogen population in commercial fields. Races 3, 4, 5, 6, 7, 9, 28, and 45 were commonly detected, each of these races represented 5 to 11% of the pathogen population. Twelve races and one IRT were identified from 44 *P. sojae* isolates obtained from the PRR nursery in Ottawa, and 12 races and one IRT identified from 52 isolates from the PRR nursery in Woodslee. Races 3, 5, 6, 7, 8, 9, 14, 22, 25, and 28 were commonly detected in these nurseries. Of the 24 races, 18, including the predominant race 25, were identified for the first time in Ontario. These results suggest that the race profile of *P. sojae* in Ontario has changed and new sources of resistance are needed for the development of resistant cultivars. The common races in the two RPR nurseries were similar to what were found in commercial soybean fields, suggesting that both PRR nurseries are appropriate and effective for screening soybean germplasm for cultivar development for Ontario.

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Host shift in the fungi of the genus *Alternaria*, sect. *Porri*

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The fungal genus *Alternaria* consists of approximately 280 species. Approximately 120 *Alternaria* species are assembled in the section *Porri*. This section includes only plant pathogenic species with rather narrow host specialization. The host range of many studied species is restricted to one plant species or a few species from one genus. Host specialization does not correlate with five-gene-based phylogeny of this section that was recovered using 46 species. These species were found on plants from 15 families which belong to different orders and subclasses. In a number of cases closely related fungal species were associated with non-related plants. One species, *A. porri*, has a host from monocots when all other species were found on dicots. *Alternaria* species associated with *Asteraceae* were distributed among 7 clades in a phylogenetic tree. Species from *Solanaceae* appeared as 6 clades, *Euphorbiaceae* – 4 clades, *Passifloraceae* – 3 clades, *Convolvulaceae* – 2 clades. Species from other 10 families (*Alliaceae*, *Apiaceae*, *Cucurbitaceae*, *Fabaceae*, *Linaceae*, *Malvaceae*, *Pedaliaceae*, *Primulaceae*, *Rubiaceae*, *Rutaceae*) appeared in the tree only in one clade each. Thus the comparison of phylogeny and host specialization reveals that *Alternaria* species have mechanisms for abrupt host shift. These fungi adapted to plants from *Asteraceae*, *Solanaceae*, *Euphorbiaceae*, *Passifloraceae*, and *Convolvulaceae* several times.

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Tilletia controversa JG Kühn on winter wheat in Ukraine

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In 2013 bunt disease similar to common bunt was observed on winter wheat in different regions of Ukraine. Disease incidence in Ternopil and Khmelnytsky regions reached 10–15%. Infected plants produced high number of tillers and their length was 2–3-times shorter. Ears looked more dense and had an atypical form. Sori were spherical, with hard mass of teliospores and emitted an odor of trimethylamine. Initially ears and grain samples collected from commercial fields were examined using light microscopy. Teliospores were yellow-brown, globose, 20–24 μm in diameter, with 1.5–3 μm hyaline sheath and net-like reticulation. Nearly 7% of cells were sterile. They were colorless, smaller with smooth rarer reticulated sheath. According to morphological characteristics the pathogen was identified as *Tilletia controversa* JG Kuhn. PCR assay was used to confirm identification. We obtained the PCR product of 201 bp in length with the primer pair $CQUTCK_2$/$CQUTCK_3$ corresponding to the PR32 sequence of *T. controversa* (No DQ266258.1 in the GeneBank database according to Yuan et al., 2009) in all cases. Weather conditions in 2012–2013 (long period with deep snow cover and especially prolonged spring) favored dwarf bunt development. Taking in account the longevity of teliospores in soil, this disease sporadically can cause serious losses in winter wheat when favorable conditions occur.

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Viruses associated with alfalfa and adjacent weeds and cultivated plants in the Kingdom of Saudi Arabia

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A total of 1166 alfalfa and 182 weed and cultivated plant samples belonging to 13 different species and showing different virus-like symptoms in a two years survey starting Jan 2012 and ending Dec 2013, from five principal alfalfa-producing regions in Saudi Arabia (SA) for detection of alfalfa viruses in NPSTI project No 10-BIO 979-02. Ten viruses were detected by DAS-ELISA in alfalfa. These were AMV, BCMV, BLRV, BYMV, CMV, LTSV, PeSV, RCVMV, TSV, WCLMV and. AMV and BLRV were the most prevalent ones accounting for 58.4% and 12.5% of detection respectively. The percentage of detection for each of the 8 other viruses was less than 3%. PSV was detected in 26 out of 45 samples that were negative to all above viruses. To our knowledge this is the first report of BLRV, RCVMV, WCIMV PSV and LTSV in Saudi Arabia. These viruses were also detected in weeds and cultivated plants growing in vicinity of alfalfa fields. *Sonchus oleraceae,* which was the most prevalent weed, found to be infected with ten viruses with AMV being the most frequently detected in it. Alfalfa plants, weeds and cultivated plants were found to be multiply infected with several viruses. Selected samples that were positive to ELISA were confirmed by RT-PCR. Phylogenetic tree for two groups of 16 AMV isolates from alfalfa and 17 isolates of weeds and cultivated plants indicated 89.4–100% and 92.9–100% similarity among them for each group, respectively. Comparing these two groups with 15 AMV isolates from GenBank for each group, indicated a phylogenetic relationship that had the same range for alfalfa isolates of the virus and 91.5–100% for the weeds and cultivated crops isolates of this virus. Eight BLRV isolates detected in alfalfa in SA showed phylogenetic relationship of 99.3–100% among themselves and 93.1–100% similarity with 11 isolates from the GenBank.

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Characterization of *Colletotrichum fuscum*, a new causal agent of oregano leaf spot disease in Poland

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In 2012–2013 on plantations of oregano (*Origanum vulgare* L.) grouped in south-eastern Poland *Colletotrichum fuscum* Laub. was detected. This is the first report of the presence of *C. fuscum* in Poland. Identification of isolates was based on morphological and genetic characteristics and pathogenicity test. The morphological and genetic studies showed similarity of polish isolates of *C. fuscum* to reference isolate CBS 102 189 received from the Netherlands. Moreover, the sequence analysis of ITS regions was conducted. The pathogen was previously recorded in Japan as the causal agent of anthracnose of nemesia (*Nemesia strumosa*) and in United States as the pathogen of foxglowe (*Digitalis lanata*) (*Digitalis purpurea*). On the leaves of oregano *C. fuscum* causes necrotic, regular, concentrically zoned spots with lighter center and the slightly raised edge. The harmfulness of the disease involves premature defoliation of infected leaves of oregano which decreases the amount of herbal material.

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Genetic variability within *Septoria carvi* Syd. a new causal agent of caraway in Polish conditions

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Isolates of *S. carvi* obtained from various organs of caraway cultivated in south-east and central Poland were examined using RAPD-PCR analysis. The studies were carried out using randomly chosen primers from Genomed corporation. The DNA profiles obtained with 4 primers were useful to determination of genetic variability among the genotypes of studied isolates within studied species. A total number of 67 bands across *S. carvi* isolates were analysed. The number of DNA fragments generated using single primer ranged from 13 to 22. On average 16.75 amplicons were produced by single primer. As results of RAPD-PCR reaction 60 polymorphic products were obtained and the number of banding patterns scored for each primer ranged from 12 to 19. The average 15 banding patterns were generated by single primer. Specific products were also obtained from three starters. Totally seven specific banding patterns were obtained, their number ranged from 1 to 3 depending on the isolate. No monomorphic markers were observed. The matrix of Dice genetic similarity indicates the similarity between the isolates from 0,254 to 0,607. Additionally, the average similarity of all isolates was 0,48. Cluster analysis using UPGMA method indicated on the presence of two main groups of isolates. The first group consisted of isolates obtained from central and south-eastern part of Poland, while the second group contained mainly isolates from south-eastern part of Poland. Considering the high genetic variability of *S. carvi* isolates, the analysis of the sequence within ITS1 and ITS2 regions are carried out.

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Morphological and genetic characteristics of *Colletotrichum* spp. isolated from newly emerging fruit spot disease on apple

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In 2013, fruit spot symptoms were observed on apples in Korea. A small spot lesion is observed at the beginning of the growth period. The spot doesn't expand further, is remaining static until the harvesting season. We found that the shape and size were similar with these two types fungi (typical anthracnose symptoms and statics fruit symptoms). The conidia of the two types fungi were straight, cylindrical, with an obtuse apex. A pathogenicity of these two types was performed on immature apples (cv. Fuji) by inoculating with conidial suspensions (10^5 conidial/ml). In a typical anthracnose symptoms fungus-inoculated apple, anthracnose symptoms were progressed and also soft and sunken. However, with static symptoms fungal inoculation, there was no observed expansion in the size of static spots. Although the nucleotide sequences of the actin, chitin synthase, and β-tubulin were 100% consistent, Polymerase chain reaction (PCR) analysis by the specific primers was observed two types isolates consistent *Colletotrichum gloeosporioides*. The result of random amplified polymorphic DNA-PCR was showed clearly differentiated subgroups of *C. gloeosporioides* genotypes. The clustering of these groups was highly related to the symptom types of the individual strains.

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Session 2

Toxic metabolites of pathogens

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Development of detection assays for toxic metabolites produced by fungi

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Fungi are being developed as biological control of insect pests and plants diseases. The objective of this study was to develop a protocol to screen for mutagenic secondary metabolites produced by biocontrol fungi. In this experiment, insect biocontrol fungi *Metarhizium. anisopliae* BIPESCO 5, *Beauvaria bassiana* ARSEF 5808 and *Beauvaria brogniartii* BIPESCO2, and plant disease biocontrols, *Trichoderma harzianum* JAT1977, *Clonostachys rosea ACM941* were tested to determine if they produce any toxic secondary metaboloits. Two known toxin producing fungi, *Aspergillus flavus* and *M. robertsii* ARSEF 2575, and commercial toxin (AFB1) were used as positive controls. Concentrated extracts produced in liquid culture media by these biocontrol fungi were tested using TA98 and TA100 isolates of *Salmonella typhimurium* in the Ames MPF Penta I microplate format mutagenicity assay. In this study, an aflatoxin producing strain and a non toxin producers were compared for the production of mutagenic compounds, 4-nitroquinoline N-oxide (4-NQO), 2-aminoanthracene (2-AA) and 2-nitrofluorene (2-NF). The *S. typhimurium* TA98 detects 2-nitrofluorene (2-NF) and 2-AA while the *S. typhimurium* TA100 detects 4-NQO and 2-AA. The results from the Ames MPF Penta I assay showed that the positive control *A. flavus* produced two mutagenic metabolites, 4-NQO and 2-AA, while *M. anisopliae* BIPESCO 5 tested negative for 4-NQO and 2-AA. The negative result for the metabolite 2-NF using the *S. typhimurium* TA98 tester strain, indicates that the mutagen 2-NF was not produced by both *Metarhizium* isolates. All of the biocontrol fungi tested, in this study, failed to produce mutagenic secondary metabolites that can be detected by the sensitive MPF Penta assay, while, the low concentration of the metaboloites produced by the two toxin producers and the commercial toxin (AFB1) were detected by the assay. All the experiments were repeated at least once.

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Effect of tebuconazole on mycotoxin production ability of *Fusarium langsethiae* strains

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An increased occurrence of T-2/HT-2 toxins in small grain cereals has been reported in European part of Russia over the last decade (Gagkaeva et al., 2011). Fungicide application is one of the available methods to reduce the risk of mycotoxin contamination of harvested grain. The aim of the study was to assess the efficacy of the fungicide (active substances – tebuconazole 250 g/L) on the mycotoxin production ability of 26 *F. langsethiae* strains originated from different regions of Russia.

The fungicide was added to the potato sucrose agar (PSA) medium just before pouring to get the final concentrations of 0.1, 0.5 and 1 ppm. Every strain was cultivated on fungicide-amended and fungicide-free media for 7 days at 23°C, then the amounts of T-2 toxin and diacetoxyscirpenol (DAS) were determined by ELISA. It was revealed that in the control (PSA without tebuconazole) *F. langsethiae* strains produced T-2 toxin from 5560 to 120900 ppb and DAS from 36 to 2042 ppb.

On the highest concentration of the fungicide mycotoxins in the low amounts near the detection limits were found in the medium after growing of 15% of the strains. In case of the growth on PSA containing of the fungicide 0.1 and 0.5 ppm the amount of T-2 toxin reduced in comparing with the control by 56 and 93% and the amount of DAS reduced by 29 and 77%, respectively. Comparative analysis of the mycotoxins production ability of *F. langsethiae* from different origin has showed that the strains from the central region produced significantly higher amounts of mycotoxins and were more sensitive to tebuconazole, than strains from the southern region. These data will be useful for evaluating fungicide exposure for control of mycotoxin contamination of cereals grain.

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Concentration of *Fusarium* mycotoxins in winter wheat grain

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In 2012–2013, winter wheat (*Triticum aestivum* L.), Boomer cultivar was grown in a plot experiment conducted in Tomaszkowo (randomised block design, in three replications). The experiment included seven objects: 1. control (no fertilisation), 2. mineral fertilisation NPK (90 kg N · ha^{-1}, 70 kg · ha^{-1} P_2O_5 and 100 kg · ha^{-1} K_2O) and objects with NPK fertilisation and foliar fertilisation with micronutrients: Cu, Zn and Mn at 0.2 kg · ha^{-1} applied individually (objects 3–5) and in combination (6), and with Nano Gro vaccine (7). The intensity of ear fusariosis was estimated during the vegetation period. After harvest, the content of fusarium mycotoxins in grain was determined.

Wheat ears in the object fertilised with micronutrients in combination (2012) and ears of plants in the object with Cu fertilisation and the vaccine were the most infested by *Fusarium* fungi.

A higher content of ergosterol, zearalenone and trichotecen (deoxynivalenol and nivalenol) was found in grain of winter wheat in 2012 than in 2013. Fumonisin B1 accumulated in grains in comparable amounts in both years of the study, while fumonisin B2 accumulated more frequently in 2013. Mycotoxins accumulated more frequently in wheat grain cultivated on the control plot; moreover, fumonisins in 2012 accumulated on the object with the Nano Gro vaccine and fumonisin B 1 in 2013 accumulated on the object with mineral fertilisation.

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Fusarium community and mycotoxins in asparagus plants from the long-abandoned orchard

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Asparagus (*Asparagus officinalis* L.) is a very popular vegetable because of its exceptional taste and high nutritional value. It is often colonized by various pathogenic fungi belonging to the *Fusarium* genus. *Fusarium proliferatum* and *Fusarium oxysporum* are the most frequent species occurring on cultivated asparagus plants, being well known mycotoxin producers (mainly fumonisins, beauvericin and moniliformin). Being a perennial crop, it may serve as a perfect host for weak pathogens as well as for the endophytic organisms, e.g. at least some among many *Fusarium* species. Asparagus spears were collected continuously through May–October during 2013 season from a large wasteland in Poznan city in central Poland to investigate the variety of *Fusarium* population in wild asparagus plants. None of the plants have shown any visible symptoms of infection. After isolation of fungal strains, the species were identified on the basis of genomic sequence analysis. Morover, myxotoxin concentrations were measured in the tissues of collected stems. Forty-four *Fusarium* strains were isolated from asparagus plants, belonging to eight species, of which *F. proliferatum* and *F. sporotrichioides* were the most frequent. Therefore, fumonisin analogs were identified and quantified using UPLC method, as the most likely occurring mycotoxins present in asparagus stems. Moreover, moniliformin and cyclic peptides from beauvericin/enniatin group were also analysed. The effectiveness of the in vitro biosynthesis of the same metabolites by strains tested was also analyzed using sterile rice cultures. Generally, the *F. proliferatum* strains isolated from wild asparagus plants produced low or moderate amounts of mycotoxins, while the mycotoxin levels accumulated in plant tissues were elevated when compared to the cultivated asparagus plants.

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Enzyme secretion of some pathogenic isolates of *Fusarium oxysporum* f. sp. *albedinis*, causal agent of Fusarium wilt of date palm in North Africa

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Fusarium wilt is the most serious disease of date palm in North Africa. It is caused by a soil-borne fungus, *Fusarium oxysporum* f. sp. *albedinis*. Several phytopathogenic microorganisms secrete active toxins and hydrolytic enzymes against the tissue structures of the host plant. They are mainly cellulases and pectinases, whose role in fungal disease has been discussed. Studies have shown that these hydrolases are capable of degrading in vitro cellulosic and pectic compounds. In this study, we compared both cellulase and pectinase enzymes activities from four isolates of *F. oxysporum* f. sp. *albedinis* that have already shown an aggressiveness on date palm seedlings in inoculation tests. An isolates culture in a liquid medium to give a filtrate was performed. The determination of cellulolytic and pectinolytic activities were performed according to the technique described by Miller. This technique quantifies the reducing sugars released in the reaction medium. Enzyme assays revealed in these filtrates a significant cellulotic and pectinolytic activities for strain 15, whereas the strain 52 has a significant pectinase activity. Nevertheless, the strain 13 shows a high cellulase activity compared to others.

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Session 3

Pathogen identification, detection and monitoring

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Significance of the ITS rDNA and 18S rRNA regions as the genetic taxonomical marker for studies of the soil microbiota

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Current knowledge on the composition and function of soil fungal communities is limited. Application of a few independent but supplementary methods of isolation and identification of microorganisms is necessary for broadening our knowledge about density, diversity, activity and function of microorganisms in soils. The application of the molecular method helps to recover microbiota directly from environmental samples, and to detect particularly these microorganisms which due to the limitations of microbial isolation methods, have been gone unnoticed with traditional culturing techniques. The results of the molecular method depend on the specificity of region studied. The usefulness and significance of the ITS rDNA and 18S rRNA regions as the genetic taxonomical marker for studies of the soil microbiota was analysed with sequencing of rDNA after extraction and cloning of the environmental DNA from the forest soil. Fungal communities studied with ITS rDNA and 18S rRNA regions differed in terms of diversity. Communities contained fungi from *Zygomycota, Ascomycota* and *Basidomycota*. There were no common species in fungal communities studied with ITS rDNA and 18S rRNA. Organisms from kingdoms Animalia and Protista were obtained after application of 18S rRNA region.

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The use of *in vitro* cultures to micropropagation and releasing horseradish (*Armoracia rusticana* L.) from TuMV

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Application of tissue culture enable to obtain healthy plants from initial material affected by viruses. Viral diseases, especially TuMV, constitute a serious economic problem due to losses in horseradish production. Elaboration of multiplication process will increase the intencity of healthy plant propagation in *in vitro* cultures. Buds collected from horseradish roots during introducing to tissue culture was sterilized in 5% PPM solution. The use of PPM allowed to obtain 65% sterile cultures. From all tested media, the most effective for horseradish meristems was MS medium containing kinetin, IAA and 0,1% v/v PPM. Rosettes and leafs received from meristem cultures were used in multiplication process. The largest number of rooted plants was obtained on MS medium supplemented with BA, NAA and putrescine using rosettes as initial explants. In case where leafs were used as initial explants, the best multiplication was gained on MS medium with TDZ and NAA. Obtained rosettes were next rooted in *in vitro* conditions and a greater percentage of rooted rosettes (83%) was achieved on MS medium containing sucrose and NAA. Well developed plants were then adapted to *ex vitro* conditions. In this process 100% of horseradish plants adapted to external conditions. After testing with Elisa and RT PCR method, it was found that among horseradish plants obtained in *in vitro* cultures of meristems collected from plants infected with TuMV virus 13% of plants were virus-free.

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Genetic variability of hazel isolates of *Apple mosaic virus*

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Apple mosaic virus (ApMV) is a member of the subgroup III of the genus *Ilarvirus,* family *Bromoviridae*. The virus infects over 65 plants species including: apple, hazel, strawberry, apricot, black cherry, almond, red currant, raspberry, rose, hop and birch. ApMV causes chlorotic or yellow patterns, rings and mosaic on the hazel leaves and losses in yield.

The aim of this study was to characterize the molecular properties of ApMV isolates from hazel.

Silica capture (SC) method was used for extraction of total nucleic acids from the samples of leaves collected from 125 hazel plants growing in commercial orchards and private gardens in south-west Poland. Several primer pairs, including primers designed during this study, were used for RT-PCR to amplify the coat protein (CP) and movement protein (MP) genes of the ApMV.

The virus was detected in samples from seven hazel trees of 'Trapezundzki' cv. and five samples of 'Negret' cv. Molecular characterization of the amplicons was determined by RFLP, sequencing and phylogenetic analyses. The RFLP analysis of the amplicons singly digested with *Alu*I, *Mse*I, *Rsa*I resulted in significant variability of the ApMV isolates found in hazel plants. The analysis of nucleotide sequences of CP genes confirmed their genetic diversity. The similarity of nucleotide sequence of movement protein gene and coat protein gene of the virus isolates was 87.7–98.8% and 90.7–99.7%, respectively. Phylogenetic analysis showed that ApMV isolates from hazel grouped separately from the virus strains found in other plant species.

The efficiency of the three methods for nucleic acids extraction was compared: 1) the RNeasy Plant Mini Kit (Qiagen, Germany); 2) adsorption on silica gel (SC), and 3) the capture of virus particles using specific antibodies (IC) procedures. Depending on the procedure, it was possible to detect ApMV after dilution 10^{-4}–10^{-6} of the extracts from infected leaves.

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The effect of different organic fertilization on fungi colonizing plant roots and seeds of fodder galega (*Galega orientalis* Lam.)

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The aim of the study was to determine the effect of varied organic fertilization of fodder galega plantation on the species composition and number of fungi isolated from roots and seeds. Fodder galega plants were organically fertilized: I – barley straw, II – galega straw III – sewage sludge, IV – willow chips. Additionally, in the control combination – without organic fertilization, fungi colonizing the fodder galega roots were studied. Analyses were conducted in 2009–2010. In total,1168 colonies of fungi belonging to 29 species were isolated, including 602 colonies in 2009 and 566 in 2010. In the first year of the study *Rhizoctonia solanii* was the most frequently isolated species (31%), whereas *Penicillium* spp. was the most often isolated fungus in 2010 (14%). *Rhizoctonia solani*, *Penicillium* spp., *Trichoderma viride* and *T. koningii* were the most frequently occurring fungi.The highest number of fungi was isolated in combinations with barley straw and galega straw fertilization, however the least number – with sewage sludge. Ten species of fungi were identified on seeds. *Alternaria alternata*, *Aureobasidium bolleyi*, *Epicoccum nigrum*, *Cladosporium herbarum*, *Stemphylium botryosum* and *Penicillium* spp. were the most often isolated saprotrophic fungi.

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Cereal viruses' occurrence in the Central Bohemia region

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During the vegetation period the samples were collected from different locations of Central Bohemia region. The main attention was paid to the fields close to the forests and perennial meadows as well as to the margins of the fields. In total, 188 samples were collected, consisting of winter and spring barley, wheat and perennial Poaceae. All samples were analysed using common DAS-ELISA which revealed infection in 60 samples, form which 6 were positive for BYDV, 11 for WSMV and 43 for WSMV. The positive samples were subsequently confirmed by (RT)-qPCR. Also the suspected samples, in which the presence of some rare viruses was supposed, were also tested by RT-qPCR. Using this approach, we found out other viruses like ONMV, RgMV, SBCMV, BMV, SpMV and a newly detected virus, *Lolium latent virus* (LoLV).

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The effect of *Fusarium* fungi on behaviour of the rice and granary weevils

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The rice and granary weevils (*Sitophilus oryzae* and *S. granaries*) are serious stored product pests attacking many cereal crops. Fungi produce metabolites, such as mycotoxins and volatile compounds, which may influence insect behaviour. The aim of our study was an evaluation of the effects of different *Fusarium* fungi (*F. graminearum*, *F. culmorum*, *F. cerealis*, *F. poae*, *F. sporotrichioides*, *F. langsethiae, F. sibiricum*) on olfactory responses of the rice and granary weevils. The tests were carried out after growing of the strains on two substrates: potato sucrose agar and autoclaved wheat grains. The basic experimental testing unit for the insects was a dual-choice pitfall bioassay chamber.

The results showed that volatile organic compounds released by these fungi can possess neutral, attractive and repellent properties to weevils. The pathogenic to plants *Fusarium* spp. (*F. cerealis, F. culmorum, F. graminearum,* and *F. sporotrichioides*) generally stimulated a repellent effect to beetles of the weevils. The *Fusarium* species characterized as weak pathogens (*F. langsethiae, F. poae, F. sibiricum*) in most cases stimulated attractive and neutral effects on beetles.

Generally, beetles of two species demonstrated similar reaction in the presence of fungal cultures growing on the both substrates. However, the granary weevil showed more frequently a neutral response to the pathogenic *Fusarium* spp. In contrary to the rice weevil, the granary weevil lacks the ability to fly and develops only within different storages. There are substantial differences in susceptibility of the species of the weevils to volatile compounds of fungi. The results are discussed in a context of possible relationships between *Fusarium* fungi and the weevils utilising cereal grains as a common food substratum.

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Mycobiota of *Linaria vulgaris* and *L. genistifolia* containing DNA sequences of agrobacterial origin in their genomes

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Linaria vulgaris Mill (section *Linaria*) and *L. genistifolia* (L.) Mill. (section *Speciosae*) are widespread in Russia species, containing DNA sequences of agrobacterial origin in their genomes (Matveeva et al., 2012). One of the possible advantages of the T-DNA-containing plants is enhanced secondary metabolite production (Chandra, 2012), which may play role in disease resistance. The object of this investigation is to analyze species composition of micromycetes of *L. vulgaris* and *L. genistifolia* in Russia. 15 species of micromycetes of 14 genera were identified (*Peronospora flava* Gaeum., *Itersonilia perplexans* Derx, *Entyloma linariae* J. Schroet., *Gibberella fujikuroi* (Sawada) Wollenw., *Alternaria* spp., *Aureobasidium pullulans* (de Bary) G. Arnaud, *Epicoccum nigrum* Link, *Fusarium avenaceum* (Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. solani* (Mart.) Sacc., *F. sporotrichioides* Sherb., *Myrothecium verrucaria* (Alb. et Schwein.) Ditmar, *Ramularia linariae* Baudyš et Picb., *Colletotrichum destructivum* O'Gara, *Phoma exigua* var. *exigua* Desm., *Phoma* sp., *Phomopsis oblonga* (Desm.) Traverso, *Vermicularia* sp.). The evaluation of virulence against *L. vulgaris* (cDNA+) and *L. maroccana* (cDNA-) for 14 strains of 7 species (*Phoma exigua* var. *exigua*, *Phomopsis oblonga*, *Ramularia linariae*, *Fusarium sporotrichioides*, *F. avenaceum*, *F. solani*, *Gibberella fujikuroi*) indicated that *L. vulgaris* are less susceptible to pathogenic micromycetes than *L. maroccana*. Low susceptibility of *L. vulgaris* to pathogens may be connected with presence of cT-DNA in its genome.

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Fusarium species distribution on stem base of winter cereals

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Fusarium spp. distribution on the stem base of seedlings of winter wheat 'Ada', winter triticale 'Pigmej' and winter barley 'Alinghi' was studied at Institute of Agriculture in 2013–2014. Naturally infected seeds were used. Samples were taken at BBCH 13 (in autumn) and BBCH 25 (in spring) from the untreated plots and plots treated with tebuconazole (0.05 g l^{-1}) at a dose rate of 0.5 l t^{-1}. Plant stem base tissues were examined on a PDA medium and identified by morphological symptoms of *Fusarium* species. The results showed the differences in *Fusarium* spp. distribution and infection level of the pathogen between the treated and untreated plants. *Fusarium* seedling blight ranged from 25 to 41.25% in the untreated and 8.75 to 22.25% in the treated plots. In spring, *Fusarium* spp. infection level was from 15 to 43.75% in the untreated and 10 to 35% in the treated plants. *F. avenaceum*, *F. culmorum*, *F. equiseti* were most commonly detected in the winter barley untreated plots. Winter triticale plants were infested with *F. avenaceum*, *F. graminearum*, *F. culmorum*, *F. equiseti* in the untreated plots, winter wheat plants – with *F. avenaceum*, *F. poae*, *F. tricinctum* and *F. graminearum* in the untreated plots. *F. avenaceum* dominated in all plants in 2013–2014 at both assessments. Tebuconazole reduced *F. avenaceum* incidence on stem base tissue by on average 71.5% at BBCH 13 and by 50% at BBCH 25 compare to the untreated.

The abstract presents research findings obtained through the long-term research programme "Harmful organisms in agro and forest ecosystems" implemented by Lithuanian Research Centre for Agriculture and Forestry.

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Fungi colonization of population and hybrid oilseed rape cultivars

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Fungi colonizing oilseed rape seeds may decrease their quality and may affect health status of the seeds. Seeds healthiness is one of the most important factors influencing the quality and amount of potential yield. The aim of the study was to determine the settlement of seeds of selected population and hybrid oilseed rape varieties by pathogenic and saprophytic fungi . The seeds of the Department of Plant Breeding Strzelce Borowo and Plant Breeding Department Strzelce Małyszyn were used. Population and hybrid rape varieties were examined. Rapeseed were sanitized for 3 minutes in 5% sodium hypochlorite, and then were placed on Petri dishes with PDA medium (Potato Dextrose Agar) acidified with a few drops of 5% lactic acid. Experiment was carried out in 4 replications, 100 seeds each. The plates were incubated at room temperature for 7 days and then colonization by fungi was rated. With the help of key fungal colonization of oilseed rape was determined by a fungus. Following species of fungi were isolated: *Alternaria alternata, Alternaria brassicicola*, *Alternaria brassicae*, *Fusarium* spp, *Cladosporium* spp, *Penicillium* sp, and others.

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Incidence and distribution of fungi from Diaporthaceae and Botryosphaeriaceae on grapevine in Croatia

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Fungi from families Diaporthaceae and Botryosphaeriaceae are regarded as important plant pathogens worldwide, among which 19 and 27 species, respectively, are known to infect grapevine. However, incidence and distribution of those species considerably varies between countries and between different vine-growing regions (VGRs). To determine incidence and distribution of fungi from Diaporthaceae and Botryosphaeriaceae on grapevine in Croatia, from years 2009 to 2013 surveys at 77 localities in 12 VGRs were conducted. Symptomatic grapevine wood samples were collected, from which fungal species were isolated and identified based on their morphology and molecular markers ITS and EF1-α. From 322 isolates in this study which were determined to belong to family Diaporthaceae, the following species were identified: *Diaporthe ampelina* (97%), *Dp. eres* (1.5%), and *Dp. foeniculacea* (1.5%). From another 155 isolates in this study, determined to belong to family Botryosphaeriaceae, these species were identified: *Diplodia seriata* (62%), *Botryosphaeria dothidea* (24%), *Neofusicoccum parvum* (13%), *D. coryli* (0.5%) and *Dothiorella sarmentorum* (0.5%). Species *Dp. ampelina* and *D. seriata* were widely distributed in all VGRs, and with high incidence, while species *D. coryli* and *Dh. sarmentorum* both had very low incidence, and were distributed only in Coastal and Western continental VGRs, respectively. Species *Dp. eres* was distributed mostly in Coastal VGRs, to which species *Dp. foeniculacea* was exclusive, while both species had low incidence. Species *B. dothidea* was distributed in almost all VGRs with moderate incidence, while species *N. parvum* was also distributed in all VGRs but with moderate incidence in Eastern continental and Coastal VGRs, and low incidence in Western continental VGRs. It was concluded that species *Dp. ampelina, B. dothidea* and *N. parvum* could have important role in aetiology of associated grapevine diseases in Croatia and should be the focus of future research on their epidemiology and control strategies.

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The healthiness of leaves of selected oat genotypes

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The investigations of leaves healthiness of 15 oat genotypes: DC 06011-8, DC 06182/4, DC 07116-11/2, DC 14-6, DC 14-8, POB 525/10, POB 961-1344/13, POB 4109/10, POB 5733/10, POB 6020/10, STH 1.201, STH 1.230, STH 2.388, STH 3.695 and Denar were carried out in 2014 in the fields of the Strzelce Plant Breeding Company Ltd. Disease symptoms on leaves were recorded in the late milk stage (77 in the Zadoks scale). 200 leaves of each oat genotype were evaluated. The infection was estimated according to a 9° graphical scale (0° – leaves without disease symptoms, 8° – the highest infection) and the disease index was calculated. The percentage of leaves with spots on leaf blades varied from 24,5 (POB 4109/10) to 77,5 (DC 06011-8). The highest disease index for leaves of oat genotypes was recorded in the case of the breeding line POB 6020/10 (30,44) while the lowest in the genotype POB 4109/10 (5,06). From leaves with necrotic spots: *Bipolaris sorokiniana, Drechslera avenae, Exserohilum* spp.*, Septoria* spp. and *Alternaria alternata* were obtained.

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Diversity of *Puccinia triticina* fungus in Russia in 2002–2013

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3322 single-uredinial isolates of *Puccinia triticina* collected from wheat in seven regions of Russia in 2007–2013 were tested for virulence with 20 near-isogenic wheat differential lines (set 1: *Lr*1,2a, 2c,3a; set 2: *Lr*9,16,24,26; set 3: *Lr*3ka,11,17,30; set 4: *Lr*19,20,14a,18; set 5: *Lr*2b,3bg,14b,15). 331 virulence phenotypes were identified – 97 in the Volga region, 58 in the Central, 101 in the Black Earth, 99 in the North-Caucasian, 37 in the Western Siberian, 53 in the Ural and 135 in the North-Western region. THTKT, TGTKT, FGTKT were the predominant phenotypes in all regions with frequency ranging from 2 to 100%. Variation in virulence of *P. triticina* depended on selection pressure of resistance genes deployed in wheat varieties cultivated in regions. For example, over wide growing varieties with gene *Lr9* in the Western Siberia virulent isolates were revealed in the end of 2000 and later it frequency increases annually. The increase in the frequency of virulence to *Lr1* in the European Russian regions during the last decade is also likely due to the massive cultivation of wheat varieties with *Lr1* gene. Virulence to *Lr26* was the most variable among the regional populations and between years that might be caused by broad range of cultivation of varieties with *Lr26* gene and their uneven proportion in the regions in different years. In contrast, molecular markers are generally assumed to be neutral to host selection. 23 SSR-markers (Kolmer et al., 2013) were examined for detection of variability in Russian *P. triticina* isolates and selected the more informative.

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Exploring the host range of *European mountain ash ringspot-associated virus* and its distribution in the Czech Republic

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It has been previously shown that *European mountain ash ringspot-associated virus* (EMARaV), a multipartite, negative-strand RNA plant virus, is pathogenic for European mountain ash (*Sorbus aucuparia* L.), but its transmissibility to and replication in other host species is poorly understood. In this work, we performed experimental inoculations of seventeen virus-free species and inter-species hybrids of subfamily *Maloidae* (family: *Rosaceae*) with buds from EMARaV-infected *S. aucuparia* trees. Surprisingly, a majority of graft-inoculated tree species, for instance *Sorbaronia*, *Aronia* and *Amelanchier* developed symptoms typical for EMARaV infection. These symptoms included chlorotic spots of various size and shape and mottling on the leaves, but not on flowers and fruits. Propagation of EMARaV in graft-inoculated tree species was subsequently confirmed by molecular detection tools. Based on these facts, we carried out a systematic survey throughout the Czech Republic, where we identified presence of EMARaV in wild growing trees in all explored regions, with massive disease infestation in several locations. More importantly, EMARaV infection was newly detected in *S. aucuparia* subsp. *moravica*, *S. aucuparia* × *Crataegus sanguinea* cv. Granatnaja, (*Sorbus aria* × *Aronia arbutifolia*) × *S. aucuparia* cv. Burka, cv. Finskaja (a *Sorbus* hybrid of unknown origin) and *Aronia melanocarpa*, all these EMARaV-infested trees were grown in production orchards. Together, our biological findings extend our knowledge on the host range of EMARaV, and provide first evidence of its natural occurrence in different hosts unlike *S. aucuparia*.

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Monitoring of *Rhizoctonia cerealis* causing sharp eyespot on winter wheat in Poland

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Observations of the occurrence of sharp eyespot were performed over the time period of 2004–2013, on production fields of winter wheat. We evaluated a total of 533 samples, derived from fields located in different regions of Poland. At the BBCH 75–77, along the diagonal of the field, random samples were taken. One sample, consisting of 100 plants, was taken from each farm field. In the laboratory, samples were washed and the ear-bearing shoots were torn off. Then, the percentage of stems with symptoms of sharp eyespot was evaluated. The degree of the intensity of sharp eyespot was determined, applying the 0–4° scale. The degrees of infection were transformed into the Disease index. The evaluation of the plants' health status was supplemented by mycological analysis and PCR assay using the specific SCAR primers and Real-Time PCR. The incidence of sharp eyespot was tested depending on the year, preceding crop, fungicide plant protection, cultivar, dose of nitrogen fertilization and soil type. There were noted significant differences in the occurrence of sharp eyespot in respective years. Most infected stems were noted in 2007 in which it occurred, on the average, on 18.1%, and least in 2005 – on 2.1%. The intensity of the disease varied across the fields (locations). In some fields the disease was not reported at all. The cultivars with least intense disease were Pegassos, Fregata and Turnia. The cultivars with most intense disease were Smuga and Cubus. Greater intensity of the sharp eyespot was observed on lighter soils. Under production conditions, there was found no significant effect of the preceding crop, dose of nitrogen fertilization and the fungicide protection applied on the occurrence of sharp eyespot in winter wheat. *Rhizoctonia* culture analysis on PDA medium and PCR assay confirmed that *R. cerealis* was the main causal organism.

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The first registration of strobilurin resistance in *Mycosphaerella graminicola* in Belarus by PCR-RFLP method

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The most important disease of wheat in Belarus is *Septoria* leaf blotch. Mainly, the fungicides are used to protect plants from pathogen. However, the fungus adapts to different chemicals. In this connection, the monitoring *Mycosphaerella graminicola* populations for the presence of resistant isolates to fungicides is substantial. The G134A mutation in mitochondrial gene *cytb* of *Mycosphaerella graminicola* that defines the pathogen resistance to fungicides of strobilurin (QoIs) group has been studied for the first time in *M. graminicola* isolates from different Belarusian regions. The analysis has been carried out using PCR-RFLP marker Mgcytb/*Fnu4*HI (*Sat1*) (Torriani et al., 2008). The assessment of G134A mutation distribution provides appropriate strategy for wheat protection against *M. graminicola* by means of fungicides. In this context, the developed methology of PCR identification of the genetic factor associated with pathogen resistance to QoIs has essential significance as far as it provides us with relatively easy, cheap, rapid and reliable mode of monitoring fungicide resistance in pathogen populations.

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Biological features of selected *Alternaria* and *Colletotrichum* species after 8 years of cryopreservation in –80°C.

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After 8 years of cryopreservation 104 *Ascomycetes* strains have been examined for their biological features (viability, sporulation, radial growth). Among them were 21 isolates of *Alternaria alternata*, 27 *A. radicina*, 1 *A. brassicicola*, 41 *Colletotrichum gloeosporioides*, 7 *C. lindemuthianum*, 5 *C. coccodes*, 2 *C. acutatum*.

Viability of the *A. alternata* and *A. radicina* was 100%; among 41 isolates of *C. gloeosporioides* only 1 strain (2%) did not survive. "Recalcitrant" strains were found among *C. coccodes* (20%) and *C. lindemuthianum*, which showed poor viability.

Most of strains produced spores within 15 days. Very few strains changed their biological features like colony morphology and former microscopic description.

Randomly selected strains of *A. radicina* and *C. gloeosporioides* were examined for pathogenicity. *A. radicina* showed high pathogenicity on *Daucus carota* leaves petiols. Also some strains of *C. gleosporioides* were virulent to *Lupinus angustifolius* leaves during *in vitro* test.

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What is new for nomenclature of fungi?

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The International Botanical Congress (IBC), held in Melbourne, Australia, during July 2011, ratified the Amsterdam Declaration on Fungal Nomenclature prepared under the auspices of the International Commission on the Taxonomy of Fungi (ICTF). The most important change, to be proposed in the declaration, was radical modification of Article 59 establishing the principle of "one fungus – one name", valid publication using English description or diagnoses and effective electronic publication. Also a new title of code was introduced – the International Code of Nomenclature (ICN) for algae, fungi, and plants.

Many fungal taxonomists approved the proposals, but others gave critical response since for those changes mycologists have not yet been ready. They stated that even after introducing a "one fungus – one name" rule, mycologists would need to understand the so far existing system of dual nomenclature *when studying the taxonomic literature*.

Nevertheless different opinion of mycologists soon new rules started to be introduced. The ICN provided for the development of lists of accepted and rejected names out of validly published fungi which should be approved by the Nomenclature Committee for Fungi (NCF). To produce such lists international working groups concerned with particular families or genera have recently been preparing list for formal adoption. A revised draft of the list will be made available for father discussion at the 10th International Mycological Congress in August 2014, at Bangkok, Thailand. A list for adaptation by the IBC is expected to be ready in July 2017 in Shenzhen, China.

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Characterization of *Phytophthora infestans* (Mont.) de Bary isolates in the Czech Republic from 2012 to 2013

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Late blight diseased potato plants were collected from different regions of the Czech Republic in the years of 2012 and 2013. A collection of *Phytophthora infestans* isolates was used in studies of the population structure of the pathogen. Mating type determination via conventional pairing test and the cleaved amplified polymorphic sequence (CAPS) analysis revealed that 45% and 55% of isolates represented the A1 and the A2 mating type, respectively. The response of the isolates to active ingredients (metalaxyl, metalaxyl-M, propamocarb-HCl and dimethomorph) of frequently used fungicides was examined using the *in vitro* amended-agar method. The majority of the isolates were intermediate or resistant to metalaxyl, metalaxyl-M and propamocarb-HCl. All the isolates appeared to be sensitive to dimethomorph. Virulence of the isolates was tested on 11 genotypes with single genes R1–R11 from *Solanum demissum* (Black's differential set), dihaploid genotype *S. tuberosum* ssp. *tuberosum* (DH 165) and *S. bulbocastanum* with Rpi-Blb1 gene, using a detached leaflet test. Virulence factors against potato R genes R1, R2, R3, R4, R6, R7, R10, R11 and genotype DH165 were present in most isolates. Virulence complexity was significantly different between localities in both years.

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Development of quantitative PCR techniques for plant pathogens diagnostic research

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A wide range of diagnostic procedures has been employed for identification, differentiation and quantification of plant pathogens. Real-time, or quantitative, polymerase chain reaction (rtPCR), offers a rapid, sensitive, and specific method for the diagnosis of plant pathogens in various environmental samples, including hosts tissues, soil, water and air. They combine the sensitivity of conventional PCR with the generation of a specific fluorescent signal providing real-time analysis of the reaction kinetics and allowing quantification of specific DNA targets. Four main chemistries are currently used for the application of this technique in plant pathology. These chemistries can be grouped into amplicon sequence non-specific (SYBR Green I) and sequence specific (TaqMan, Molecular beacons, and Scorpion-PCR) methods. Fluorescence is used to monitor the accumulation of the PCR product after each PCR cycle. The fluorescence data are used to extrapolate the amount of target DNA present in the sample before amplification so that, detection and quantification are achieved in a single assay thus, opening new research opportunities for the study of diagnosis, inoculum threshold levels, epidemiology, disease management and host–pathogen interactions. In the present review, we discuss current applications of real-time PCR based on major chemistries to the diagnosis of plant pathogens (e.g. oomycetes, fungi, bacteria, phytoplasmas, viruses and viroids) in soil and plant samples.

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Microcyclosporella mali as a causal agent of sooty blotch in Poland

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Sooty blotch is one of the most common diseases of apples in organic orchards in many countries. Results of molecular studies performed in USA indicated approximately 30 different fungi species associated with this disease. The goal of this work was to identify and describe *Microcyclosporella mali* J. Frank, Schroers et Crous fungus causing apple sooty blotch in selected regions of Poland. Fruits of different apple cultivars and breeding lines as well as various cultivars of pear and plum showing sooty blotch symptoms were used for experiment. Fruits were collected from trees growing in orchards and small gardens non-chemically treated located in Northern, Central and Eastern Poland. In the studies conducted between 2006–2010 over 1500 fungal isolates were obtained. Among them 228 proved to be responsible for sooty blotch. Majority of obtained isolates were identified on the basis of morphology and nucleotide sequence of the rDNA internal transcribed spacer region (ITS). DNA was extracted from representative isolates and used as matrices for PCR amplification with ITS1F and ITS4 primers. The isolates most often occurred were identified as *Microcyclosporella mali*. Conidia of *M. mali* were hyaline, almost cylindrical with numerous septa. Secondary conidia of this species were often formed according to microcyclic conidiation, i.e. after germination of primary conidia. Growth dynamic of some isolates was measured on six media: Czapek, CMA, MEA, PCA, PDA and apple medium. Daily rate growth of culture area was strongly differentiated depend on a medium and an isolate. Anatomical analysis of interaction between sooty blotch fungi and epidermal cells of apple fruit was observed with light and electronic microscopes (scaning and transsmition). Hyphae of *Microcyclosporella mali* developed on the surface of epicuticular waxes and did not form appressoria but they were able to penetrate the deeper layers of cuticule.

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Colletotrichum graminicola as a causal agent of anthracnose of southern sweet-grass

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In 2013 on the leaves of southern sweet-grass (*Hierochloë australis* (Schrad.) Roem. et Schult.) symptoms of anthracnose were observed. The plant grown in collection on experimental fields of Department of Vegetable and Medicinal Plants. Anthracnose commonly occurs in many areas and climate zones. It is observed on 42 plant genera of *Poaceae* family. Recently it has been noticed on southern sweet-grass as well. Initially symptoms occur as oval-shaped, water-soaked spots with dark green color. The disease develops quickly on the leaves causing yellow and reddish-brown lesions.

The aim of this study was to investigate the fungus causing anthracnose symptoms on *H. australis* leaves. To identify the pathogen traditional methods and techniques of molecular biology were used. On the collected leaves with visible symptoms of the disease black acervuli with setae developed around brown spots. From these leaves 7 isolates of the fungus were obtained on PDA (Potato Dextrose Agar) medium. To molecular identification of isolates amplification of selected rDNA fragments (ITS1, 5.8S, ITS2) with primers ITS1 and ITS4 was performed. Amplicons were sequenced and analyzed by ClustalW 2EBI and BLAST program. The compared sequences showed 100% similarity and homology with the sequences of *Colletotrichum graminicola* (Ces.) G.W. Wilson stored in GeneBank under access numbers: AF059676, KC106722 and KC106723. Koch's postulates gave positive results. Than growth and sporulation on media PDA, Czapek-Dox and CMA was examined. Cultures produced gray mycelium. Conidia were falcate-shaped. Their size varied between 22.59–22.99 μm long and 4.37–4.42 μm wide. These isolates were identified as *Colletotrichum graminicola*. There is the first report concerning the presence of this pathogen on *Hierochloë australis* plants in Poland.

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Emerging sea buckthorn diseases and associated pathogens

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The cultivation of the sea buckthorn (*Hippophae rhamnoides* L.) in Europe is relatively new in comparison with traditional fruit crops grown for centuries. Despite its importance as an agricultural plant species, the research on diseases is limited not only in Europe, but also in the world. In general, only few diseases have been recorded, such as canker caused by *Stigmina* sp., wilt (*Verticillium* spp.), bud bacteriosis (*Pseudomonas syringae*) and dry shrink disease (*Fusarium* spp.). During the recent years concerns of growers have raised on diseases spreading in sea buckthorn plantations in Latvia. As a response to grower concerns, the research was initiated on identification and characterization of sea buckthorn diseases prevailing in Latvia. In order to identify the causes of observed problems, surveys and samplings were performed from June to September. The samples from branches and trunks with diverse symptoms were collected. Fungi and bacteria were isolated in pure cultures, and the potential pathogens were preserved. Identification of fungi was carried out by means of morphological characters and the sequencing of ITS region and 28S rDNA fragments. Presumptive identification of bacterial isolates was done based on colony morphology and biochemical tests, including LOPAT and GATTa for *Pseudomonas*. Severe canker and dieback symptoms often causing death of the infected plants were observed in several orchards. The symptoms, characteristic for bud bacteriosis, were also noticed. So far a number of isolates belonging to the known pathogenic genera causing tree cankers and dieback, such as, *Stigmina* sp., *Phomopsis*, *Cytospora*, have been identified. Pathogenic *Pseudomonas syringae* was isolated from symptoms resembling bud bacteriosis. However, the significance and the role of these associated pathogens should be further studied. The study is in progress for more detailed characterization of the isolates and evaluation of their pathogenicity on the host.

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Diversity of strawberry pathogen *Gnomonia fragariae* Kleb.

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Gnomonia fragariae Kleb. is a pathogenic diaporthalean fungus, which causes root rot and petiole blight of strawberry. The disease is widespread in Latvia and severe in perennial cultivation. The fungus has been also reported on cultivated strawberry in Sweden, Switzerland, Germany, United Kingdom and Lithuania. The aggressiveness and virulence of the pathogen were evaluated in two detached-leaf assays in the laboratory and two greenhouse experiments for two years. In detached-leaf assays performed in laboratory 57 strawberry genotypes were inoculated with young mycelial plugs of seven isolates originated in Latvia and Sweden. Development of necrosis was monitored daily, and total necrotic area was measured after 10 days. In the greenhouse experiments, micro-propagated strawberry plants of eight genotypes were inoculated around crown by mycelial plugs of the same isolates used for laboratory tests. Development of symptoms was recorded once a week. At the end of the experiments, disease severity was evaluated, and root and shoot weight measured. The diversity of *G. fragariae* was also examined through identification of vegetative compatibility groups among isolates of different geographic origin from Latvia, Sweden and United Kingdom. The tested isolates of *G. fragariae* showed substantial variations in their aggressiveness in bioassays and specific interactions were observed in combinations of isolates and cultivars. Several vegetative compatibility groups were detected, and incompatible and intermediate interactions were characterized. In most cases, isolates that were in same vegetative compatibility group were originated from the same location or were associated with movement of planting material. This study suggests that the Latvian populations of *G. fragariae* have considerable diversity and further genetic analyses are needed to elucidate the population structure of the pathogen in strawberry cultivations.

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Phytophthora spp. invasions in post-communist economies – the example of the Czech Republic

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Phytophthora spp. belong among the most important pathogens of ornamental and forest woody plants in Europe. Many of them are considered to be alien or cryptogenic. The number of their introductions depends on the globalization and level of imports of goods. That's why Central- and East European economies were partially protected from invasions before collapse of Eastern block. The diversity and distribution of *Phytophthora* spp. pathogenic to woody plants in the Czech Republic support this claim, because up to one half of *Phytophthora* spp. described in the area could be probably introduced after coup d´état in 1989.

The intensive investigation of *Phytophthora* spp. diversity in different environments (from ornamental nurseries, gardening centres to forest stands) started in 2006. Hundreds of *Phytophthora* isolates were collected, determined and their distribution was analysed.

There were found 21 taxa belonging to *Phytophthora*. Only 4 taxa are probably native (19%: *P. gallica*, *Phytophthora lacustris*, *P.* taxon oaksoil, *P. polonica*). The other 17 taxa (81%) are probably alien or cryptogenic. The distribution analysis of them shows that 6 taxa (29%) are more or less regularly distributed in natural stands, thus their introductions are probably of older date (*P. plurivora*, *Pau*, *P. gonapodyides*, *P. cambivora*) or their natural spread is extraordinarily effective (*Paa*, *P. multivora*). The 11 other species (52%) are regularly distributed in anthropogenic environments and only some of them are distributed very occasionally in highly invasible riparian stands. Moreover, the distribution of highly pathogenic *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. palmivora* and *P. ramorum* is limited only to ornamental nurseries, gardening centres and ornamental plantings.

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Detection of six *Allexivirus* species infecting garlic in Poland

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The viruses transmitted by mites*: Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), *Garlic virus X* (GarV-X) and *Shallot virus X* (ShVX) of the *Allexivirus* genus (*Tymovirales* order, *Alphaflexiviridae* family) (King et al., 2012) were the objects of the presented research. The harmfulness of allexiviruses in garlic cultivation is mainly connected with the worsening of the crop quality i.e. the drop of the bulb weight and the reduction of their diameter. Allexiviruses were detected in the 1990s in the region of China, Japan and Korea. Now, it is known that they occur in various parts of the world: Asia, South America, North America, Australia, New Zealand and Europe. The aim of the study was the detection and identification of GarV-A, GarV-B, GarV-C, GarV-D, GarV-X and ShVX in garlic plants collected from Poland (10 different parts) as well as from Egypt, Hungary, Mexico, Spain, Turkey and Ukraine. ELISA test (*enzyme-linked immunosorbent assay*) was carried out with extracts from garlic leaf samples to detect GarV-A, GarV-B, GarV-C and ShVX using commercial antiserum (DSMZ, Braunschweig, Germany). RT-PCR (*Reverse transcriptase-polymerase chain reaction*) was used with primers, designed in this study, specific to the whole coat protein gene of GarV-D and GarV-X detected. The results of the ELISA tests revealed the presence of the GarV-A, GarV-B, GarV-C, GarV-D and GarV-X in plant materials. All the plants tested were ShVX-free. GarV-X and GarV-D were the most commonly detected viruses in materials collected from garlic production fields in Poland and other regions of the world.

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The occurrence of Verticillium wilt in winter and spring oilseed rape in Lithuania

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Verticillium wilt (*Verticillium longisporum*) in Brassica crops is caused by the soil born fungus and has become a widespread problem in oilseed rape in Lithuania. The disease incidence was very low (up to 0.8%) in winter oilseed rape and it was not found in spring oilseed rape crops from 2000 to 2007. However, oilseed rape production area is annually expanding in Lithuania – during the period from 2000 to 2013 it has increased more than 23-fold. A high concentration of oilseed rape in a crop rotation has resulted in high pressure of the most important diseases such as *Leptosphaeria maculans* and *Sclerotinia sclerotiorum*. Since 2008 the incidence of Verticillium wilt has been on the increase and now it has become one of the most common diseases of oilseed rape. Research evidence obtained in other countries suggests that crop rotation is the most important factor affecting the occurrence of this disease. Field experiments, carried out at Institute of Agriculture during 2011–2013, showed a clear negative influence of oilseed rape concentration increasing in the crop rotation. The highest number of disease-affected plants was established in winter oilseed rape grown in a 3-year monocropping. The incidence and severity of Verticillium wilt in the third year were 78 and 33.1%, respectively. In winter oilseed rape monocropped for 2 years the disease incidence reached 48% and severity 14.7%. The lowest incidence (13–27%) and severity (0.6–7.8%) of the disease was determined when winter oilseed rape had been grown after a 4-year break. This study has highlighted the benefits of a crop rotation as an important tool for Verticillium wilt control.

The study findings were obtained through the long-term research programme “Harmful organisms in agro and forest ecosystems” implemented by Lithuanian Research Centre for Agriculture and Forestry.

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Contamination of rivers, canals and water reservoirs by *Phytophthora* species in Poland

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In the years 2008–2013 the occurrence of *Phytophthora* species in different water sources was monitored. For microorganism isolation baiting method with *Rhododendron* leaves cultivar 'Nova Zembla' was used. Studies were carried out in rivers flowing through agriculture, horticulture and forest area, in drainage canals and in water reservoirs localized in ornamental nurseries. *Rhododendron* baits were held in water for 4–6 days depends on the season. After taking out of the baits characteristic dark necrotic spots were observed. Small sections between healthy and necrotic tissues were plated on PDA medium or PARP. Additionaly, water sediments were also checked for the presence of *Phytophthora* species. Isolated microorganisms were identified on the basis of morphological features and using molecular techniques (PCR with species – specific primers or sequencing the ITS regions of rDNA). During 6 years studies *Phytophthora* species were detected in all analysed water sources all over the year as well as in water sediments with domination of *P. lacustris* and *P. plurivora*. Exept them *P. citrophthora*, *P. cryptogea*, *P. cambivora*, *P. gonapodyides* and *P. megasperma* were frequently isolated whereas *P. cinnamomi* and *P. syringae* only sporadically. The pathogenicity of isolated species was confirmed in laboratory conditions on detached *Rhododendron* leaf blades. Obtained results indicated on water as an important source of *Phytophthora* species.

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Long-term storage of selected fungi-like organisms of *Phytophthora* spp.

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In the Collection of Plant Pathogens are collected almost 2000 plant pathogens (bacteria, fungi and *Oomycota*); 74 of them belonged to 12 species of the genus *Phytophthora*. The most numerous species were *P. infestans*, *P. cactorum*, *P. cinnamomi*, and *P. citricola*. These cosmopolitan organisms infect over two thousands of plant species. It is necessary to understand their physiology and variability (morphological, physiological and genetic). *Phytophthora* spp. cultures stored in the collection have different origin and hosts, and they have been isolated in different years. To provide the best survival and physiological stability of these microorganisms were used several different methods of maintenance. The cultures of *Phytophthora* spp. were kept for a period of 2–19 years under mineral oil on the slant agar at 16°C, frozen at –196°C in liquid nitrogen, on the agar discs in water at 16°C and *P. infestans* on potato tubers in water at 16°C. All cultures stored on potato tuber did not survive after 6 months. Similarly, the cultures in water dried up after a few months. These methods proved to be useless, time-consuming, and the hyphae were easily infected with bacteria. Isolates of *Phytophthora* spp. (particularly *P. cactorum*, *P. cinnamomi*, *P. citricola*) can be stored for many years protected under oil (some culture survived 19 years). However, this method is laborious, requires renewal every 1–2 years, the culture is exposed to contamination and their growth is slower. In this method, two media were used (RA-Rye Agar and V8-Vegetable Agar) and RA was better for the studied species. Cryopreservation was the most effective and least laborious method for *Phytophthora* spp. but it requires the largest financial investment. Among the tested species *P. infestans* was most difficult to maintenance (59% survival after 15 years of storage). Isolates of *P. cactorum* survived both in oil storage and in liquid nitrogen. After recovery they grew very quickly and retained pathogenicity. This confirms, that the genus of *Phytophthora* is very difficult to maintenance for long periods.

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Occurrence of *Fusarium* spp. in wheat grains from different regions in Poland

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Fusarium head blight (FHB), caused by the fungi from the genus *Fusarium,* mainly *F. avenaceum*, *F. graminearum*, *F. culmorum* and *F. poae* is a damaging disease which leads to the loss of quality and quantity of cereal grains. In addition, toxins produced by these fungi render grains as unusable for human and animal food. To investigate the level of seed contamination and the composition of *Fusaria* in grains we have analysed wheat grains collected in 2011–2013 in four regions of Poland. Seeds of wheat cultivars differing with susceptibility to FHB were tested, using fungicide sprayed and unsprayed variants. Seed health was tested with standard ISTA seed health protocols. The identification of species was done based on morphological traits and PCR-RFLP, CAPS and dCAPS molecular methods. The level of wheat seed infestation varied depending on year, location, cultivar resistance and the use of fungicide application. Predominant species found on grains were *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. poae*. Other species, such as *F. cerealis*, *F. sporotrichioides* or *F. equiseti* were found less often. Fungicide application at flowering time reduced the level of *Fusarium* infestation of seeds by approximately 20 percent. The most susceptible was the cultivar 'Bogatka', while 'Arina' was the most resistant one. The region with the highest number of infected seeds was Debina located in Pomerania region (central-north Poland), whereas the lowest number of FHB symptoms was found in Charbielin located in Opole region (south-west Poland). The highest percentage of infected grains was found in 2013, and the lowest level of *Fusarium* occurrence on wheat grains was one year before. The collected material serves as a useful dataset for modelling of FHB in wheat, in relation to the occurrence of *Fusaria* in air samples.

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Exploring the host range of *Polymyxa graminis* originating from the Czech Republic and its distribution in the country

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Polymyxa graminis is an obligate root-infecting endoparasite of many cereals and grasses which has been found in several parts of the world. It is non-pathogenic, but has the ability to acquire and transmit a range of plant viruses which cause major diseases and significant yield losses in cereals. Molecular and ecological characterizations have shown a high level of diversity among *P. graminis* isolates, leading to the distinction of its five special forms. On the basis of our previous results in which was confirmed that *P. graminis* is present in the Czech Republic, its survey and following determination of its special forms were carried out in 2012 and 2013. Soil samples from fields as well as from forests and pastures served as testing material. Two special forms occurring in temperate areas, namely *P. graminis* f. sp. *temperata* and *P. graminis* f. sp. *tepida*, were detected by PCR using specific primers Pg.F1/Pg.R1 and Pg.F2/Pg.R2 (Ward et al., 2004). Both special forms were found without much difference in the frequency and co-existed in some soils. Further, host range studies involving 31 species and interspecies from the family *Poaceae* revealed different levels of susceptibility to *P. graminis* forms. Moreover, in order to evaluate whether or not host specialization is associated with the special form, the occurrence of infection of both forms on different hosts was studied.

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Pathogen identification by DNA sequencing ITS-rapeseed plants for interspecific crosses

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During the period of spring, dying *B. napus* plants can be observed on fields of rapeseed oil plants. This can be caused either by autumn damages by insects, damages caused by game animals or spore invasions of sac fungi from the genus of *Leptosphaeria* spp. In conditions of the climate in Poland, infestation with the above-mentioned pathogens is recorded every year and this fact is frequently connected with yield losses of rape seeds. During a period of four years (2009–2012), experiments were carried out to study the occurrence of microorganisms which settled inside infected fragments of *B. napus* plants, i.e. root necks and dying apexes. Following external sterilisation, the dying parts were appropriately prepared and then applied onto PDA medium. After the period of 6 days, growing hyphae of mycelium were transferred into Eppendorf tubes and frozen. Next, the isolated species were identified with the assistance of sequencing of DNA, ITS-1 or ITS-2 fragments. The species composition of the examined microorganisms differed in individual years. In 2009, the following species were identified: *Alternaria* sp., *Fusarium* sp., *Verticillium*, *Leptosphaeria maculans*. In 2010, in the same location: *Alternaria alternata*, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, *Fusarium* sp. species were recorded. In the following year (2011), *Botryotinia Botryotinia fuckeliana*, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, *Fusarium* sp. and *Sclerotinia sclerotiorum*. In order to detect the above-mentioned species, the total of 350 DNA ITS sequencing analyses were performed. During the 3-year period of analyses (2009–2011), the proportion of the most dangerous rapeseed oil pathogens, i.e. fungi from the *Leptosphaeria* sp. genus was found to be at approximately 10%.

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Distinct sub-populations of *Fusarium proliferatum* isolated from various host plant species

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Fusarium proliferatum is a casual plant pathogen, known to be able to synthesize a wide range of important and harmful mycotoxins, including fumonisins, beauvericin, moniliformin, fusaproliferin, fusaric acid and fusarins. The species used to be associated mainly with maize but it has been also frequently found in tissues of other crop plants, such as rice, wheat, asparagus, soybean, garlic and onion. Recently, during continuous research started in 2009, *F. proliferatum* strains have been isolated from various plant species, until now not being considered as common hosts for this pathogen. These included rye, *T. durum* wheat, tropical crops (pineapple and date palm), two ornamental orchid species (*Oncidium* sp. and *Cambria* sp.), and some crops of average or local importance, e.g. rhubarb and pea. At present, after five years of strain isolation, 84 *F. proliferatum* strains from 13 various host plant species are stored at the KF Collection of Pathogenic Fungi at the Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland. Genomic sequence and *in vitro* mycotoxin biosynthesis analyses were incorporated to characterize the differences between those strains. Majority of them have been clearly separated into distinct clades based on the genetic diversity analysis being in good accordance with the host plant origin (particularly garlic, asparagus, pea and pineapple strains). However, the abilities to synthesize fumonisins varied significantly and were independent on the origin. The strains isolated from the tissue of pineapple, garlic and asparagus plants displayed the highest mycotoxigenic abilities. Strains derived only occasionally from *Cambria* and *Oncidium* orchids, as well as from rhubarb and pea, presented rather medium and low toxigenicity (they produced FBs in amounts ranging between 46 and 798 μg/g in sterile rice cultures).

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Mycotoxigenic *Fusarium* species in *Triticum durum* grain grown in southern Poland during 2012 and 2013 seasons

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Triticum durum L. is a crop typically grown in countries of warm climate, *e.g.* in Italy and other Mediterranean countries. However, it can also be cultivated in southern parts of Poland, where the weather conditions are mild and somehow similar to those from the Southern Europe. Like other cereals, the species can be infected by a number of fungal pathogens, of which *Fusarium* species are among the most dangerous ones.

Three *T. durum* cultivars were grown in field trials in southern Poland. The genotypes have been bred Poland, Slovakia and Austria and were adjusted to maritime climatic conditions. Two sowing dates (optimum and delayed) and three different seed amounts (400, 500 and 600 seeds/m^2) were used, the experiments were run in triplicates in 2012 and 2013 seasons. *Fusarium* incidence was evaluated (disease score) and pathogenic species present in infected heads were identified using molecular analyses. Furthermore, mycotoxin (deoxynivalenol, zearalenone and moniliformin) levels were measured in the harvested grain.

Fusarium graminearum and *F. avenaceum* were found as prevailing species among all tested samples and there were significant differences in disease severity among the cultivars. *F. culmorum*, *F. poae*, *F. tricinctum* and *F. sporotrichioides* were found only occasionally. It can be concluded that the species composition is rather typical for this region of Poland.

In 2013 the infection was heavier than in 2012, which was confirmed by the analysis of mycotoxin contents, e.g. top values for DON content in 2012 reached 1 μg/g, while in 2013 there were 10 times higher. Similar observations were stated for ZON and MON contents. In fact, in 2012 season MON amounts were below detection limit in all grain samples.

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Trichothecene-producing *Fusarium* species in Lithuanian wheat

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Trichothecenes (TRI) are the main mycotoxins associated with Fusarium head blight (FHB) of wheat and other small-grain cereals, with worldwide economic and health impacts. *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae* and *Fusarium sporotrichioides* are the chief causal agents of FHB and TRI producers.

This work was aimed to monitor the distribution of the major trichothecene-producing *Fusarium* species in the Lithuanian commercial fields of spring and winter wheat. In 2013, surveys of FHB incidence and severity were conducted at milk maturity stage (BBCH 77) in 56 spring wheat fields in 11 districts. *Fusarium* species composition, deoxynivalenol (DON) and T-2/HT-2 toxin contents were quantified in harvested spring and winter wheat grain (95 samples from 19 districts).

The data showed that FHB incidence varied from 3.4% to 80.3%, severity from 0.7% to 12.7%. On average 50.5% of spring wheat and 26.5% of winter wheat harvested grains were affected by *Fusarium* fungi. In the grain of both wheat types, *F. graminearum* and *F. avenaceum* were the dominant species, followed by *F. poae* and *F. sporotrichioides*. *F. culmorum, Fusarium tricinctum, Fusarium equiseti* and some other species were rarely found. *F. graminearum* infected from 0 to 51.6% (average 14.9%) of spring wheat grain and the infection level correlated ($r = 0.51$; $p \leq 0.1$) with DON content. In Lithuania, like in other European countries, there was found a relationship between FHB disease, *Fusarium* infection and DON concentration and the amount of rainfall during wheat flowering – maturity period.

The abstract presents research findings obtained through the long-term research programme "Harmful organisms in agro and forest ecosystems" implemented by Lithuanian Research Centre for Agriculture and Forestry.

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Newly detected plant pathogenic viruses and their collection in Crop Research Institute, Prague

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Pathogenic viruses are emerging throughout the Europe. Subsequently in the Czech republic, *Pepper mild mottle virus*, *Squash mosaic virus* and *Broad bean wilt virus-2* were detected on vegetables, and *Grapevine leafroll-associated virus 1* strains A and E, *Grapevine virus A*, *Grapevine virus B* and *Rupestris stem pitting associated virus* on grapevine in the Czech republic for the first time recently. All the determined viruses were collected from field grown plants in the Czech republic and deposited in the Virus collection in Crop Research Institute in Prague. This collection consists of sixty-six plant viruses and strains maintained on indicator plants in a greenhouse: *Barley yellow dwarf virus*, *Brome mosaic virus, Potato leaf roll virus, Turnip mosaic virus*, *Wheat dwarf virus* and *Wheat streak mosaic virus* or stored frozen and dried in host plant leaves: *Apple chlorotic leaf spot virus, Alfalfa mosaic virus*, *Arabis mosaic virus, Apple stem grooving virus, Broad bean wilt virus-1*, *Broad bean wilt virus-2*, *Bean common mosaic virus, Cherry leaf roll virus, Cauliflower mosaic caulimovirus, Cucumber mosaic virus, Hop mosaic virus, Lettuce mosaic virus, Myrobalan latent ring spot virus, Pepper mild mottle virus*, *Potato potyvirus Y*, *Strawberry latent ring spot virus, Squash mosaic virus*, *Tomato aspermy virus*, *Tomato black ring virus*, *Tomato mosaic virus*, *Turnip yellow mosaic virus*, *Watermelon mosaic virus 2* and *Zucchini yellow mosaic virus*. Fruit tree and grapevine viruses: *Apple stem pitting virus, Grapevine virus A, Grapevine virus B, Grapevine leafroll-associated virus 1*, *Rupestris stem pitting associated virus*, *Grapevine fleck virus*, *Grapevine Red Globe virus* and *Plum pox virus* are maintained on woody plants in a technical isolate. The viruses are regularly inoculated to new plants and their presence is proved by symptoms, ELISA, RT-PCR and electron microscopy. The collection details are available on the web page: http://www.vurv.cz.

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Identification and assessment of genetic diversity of the fungal pathogen *Mycogone perniciosa* using PCR method

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Mycogone perniciosa (Magnus) Delacroix is the fungal pathogen causing wet bubble disease of white button mushrooms *Agaricus bisporus*. The disease results in severe crop loss. The pathogen may infect *A. bisporus* at various stage of development. The main symptoms of infection are undifferentiated forms of mushrooms tissue, cap spotting and development of amber liquid droplets on the distorted mushrooms. The aim of research was the morphological characteristic and molecular identification and assessment of the genetic diversity among *M. perniciosa* isolates collected during 2008–2011. The pathogens were isolated from diseased fruiting bodies of *Agaricus bisporus* from different polish mushroom houses. The pathogenicity of isolates was proved in accordance with the Koch's postulates *in vivo*. Morphological identification of isolates was done on the basis of colony structure, growth rate on different agar media (PDA, YGC, CYA, Sabouraud). Seventeen isolates of *Mycogone perniciosa* isolated from diseased fruit bodies of *A. bisporus*, two reference isolates *Hypomyces pernicious:* CBS 322.52, CBS 815.73 and one reference isolate *Mycogone rosea* were studied.

Based on morphological characteristics, the mycelium developed best on Sabouraud and YGC medium and most strains reached 75–80 mm after 10 days. Mycelium of tested isolates was copious and flocculent on these media and around 6 day of incubation mycelium turned dark brown upon the production of chlamydospores. Moreover, on Sabouraud and YGC media earlier sporulation was observed than on CYA.

PCR method and ITS region sequencing were done to identification of the isolates. To amplification of ITS region was used specific primers ITS1 and ITS4. PCR amplification of all *M. perniciosa* isolates with ITS1and ITS4 primers yielded an estimated 600-bp product. Most isolates showed 96,1–97,7% sequence similarity with reference isolate CBS 815.73 and 96,3–98,2% with isolates CBS 322.52.

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Seasonal changes in the concentration of *Apple mosaic virus* in apple trees

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The status of *Apple mosaic virus* (ApMV), causal agent of apple mosaic disease, was investigated in the Czech Republic by examining plants of different species mainly belonging to the *Rosaceae* family. A survey was carried out in 2011–2013 during the growing season and a total of 200 plants were sampled and tested by RT-PCR. Based on our studies, in which the ApMV detection in several plant species (e.g. *Sorbus aucuparia*, *Chaenomeles japonica* and others) was often less clear-cut, in terms of amplicon amount, the titer of the virus in its hosts within the vegetation period was studied subsequently. Buds, petals, symptomatic and symptomless leaves from three different apple trees were tested by RT-PCR and ELISA using serial dilutions of the plant material. The highest virus concentration was observed in petals. ApMV was also detectable in buds, but there were no signs of the virus in bark. Only minor concentration differences were shown between symptomatic and symptomless leaves from the same tree. It was confirmed that virus content was relatively low in all trees and was changing during the vegetation. The virus concentration reached a peak in April to decrease slowly in the following months. This information should be taken into consideration when designing and implementing a certification schemes so as to reliably detect ApMV in plant material.

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The spike morphology and microbiome of grain of hybrids between *Triticum aestivum* and *T. spelta* and their contamination by toxigenic *Fusarium* pathogens

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Spelt (*Triticum spelta* L.) is a hexaploid wheat relative of common wheat (*Triticum aestivum* L.). Hybrids between spelt and common wheat may combine positive features of parental components and create forms which are tolerant of biotic and abiotic stressors yet maintaining high quality of flour. The aim of the study was to analyze chosen morphological features of spikes and the structure of grain/spikelet microbiome of *T. aestivum* and *T. spelta* as well as hybrids *T. aestivum* × *T. spelta* and *T. spelta* × *T. aestivum* in order to select genotypes which are least prone to contamination of grain with the toxigenic *Fusarium* fungi. The microbiological analyses were carried out using the method of washing off the bacteria and fungi from the surface of grain/spike and laying the grain/chaff out on selective agar medium. It was the *Azotobacter* and *Pseudomonas* bacteria which were the genera most often washed off from the grain/spikes. The communities of filamentous fungi and yeasts were not so numerous. Fungi belonging to genus *Fusarium* constitute on average over 50% of filamentous fungi. On *T. aestivum* kernels the *Pseudomonas* bacteria were dominant, while on the *T. spelta* spikes it were fungi of genus *Cladosporium* and *Fusarium*. Additionally from the grain and spikes were isolated *Alternaria alternata* and *Pyrenophora tritici-repentis*. Some *T. spelta* parental lines stand out with grain significantly less infected by the toxigenic *Fusarium* fungi than cv. Zebra (*T. aestivum*). The differentiated genotypes of hybrids determined the structure of grain's microbiome, which in some cases resulted in decrease in contamination with *Fusarium* pathogens. From the unhulled grain of hybrid UWM-13 (spelt) × Torka (common wheat) there were least *A. alternata* and *P. tritici-repentis* and no *Fusarium* colonies isolated.

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Genetic variation between *Fusarium anguioides*, *F. avenaceum* and *F. arthrosporioides* isolates

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F. anguioides isolates are found in different plants including cereals and they are closely related to *F. avenaceum* and *F. arthrosporioides*. Beta tubulin DNA sequences and ISSR (inter simple sequence repeat) fingerprinting patterns of 13 *F. anguoides* isolates were compared to those of eight *F. avenaceum* and two *F. arthrosporioides* isolates. All isolates are single spore isolates and they were originally identified morphologically. According to beta tubulin sequences there are three groups: *F. anguioides* isolate 47 (Vladivostok, from *Rudbeckia* sp.), which has some similarities with *F. tricinctum*, *F. anguioides* isolates 57, 53, 50, 49, 48, 41 and 52, which have similarities with *F. avenaceum* isolates of main group I, and the rest of *F. anguioides* isolates, which have similarities with *F. avenaceum* isolates of main group II.

According to the preliminary ISSR results *F. anguioides* isolates 37, 39, 42 and 45, which have identical beta tubulin sequences, are closely related to each other and form their own phylogenetic group. We will repeat the ISSR work and compare the beta tubulin sequences of the 23 isolates to known sequences to find out, if *F. anguoides* isolates form their own phylogenetic group.

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Applications of flow cytometry in plant pathology

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Flow cytometry (FCM) is multiparametric research technique of cells and micro particles which is more and more commonly used, both in clinical diagnostics and scientific research. Available FCM methods allowed to collect a wide variety of data including determination of physiological parameters and biochemical characteristics of cell population in short time. On the basis of flow cytometry it is also possible to provide information about the size, shape and complexity of both studied host and pathogen cells. These traits makes FCM as very promising tools in general microbiology as well as phytopathology. Performed preliminary tests confirmed the possibility of FCM applying in cell cycle analysis of plant pathogenic fungi, analysis of their genome size and evaluation of cytotoxic effect of plant compounds on spores of selected fungal species.

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Monitoring of Hosta virus X in Ukraine

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Hostas are popular shade plants. Gardeners love these plants for their wide range of sizes, textures and colors. Hostas are usually easy to grow. Viruses are an emerging and important issue in growing hostas and HVX is generally considered to be the most economically important virus infecting hostas. Despite the observation of virus like disorders on hosta plants the occurrence of HVX in Ukraine has not been investigated. Considering this, collection of hostas maintained in M. Grishko's National Botanical Garden (NAS of Ukraine) was investigated for the presence of this virus.

During 2012–2014, a total of 73 hosta plants of 35 varieties displaying various virus-like symptoms including mosaic, mottling leaf chlorosis and leaf distortion were collected in botanical garden. Thirty-three of the 73 samples gave a positive result.

The total viral RNA was extracted from leaves of hosta Sum and substance with symptoms and subjected to RT-PCR using primers PHVXCP5 upstream primer, 5'-AGTCTCGAACTAAC-TAACAGG-3, and PHVXCP3 downstream primer 5'-TCGGTGGAGCCTTGTTTATTG-3 (M. H. Park & K. H. Ryu 2003). The amplification products was sequenced in both directions. The consensus sequence from obtained clone was deposited in the GenBank (ID BankIt1695170). The next step in our work was alignment of sequences. Comparison of deduced nucleotide sequences of CP with the isolates from GenBank, showed 100% identities with USA's isolate (XVH USA Sum and substance).

Hosta is a popular perennial ornamental plant and is in high demand for landscaping. As HVX has had a significant economic impact on hosta growers and can be easily spread the EPPO Secretariat added this virus to the EPPO Alert List in August 2013 (EPPO Reporting Service, 2013). So it is necessary to take measures to reduce the spread of HVX. The use of ELISA or PCR for routine screening of plants can help detect infection before it becomes a major problem. Early detection is critical so that infected plants can be removed from collections, preventing further distribution of the virus.

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Health status and yielding of durum wheat in climatic-soil conditions of selected research region in Poland

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Three years field research aimed at an assessment of health status and assimilation area, WTG and yield of three Slovak durum wheat cultivars, cultivated using intensive and moderate-intensive agrotechnology in climatic-soil conditions of Prusy localized near Krakow (Malopolskie Voivodeship, Poland) was performed. Conducted analysis of plants health status demonstrated an occurrence of significant intensity of Fusarium foot rot, eyespot, powdery mildew, leaf septoria, stripe rust as well as black molds, Fusarium head blight and septoria nodorum blotch. The factors considerably affecting plants health status, as well as LAI, WTG and the yield, were climatic conditions of the vegetation season and an intensity of cultivation technology. An application of intensive agrotechnology caused a limitation in an occurrence of most of fungal diseases and improvement in the quantity and quality of the yield obtained. Among the examined cultivars (Istrodur, Pentadur and Riveldur), Istrodur may be evaluated as yielding the best in the research conditions. Generally, the level of examined cultivars susceptibility on the diseases may be accepted as similar. Riveldur cultivar appeared to be susceptible on the strip rust, but is was less infested with the cause of eyespot. Istrodur was characterized by the lowest infestation by black molds cause and the highest susceptibility on leaves septoria. The symptoms of other diseases examined in this study were on a little differentiated level, and the differences noted between the cultivars were insignificant statistically. Compared cultivars of durum wheat may be recommended for cultivation in the research region and regions with similar climatic conditions.

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Effect of various kinds of yerba mate and nanosilver on selected phytopathogenic fungi under *in vitro* conditions

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Fungistatic effect of water extracts from four kinds of dry yerba mate (*Illex paraguariensis*): Pajarito instant, Pajarito con palo, Rosamonte despalada and Rosamonte con palo, applied in concentrations of 50 and 25 mm^3/cm^3 and effect of nanosilver (colloidal silver of 1–5 nm particles) on phytopathogenic fungi biomass: *Fusarium solani*, *var. coeruleum, Fusarium culmorum*, *Fusarium heterosporum* and *Sclerotinia sclerotiorum* was researched under laboratory conditions. Under *in vitro* conditions the effect of water extracts of Yerba mate and nanosilver on biomass of the analyzed fungi depended on the kind and applied concentration of the extract/nanosilver in the medium, and on fungus kind. Irrespective of applied concentration, all water extracts of *Illex paraguariensis* inhibited growth of tested fungi biomass more strongly than nanosilver. Considering the tested fungi species, *S. sclerotiorum* proved the most sensitive. Analyzed kinds of water extracts and nanosilver were inhibiting its biomass increments within the range from 62.5–88.5%. Water extracts of Yerba mate revealed the weakest fungistatic effect on *Fusarium solani var. coreuleum*, limited growth remained within the range from 2.5 to 34.5%. On the other hand, 10 mm^3/cm^3 concentration of nanosilver in the medium was stimulating this species biomass increment in 34%. Generally, higher concentrations (50 mm^3/cm^3) of water extracts of *Rosamonte con palo*, *Rosamonte despalada* and Pajarito instant inhibited increases in *F. culmorum* and *F. solani var. coreuleum* more strongly, whereas lower concentrations reduced the increments of *S. sclerotiorum* and *F. heterosporum* biomass to a greater degree.

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Assessment of the impact of chitosan on the selected plant pathogenic fungi

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Under *in vitro* conditions there was compared the effect of the fungistatic influence of Biochikol 020 PC (chitozan) and Biosept 33 SL (an essence of the grapefruit's pulp) on fungus such as: *Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium vermiculatum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Verticillium* spp., into PDA culture medium there were cultured the biopreparations in the quantity of 2,5, 1,0, 0,5 mm^3/cm^3 and their fungal culture test was carried out. Based on the survey found that irrespective of concentration applied Biochikol 020 PC has a much smaller fungistatic impact for fungi such as: *F. culmorum*, *F.oxysporum*, *F. solani*, *Verticillium* spp., *R. solani* and *P. vermiculatum* than Biosept 33 SL. In relation to the above-mentioned species higher concentration of Biosept 33 SL in culture medium (2.5 mm^3/cm^3) shows a stronger inhibition of fungal growth than lower concentrations. Among the tested fungi: *F. culmorum* and *P. vermiculatum* proved to be most sensitive to Biochikol 020 PC. In all concentrations used the inhibition effect of area growth of colonies were respectively at: 60.78–68.43% and 52.65–63.67%. The species of fungi *Verticillium* spp, *R. solani*, *F.oxysporum*, *F. solani* were averagely resistant to Biochikol 020 PC and its concentration. Reflection of this is the average factor value of linear mycelium growth inhibition ranging from 44.02% to 30,09%. However, there was no fungistatic influence of Biochikol 020 PC concentrations on a species *S. sclerotiorum*.

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Infestation by pathogens of leaves and stem base of *Triticale* and *Secale cereale* fertilized with microelements

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Winter triticale (*Triticale*), Dinaro cultivar and winter rye (*Secale cereale* L.), Dańkowskie Diament cultivar, were cultivated in 2012 and 2013 in a strict plot experiment in Tomaszkowo near Olsztyn. Seven fertilisation objects were included in the study: 1. control (no fertilisation); 2. NPK (90 kg N · ha^{-1}, 70 kg · ha^{-1} P_2O_5 and 100 kg · ha^{-1} K_2O); 3. NPK (as in object "2") + foliar fertilisation with 0.2 kg Cu · ha^{-1}; 4. NPK + 0.2 Zn kg · ha^{-1}; 5. NPK + 0.2 kg Mn · ha^{-1}; 6. NPK + Cu + Zn + Mn; 7. NPK + Nano Gro. The degree of infestation of the cereal leaves and stalks by pathogens during the period of vegetation was assessed.

Powdery mildew of cereals and grasses appeared with small intensity only on leaves of triticale and winter rye solely in 2013. The weather conditions in the 2012 season favoured the development of septoria tritici blotch and brown rust on triticale (the largest infestations were 41.6% and 23.8%, respectively) and rye scald and brown rust on rye (29.0% and 38.1%). Foliar fertilisation with microelements differentiated the extent of infestation of triticale leaves by *Septoria tritici* and *Puccinia recondita*. The stalks of both species were used to identify brittleness symptoms of cereal stalks and fusariosis. Sporadic symptoms of root rot of cereals and rhizoctoniose were also recorded. Significant differences were found between the experiment objects in terms of the intensity of infestation of the triticale culm base by *Gaeumannomyces graminis* and of rye by *Pseudocercosporella herpotrichoides* and *Rhizoctonia* spp.

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Influence of cultivation system and forecrop on colonization of spring wheat grain by fungi

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Cereal grain colonization by pathogenic fungi is affected among other things by availability of pathogen inoculum, weather conditions during plant vegetation and employed cultivation systems. The aim of the study was to determine influence of cultivation method and forecrop on colonization of wheat grain by fungi. The experiment was carried out in the years 2012 and 2013 in the fields of the Field Experimental Station of the Institute of Plant Protection of the National Research Institute (PSD IOR PIB) in Winna Góra. Spring wheat cultivar Żura was sown after four different forecrops: rape, beet, corn and wheat. Soil was prepared for the experiments with the traditional and reduced tillage system. The wheat grain collected in the experiments was placed on medium to determine grain colonization and share of species of pathogenic fungi. The greatest colonization of spring wheat grain by fungi of the genus *Fusarium* in the both years of the research was observed in cultivation after beet. Predominant species included *Alternaria alternata*, *Epicocum purpurescens*, *Cladosporium herbarum* and *Botrytis cinerea*.

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Insights on the interactions between the nut rot agent *Gnomoniopsis castanea* and the Chinese gall wasp *Dryocosmus kuriphilus* on chestnut

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Gnomoniopsis castanea is an emergent fungal pathogen causing nut rot in chestnut trees.

In Italy its occurrence was detected in conjunction with the invasion of *Dryocosmus kuriphilus*. This exotic pest lays eggs inside the chestnut buds inducing galls formation and reducing the development of leaves and flowers. To assess if an ecological association between the fungus and the insect exists, the following hypotheses were tested: I) whether adults of *D. kuriphilus* may be vectors of viable inoculum of *G. castanea*; II) whether the presence of *G. castanea* in the galls is correlated to the number of inhabiting insects; III) whether the presence of *G. castanea* in the buds and oviposition may be related.

I) From 323 galls sampled in three different sites, 339 emerging adults of *D. kuriphilus* and five fragments per gall were plated to isolate *G. castanea*. The fungus was not isolated neither from the 53% of insects coming from galls colonized by *G. castanea*, nor from the others. This finding suggests it is unlikely that adults of *D. kuriphilus* could carry viable inoculum of *G. castanea*.

II) The above experiment showed also that, on average, galls colonized by the fungus host a significantly higher number of insects if compared to the others (3.76 *vs.* 2.54; $P < 0.05$). This may indicate a possible synergy between *G. castanea* and *D. kuriphilus*.

III) Before oviposition time from 350 buds sampled in the same sites *G. castanea* was isolated on average in 33% of samples. After oviposition time other 350 buds were inspected both for *D. kuriphilus* eggs and for *G. castanea* presence. The odds ratio of 0.98 with a 95% confidence interval between 0.71 and 1.33 indicates the absence of association between the two phenomena. This demonstrates that the fungus can colonize galls tissues before and independently from *D. kuriphilus* oviposition.

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Bacterial and fungal communities in the rhizosphere of pea (*Pisum sativum* L.) after applying of Miedzian 50 WP and grapefruit extract

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The present studies determined the effect of fungicide Miedzian 50 WP and grapefruit extract on the microorganism population in the rhizosphere of pea. Before the sowing, the seeds of 'Sześciotygodniowy TOR' cv. pea were dressed with 0,2% Grevit 200 SL (grapefruit extract 200 g · dm^{-3}) and the chemical preparation Miedzian 50 WP (50% oxychloride of copper) in the quantity of 2g · kg^{-1} seeds. The seeds that were not dressed constituted the control.

The microbiological analysis of the rhizosphere soil of pea showed that the population of bacteria in 1g d.w. of soil in the treatment with Grevit 200 SL and Miedzian 50 WP, both in the seedling phase and at anthesis, was significantly higher than in the control treatment. The total amount of bacteria ranged, on an average, from 2.15 × 10^6 to 3.57 × 10^6 cfu · g^{-1} d.w. of soil at the seedling phase and from 2.85 × 10^6 to 4.96 × 10^6 cfu at anthesis. The total amount of fungi was, on an average, from 4.34 × 10^3 to 8.86 × 10^3 cfu at the seedling phase and from 5.12 × 10^3 to 9.98 × 10^3 cfu at anthesis. *Bacillus* spp. were more frequently isolated from the rhizosphere of pea than *Pseudomonas* spp. More studied microorganisms were isolated from the rhizosphere of pea at anthesis than in the phase of seedlings.

Fewer fungi isolates were obtained from the rhizosphere in the seedling phase than at anthesis. *Fusarium oxysporum* and *Thanatephorus cucumeris* were most frequently isolated. Besides, the following fungi were often isolated: *Alternaria alternata, Botrytis cinerea, Fusarium culmorum, Sclerotinia sclerotiorum, Mucor* spp., *Rhizopus nigricans, Acremonium strictum*. *Trichoderma* spp. dominated among saprophytic fungi in the rhizosphere of pea, and they were more abundant in the treatment with Grevit 200 SL and Miedzian 50 WP as compared to the control.

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Air temperature in vertical profile of winter rape stand

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Air temperatures were monitored in oilseed rape canopy in Zabcice (Central Europe, South Moravia) during the main growth season in 2010–2012. Automatic sensors were positioned at three levels (on the ground, at the effective height and at 2 meters above the ground) in order to cover the whole vertical profile. The differences in vertical stratification of air temperature were pronounced especially during the light part of the day. The highest temperatures were recorded in rape effective height, usually. The air temperature in this level was by 1–2°C higher in all rape developmental stages. The ground temperatures were significantly lower in comparison with temperatures measured at 2m in dependence on year, rape developmental stage and part of the day. The differences were 2°C in the cold period of 2010 and 5°C or 3°C in warm period in 2011 and 2012 during the stage of flowering, respectively. Similar differences were recorded also in the developmental stages of fruits and ripening. These findings can be used in making more accurate prediction models of pathogens and pest occurrence on winter rape.

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Micromycetes on infested flowers and seeds of evergreen rhododendron *Rhododendron* L.

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In 2010–2012, research was conducted on 10 taxa of evergreen rhododendron *Rhododendron* L. that were infested by fungus growing on flower buds, flower petals and seeds. Mycological analysis was conducted on 1,500 specimens of buds and petals and 500 rhododendron seeds. It was shown that necrosis and dieback of buds were caused by complex *Micromycetes* (43 species), with dominants *Pestalotiopsis sydowiana*, *Alternaria alternata*, *Truncatella truncata* and *Epicoccum nigrum*. Watery, brown spots on the flower petals, resulting in the dieback of flowers, were caused by 38 species, including the most common *P. sydowiana*, *A. alternata* and *Trichoderma viride*. The seeds were contaminated by 18 species, and in addition to the above, the following were numerous: *Oidiodendron tenuissimum*, *Davidiella macrocarpa* and *Phoma leveillei*. The research revealed which taxa attracted the largest number of colonies and species of fungi. The similarity coefficient was calculated for the communities of fungi isolated from the plant material of the rhododendron taxa. The results of the mycological analysis confirmed the diversity of *Micromycetes* species that inhabit the infested rhododendron flowers and seeds.

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Micromycetes colonizing and damaging leaves of evergreen rhododendron *Rhododendron* L.

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In May and October 2010–2012, mycological studies were conducted on 10 cultivars of rhododendron bushes growing in containers in the nursery of ornamental plants. Out of 3,000 specimens of infested leaf fragments 2,566 fungal colonies belonging to 41 species were isolated. The following species colonising the leaves and causing their necrosis were extracted in the largest number of colonies: *Alternaria alternata*, *Aspergillus brasiliensis*, *Epicoccum nigrum*, *Humicola grisea*, *Pestalotiopsis sydowiana*, *Phoma laundoniae*, *Sordaria fimicola*, *Trichoderma koningii*, *T. polysporum*, *Truncatella truncata*, *Umbelopsis isabellina* and others. The research showed that the *Micromycetes* colonies colonizing and damaging rhododendron leaves varied in species composition and the number of colonies in different years and dates. The study determined which rhododendron cultivars were characterised by high health and which had the greatest susceptibility to infection by *Micromycetes*.

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Development of an air dry multiplex PCR master mix in detection of two important soil borne fungi (*Rosellinia* and *Armillaria*)

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Root rot disease caused by *Rosellinia* and *Armillaria* species is one of the most destructive diseases on many woody plants worldwide. Rapid detection of these pathogens in the field prior to occurrence of significant symptoms can help to determine the precise control time. In this study, we developed an air master mix based on multiplex PCR method for preparing ready-to-use master mix to detect both of these important soil borne fungi directly from the soil. Fungal DNA was extracted from soil and amplification was carried out with R10-R7 and AR1-AR2 primers. The stability of the *Taq* DNA polymerase was improved using stabilizers such as sucrose, mannitol and trehalose in the air-dried PCR mix. Recent prepared PCR mix was able to maintain the activity of *Taq* DNA Polymerase at room temperature for 60 days, when evaluated with DNA extracted from 250 pg/kg dried mycelium of each *Rosellinia* and *Armillaria* in soil. This method enables the detection of these fungi using species-specific primers directly from soil samples without the need to prior isolation and cultivation of fungi. According to our findings, trehalose indicates more improvements on PCR reaction compared to other stabilizers when used in air drying treatment. The stabilization of *Taq* DNA polymerase can help easily handling and long storage at room temperature. Hence, supplying the air dried PCR master mix appears to be a simple and low cost technique; we report this technique, for supplying the simple clinical diagnostic detection kit.

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Analysis of Grapevine and Tree Fruit virus collections using Next Generation Sequencing

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The Canadian Food Inspection Agency in Sidney, and the Pacific Agri-Food Reseach Centre in Summerland have collections of grapevine, treefruit and small fruit virus. Over the last 2 years, several hundred specimens have been analyzed using next generation sequencing (NGS) to identify the viruses present. This work was initiated to develop a reference database as an initial step to bringing NGS diagnostic methods into plant virus testing laboratories. Many specimens contain more than one virus species or multiple variants of a species. Initial data will be presented from this work.

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Monitoring evolution over time in the Fusarium wilt of date palm (*Fusarium oxysporum* f. sp. *albedinis*) on various varieties from arid zones in Algeria and Spain

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Fusarium wilt is the most serious disease of date palm in North Africa. It is caused by a soil-borne fungus, *Fusarium oxysporum* f. sp. *albedinis*. The objective of this work is to monitor the disease over time. An experiment was conducted to study the effect of inoculation of spore suspension of two isolates of *F. oxysporum* f. sp. *albedinis* on three well known Algerian varieties (DN, TAN, TIM) and a Spanish variety Elche (VE) at two stages of young seedlings. At first, the results indicate that the most sensitive variety is the variety DN whatever the strain tested. In addition, TIM and TAN varieties have a difference of sensitivity with respect to the strain used. However, the variety (VE) has a very low sensitivity. Moreover, the second observation, two-leaf stage, shows that the variety TIM is the most susceptible variety, comes after the DN range, and the other two varieties (TAN, VE) have a tolerance of the disease.

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Monitoring of pectinolytic bacteria originating from potato (*Solanum tuberosum* L.) plants and water samples

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Pectinolytic bacteria classified to the genera *Pectobacterium* and *Dickeya* (Hauben et al., 1998; Samson et al., 2005) are causative agents of blackleg and soft rot in economically important plants such as potato, tomato or maize. Due to the fact that the control of mentioned diseases is based only on prevention methods, our research group monitors potato fields and surface waters every year, looking for bacteria from the genera *Pectobacterium* and *Dickeya*. Generally, we perform the cultures of plant homogenates and water samples on selective CVP medium in 28°C to detect and isolate pectinolytic bacteria. The identification of *Pectobacterium atrosepticum* (Pca), *Pectobacterium carotovorum* (Pcc)/*Pectobacterium wasabiae* (Pwa) and *Dickeya* sp. (Dsp) is based on multiplex PCR test (Potrykus et al. 2012, patent). Additional PCR reactions are performed for Pwa and *D. solani* (De Boer et al., 2012; Prichard et al., 2012). In 2013, we examined 124 potato stems, 74 tubers, 50 weeds and 1866 water samples, obtained from the The State Plant Health and Seed Inspection Service. We detected pectinolytic bacteria belonging to the genera *Dickeya* and *Pectobacterium* in 73 samples of plant material – 21 Pcc, 30 Pwa, 23 Pca and 7 Dsp. There were 10 cases of mixed infection. Concerning water samples analysis – 19 of them were positive for pectinolytic bacteria: 7 Pcc, 6 Pba and 6 Dsp. The most virulent species, *D. solani* and *P. wasabiae* were identified in the plant material only. The analysis of vivodenships, where we have found pathogens of interest, revealed that *Pectobacterium* spp. are widespread all over the territory of Poland, while *Dickeya* spp. are limited only to certain provinces (Greater Poland, Pomerania, Mazovia, Podkarpacie). In comparison to the results from monitoring studies performed in previous years the percentage of Pca isolates decreases, while the amount of Dsp and Pcc/Pwa fluctuates over time.

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Fusarium spp. in Norwegian potatoes

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Fusarium dry rot is one of the most important postharvest diseases in potato. The disease is caused by several species of *Fusarium*. Previously *F. coeruleum* was the most common *Fusarium* species isolated from symptomatic potatoes in Norway. In the last decade, problems with Fusarium dry rot has increased in Norway and therefore an extensive survey was started to investigate which *Fusarium* species are currently causing the problem. In three growing seasons (2010–2012), potato samples (238) were collected from fields in potato growing regions spread all over Norway. Seven different species of *Fusarium* spp. were detected and *F. coeruleum* and *F. avenaceum* were most commonly found. The identification of the species was based on morphological characterization and confirmed with molecular methods. Correlations between *Fusarium* spp. and agronomic and storage factors were examined. Cultivar was the only factor that had a significant effect on the presence of *Fusarium* spp. Regional differences were also found regarding incidence of the different *Fusarium* spp. Another important task in our project is to implement and / or develop molecular detection methods for use in postharvest diseases in potatoes, in order to detect potential disease causing *Fusarium* species just after harvest, before the tubers are put into storage. We tested previously developed real-time PCR methods for detection of species found in the *Fusarium* survey and developed a new real-time PCR method for *F. coeruleum*. Multiplex real-time PCR for detection of individual *Fusarium* species was not successful.

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Molecular phylogeny and diversity of apple pathogen *Venturia inaequalis* (Cooke) Wint.

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The apple scab caused by *Venturia inaequalis* (Cooke) Winter is an economically important disease worldwide. The pathogen populations differ among the regions due to the host genotypes grown and management strategies. *Venturia inaequalis* has a high adaptation ability resulting in breaking of cultivar resistance or forming resistance to fungicides. The collection, consisting of more than 150 monoconidial *Venturia inaequalis* isolates originating from 27 *Malus* genotypes and different locations in Latvia, was established to characterize the diversity of the pathogen. Nucleotide sequences of portion of the 18S ribosomal DNA, ITS1-5.8S-ITS2 and *tef-1a* gene from 49 *Venturia inaequalis* isolates and available homologous sequences in the databases were compared using phylogenetic analyses to characterize genetic diversity of Latvian *Venturia inaequalis* isolates. Both, introns in 18S rDNA gene containing and non-containing strains of *Venturia inaequalis*, were present among the studied isolates. The diversity in relation to geographic origin, host genotype and virulence is discussed.

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Bacterial wilt caused by *Ralstonia solanacearum* in Georgia

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Ralstonia solanacearum causing bacterial wilt is an economically important pathogen worldwide. It is a gram-negative, soil borne bacteria, known for its high polymorphism. Diverse pathogen populations are usually represented by races, sub-races, biovars and biotypes. The *R. solanacearum* 'species complex' includes five races and five biovars. Identification of *R. solanacearum* biovar is based on its ability to metabolize alcohol and carbohydrates. The most widespread component of *R. solanacearum* species complex is race 3 biovar 2. It is present in nearly 80 countries, including UK, The Netherlands, Sweden and others. The economic loss reaches 950 million USD each year. The bacterium is included into the quarantine list A2 of EPPO and also in the quarantine list of Georgia.

In 2012–2014, we obtained 67 bacterial isolates from wilting potato, tomato, eggplant, pepper and ornamental plants located in different regions of Georgia. The isolates were identified as *R. solanacearum*, based on culturing on a semi selective medium SMSA and species-specific PCR analyses using primer pair OLI1/Y2. Diversity within the Georgian isolates was determined based on biovar determination and pathogenicity on host plants.

On the basis of biochemical, phenotypic and pathogenic characteristics 3 biovars were identified. The most important was biovar 2, an equivalent of race 3. This race appeared in potato industrial region of Georgia, Akchalcikche. Biovar 2 strains are characterized by low growth temperature 20–25°C and good adaptation to Georgian cool climate. *Ralstonia solanacearum* have many different host plants in Georgia, what causes big biodiversity. This is the first report on *R. solanacearum* in Georgia.

11th Conference of the European Foundation for Plant Pathology

Session 4

Genomics, proteomics and bioinformatics

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Genetic diversity of *Puccinia triticina* populations from Romania analysed by RAPD technique

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Different molecular markers have been used to describe the worldwide virulence and molecular diversity of the wheat leaf rust fungus *Puccinia triticina*. However, little is known about the molecular polymorphism of the *P. triticina* populations from eastern part of Europe, especially from Romania. Therefore, the objective of the study was to analyse ten populations of *P. triticina* collected between 2011 and 2013 from different fields (Fundulea, Livada, Pitesti), using randomly amplified polymorphic DNA (RAPD) technique with 8 primers (UBC 402, 489, 450, 517, 519, 521, 538, 556). The molecular phenotypes distinguished by each primer generally varied by one to three bands, exception the primer UBC 521 which the RAPD profiles were identical for all *P. triticina* populations. Moreover, the molecular analysis of population DuRes 14 and the single-uredinial isolate MP 26 (developed from population DuRes 14) with primers UBC 519 and UBC 538 showed a clear intrapopulation molecular differentiation. Similar result was obtained with a simple sequence repeats (SSR or microsatellite) primer PtSSR 184 that allowed the detection of the highest polymorphism among the individual isolates.

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Molecular phylogeny of cercosporoid in native plants from Cerrado

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Cercospora and *Pseudocercospora* species are among the most prevalent and destructive of plant pathogens and they can be found on leaves, pedicels, stems, fruits, and bracts. They occur in arid as well as wet environments and in a wide range of climates including cool temperate, sub-tropical and tropical regions. In this study, Monosporic cultures of the isolates studied which were identified as members from the genera *Cercospora* and *Pseudocercospora*, obtained from the native plants from Cerrado (*Aegiphila* sp, *Annona* sp, *Eichornia* sp, *Eremanthus* sp, *Hydrangea* sp, *Palicourea* sp, *Sida* sp, and *Tabebuia* sp) were used for genomic DNA extraction. Partial sequences were derived from the internal transcribed spacer regions and intervening 5.8S nrRNA, actin, calmodulin, histone H3 and translation elongation factor 1-alpha genes were used to compile a molecular phylogeny. Phylogenetic analyses were carried out using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Phylogenetic analysis from the sequences resulted in two well-supported clades corresponding to *Cercospora* and *Pseudocercospora*, with bootstrap values greater than 60% for each clade. This work is the first report of *Cercospora* sp in the host *Vernonanthura* sp.

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Phylogenetic study of *Fusarium* species based on the *CYP51* gene analysis

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The *Fusarium* species are common pathogens of various crops, also cereals, infecting plants in all development stages. In Poland the most important species are: *F. culmorum*, *F. graminearum* (*Gibberella zeae*), *F. poae*, *F. tricinctum* and *F. avenaceum*. Sterol C14-demethylase is an enzyme of the sterol biosynthesis pathway. The fungi may have from one to three C14-demethylase gene variants (*CYP51A*, *CYP51B* and *CYP51C*). The *CYP51*C seems to be specific gene variant of the *Fusarium* genus. Understanding the structure and variability of *CYP51* gene can be useful for the evaluation of genetic similarity of *Fusarium* species. The aim of the study was to analyze the gene sequence *CYP51*C gene of different *Fusarium* species. Isolates: *F. avenaceum*, *F. culmorum*, *F. graminearum* (*Gibberella zeae*), *F. poae*, *F. tricinctum* and *F. oxysporum*, collected in Poland were selected for the study. PCR primers: F1 and R1, were used for the amplification of the part of *CYP51C* gene. Sequence analysis was performed using MEGA software. The differences in the *CYP*51C gene were found between tested *Fusarium* species. The analysis provide clear division of the isolates into four separated groups. The first include all *F. poae* isolates, the second *F. culmorum* and *F. graminearum* isolates. *F. avenaceum* and *F. tricinctum* were assigned into the third group and the fourth group consists of a single isolate of *F. oxysporum*.

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Expressional regulation of pathogenicity related an endoglucanase gene, *eglXoA* in *Xanthomonas oryzae* pv. *oryzae*

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eglXoA, eglXoB, and *eglXoC,* which encode endoglucanases are clustered in a single region of the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) genome. A pathogenicity assay revealed that the mutant strains *eglXoA*::Tn5 and *eglXoB*::Tn5 were completely virulence deficient in rice leaves, while mutant *eglXoC*::Tn5 was non-pathogenic. Reverse transcription (RT)-PCR was carried out to analyze the transcriptional linkage between *eglXoA*, *eglXoB*, and *eglXoC* within the cluster. *eglXoA, eglXoB*, and *eglXoC* did not affect transcriptional expression of each other. RT-PCR showed that the *in trans eglXoA* was transcriptionally regulated by HrpX, a type III secretion regulator, and cyclic AMP receptor-like protein (ClpXo), which is a global regulator. Western blot analysis showed that EglXoA is secreted via a type II secretion system, and the hybridized signal to anti-EglXoA antibodies was detected in wild-type strain KACC10858 but not in mutant strains *hrpX*::Tn5 and *clpXo*::Tn5. In an electrophoretic mobility shift assay, the promoter region of *eglXoA* directly bound to ClpXo. Two consensus *eglXoA* upstream regions include putative Clp-binding sites with a perfect TCACA-N block in the left arm and a 2/5 matched block, TGT, in the right arm. *eglXoA* encoding endoglucanase appears to be the first *Xoo* gene known to be activated by Clp via direct binding to the promoter region.

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The Czech National Programme on Conservation and Utilization of Genetic Resources of Microorganisms Important for Agriculture

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The Czech National Programme on Genetic Resources of Microorganisms Important for Agriculture is directed by Czech Ministry of Agriculture.

The aim:

- conservation of genetic resources of microorganisms *ex situ* in collections, safe maintaining of the genetic resources and their biodiversity,
- record keeping and documentation of genetic resources, their evaluation on molecular level and possibility of utilization for agriculture,
- enhancing the international cooperation, exchange of genetic resources.

The National Programme consists from 20 collections of genetic resources held in 12 different bodies:

- Collection of Plant Pathogenic Viruses (Crop Research Institute Prague – CRI)
- Collection of Potato-Pathogenic Viruses (Potato Research Institute Havlickuv Brod)
- Collection of Fruit Trees and Small Fruit Viruses (Research and Breeding Institute of Pomology Holovousy)
- Collection of Ornamental Plant Viruses (Research Institute for Landscape and Ornamental Gardening Pruhonice – RILOG)
- Collection of Phytopathogenic Bacteria (CRI)
- Culture Collection of Rhizobia (CRI)
- Collection of Phytopathogenic Fungi (CRI)
- Culture Collection of Basidiomycetes Important for Agriculture (Institute of Microbiology, Academy of Sciences, Prague)
- Culture Collection of Fungi Important for Agriculture and Food Industry (Charles University Prague)
- Collection of Fungi Important for Horticulture – Macromycetes (CRI)
- Collection of Rusts and Powdery Mildew (CRI)
- Collection of Phytopathogenic Microorganisms (Palacky University Olomouc)
- Collection of Brewery Microorganisms (Research Institute of Brewing and Malting Prague)
- Culture Collection of Dairy Microorganisms (MILCOM a.s. Tabor)
- Collection of Industrially Utilizable Microorganisms (Food Research Institute Prague)
- Collection of Animal Pathogenic Microorganisms (Veterinary Research Institute Brno)
- Collection of Hop Pathogens (Hop Research Institute Zatec)
- Collection of Invertebrate Crop Pests and their Natural Enemies (CRI)
- Collection of Stored-Product Pests, Mites and Fungi (CRI)
- Collection of Phytopathogenic Oomycetes (RILOG)

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Isolation and structural analysis of *Xanthomonas campestris* pv. *campestris* resistance genes in *Brassica oleracea* L.

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Xanthomonas campestris pv. *campestris* (Xcc) is a gram-negative bacterium that causes black rot, the most important disease of vegetable brassica crops worldwide. Pathogen infection in plants is often limited by a multifaceted defense response triggered by resistance genes. The most prevalent class of resistance proteins includes those that contain a nucleotide-binding site-leucine-rich repeat (NBS-LRR) domain. These genes are very numerous in the plant genome, and they often occur in clusters at specific loci following gene duplication and amplification events. To date, hundreds of resistance genes and relatively few quantitative trait loci for plant resistance to pathogens have been mapped in different species, with some also cloned. This has allowed the identification of candidate genes for resistance, and the development of molecular markers linked to R genes. This study selected 29 NBS-LRR domain containing resistance genes of *Brassica oleracea* based on previous studies and investigated their polymorphism in 5 Xcc resistant and 6 susceptible cabbage lines using their promoter regions. Among them only one gene showed polymorphism and this gene was cloned in these above resistant and susceptible cabbage lines. Detailed investigations of this gene found to have 923 bp deletions in the promoter region of susceptible lines gene. ORF region of this gene also has several single nucleotide mutations which alter amino acid as well. Taken together, this might be a strong candidate as a Xcc resistance gene for brassica crops and could be utilized for molecular marker development.

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Development of a multiplex-PCR method useful for detecting and monitoring *Trichoderma* in field soil

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Numerous *Trichoderma* strains are beneficial to plants and can support plant growth and protect plants against stress factors, mainly pathogenic fungi. Consequently, *Trichoderma* are used as biocontrol agents in agriculture. Application of biocontrol *Trichoderma* strains to soil necessitates a suitable method to confirm the presence of these fungi during plant cultivation. In this study, mixtures of *T. atroviride* and *T. harzianum* strains on organic carrier were applied to field soil before lettuce cultivation. Field experiment was performed in triplicate in the years 2012 and 2013. Soil samples were collected before *Trichoderma* application, and then during, and after lettuce cultivation. To confirm the presence of *T. atroviride* and *T. harzianum* in soil samples, a multiplex-PCR approach was developed. PCR primers specific to the *T. atroviride* and *T. harzianum* (*sensu stricto*) were designed and tested in different combinations, and the best combination was used in a single multiplex-PCR reaction. *Trichoderma* in soil was monitored by this multiplex-PCR method and by plating soil samples serial dilutions on rose-bengal medium and then counting the number of colony-forming units (CFUs). Multiplex PCR confirmed the presence of *T. atroviride* and *T. harzianum* in all soil samples collected after the fungi were applied, whereas no *Trichoderma* were detected prior to their application. The results of multiplex-PCR and CFU counting were in agreement with each other, and showed that the newly developed multiplex-PCR method was suitable for detection of *T. atroviride* and *T. harzianum* at levels higher than 10^4 CFU per gram of dry soil.

The studies were co-financed by the European Union through the European Regional Development Fund within the Innovative Economy Operational Program, 2007–2013, Priority 1.3.1. Project No. UDA-POIG.01.03.01-00-129/09-07 “Polish Trichoderma strains in plant protection and organic waste management”.

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Monitoring of *Trichoderma* in the soil environment by Illumina Miseq metagenomic sequencing

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Progress in the Next-Generation Sequencing Technologies (NGS) brings a wide range of new capabilities to study microbial populations. In environmental studies NGS makes it possible to recognize species composition of microbial populations on an unprecedented scale without need to cultivate microorganisms. Several NGS platforms are useful for metagenomic studies including 454-pyrosequencing, Illumina, or Ion Torrent. In this study metagenomic sequencing was performed to monitor *Trichoderma* strains used in lettuce cultivation to improve plant growth and health. *Trichoderma* mixtures composed of *T.atroviride* and *T.harzianum* were introduced on organic carrier to the soil before lettuce planting. Representative soil samples were collected from experimental plots before *Trichoderma* application, during lettuce cultivation, and after lettuce harvesting. DNA isolated from the soil samples was used to amplify fungal ITS1/ITS2 and bacterial 16S rDNA V4 regions. PCR amplicons for each of 24 soil samples were used to construct library, multiplexed, and then sequenced as a single run with Illumina MiSeq platform. Using bioinformatics approach bacterial and fungal taxa were analysed and compared for their presence and distribution in soil samples collected before and after *Trichoderma* application. The study confirmed that fungal population was highly dominated by *T.atroviride* and *T.harzianum* after their application during lettuce cultivation. No effect of *Trichoderma* introduction on bacterial population structure was found. This study shows that MiSeq-based metagenomic sequencing is useful to monitor *Trichoderma* application in the field environment.

The studies were co-financed by the European Union through the European Regional Development Fund within the Innovative Economy Operational Program, 2007 – 2013, Priority 1.3.1. Project No. UDA-POIG.01.03.01-00-129/09-07 “Polish Trichoderma strains in plant protection and organic waste management”.

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A comparison of the structure of the key genes in type VI secretion system of *Pectobacterium* and *Dickeya* species

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The type VI secretion system (T6SS) in pectinolytic bacteria is an important mechanism of extracellular transport that participates in plant pathogenesis. The objective of this study was to determine variations in sequence fragments of *vgrG*, *hcp*, *icmF*, *dotU* and *clpV* genes participating in the synthesis and function of protein structures that form the "molecular syringe" of T6SS. The sequences were obtained from the GenBank data bases (www.ncbi.nlm.nih.gov) and compared with the BLAST algorithm. The sequencing analysis was performed for the whole genome of *Pectobacterium carotovorum* PCC21 (NC_018525.1, CP003776.1), *Pectobacterium carotovorum* PC1 (NC_012917.1, CP001657.1), *Pectobacterium atrosepticum* SCRI1043 (NR_076344.1, BX950851.1*), Pectobacterium wasabiae* WPP163 (NC_013421.1, CP001790.1), *Pectobacterium* sp. SCC3193 (NC_017845.1, CP003415.1), *Dickeya dadantii* Ech586 (NC_013592.1, CP001836.1), *Dickeya dadantii* 3937 (NC_014500.1, CP002038.1) and *Dickeya zeae* Ech1591 (NC_012912.1, CP001655.1). A preliminary analysis of the main cluster of T6SS genes in the Mauve 2.3.1 application revealed differences in cluster size due to the presence of an additional gene at the end. The analysis involving the BLAST algorithm demonstrated a high degree of similarity in the sequences of T6SS subunits of bacterial genera *Pectobacterium* and *Aeromonas*. Phylogenetic analyses of the main cluster of T6SS genes revealed a high degree of sequence similarity in the genera *Pectobacterium* and *Dickeya*, as well as significant differences in *hcp* and *vgrG* genes outside the main cluster of T6SS genes in various regions of the genome. A map indicating the position of the majority of the analyzed T6SS subunits was developed.

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Genetic variability at the mating type locus in *Fusarium* sp. section *Liseola*

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The members of *Liseola* section are heterothallic fungi that require the presence of two genetically distinct mycelia of opposite mating types for the sexual reproduction to occur. Such dimictic mating system is defined by one mating type locus with two idiomorphic highly dissimilar alleles, MAT1-1 and MAT1-2. The MAT locus genes serve as regulatory transcription factors in the sexual process, but they may influence other fungal biological traits, such as pheromone signaling and secondary metabolites biosynthesis as well. The aim of the study was to analyse the MAT locus polymorphism on the basis of the frequency of both idiomorphs within the population of two *Fusarium* species: *F. verticillioides* and *F. temperatum* . In the course of the analysis, three genes in the MAT1-1 idiomorph (MAT1-1-1, MAT1-1-2and MAT1-1-3) and two genes in the MAT1-2 idiomorph (MAT1-2-1, MAT1-2-3) were amplified with the use of specific primers designed using the Primer3 software. The genetic variation and the evolutionary diversity of the obtained sequences were examined with the use of bioinformatic tools. The obtained results showed the higher evolutionary diversity in the MAT1-1 idiomorph among *F. verticillioides* strains, whereas the opposite trend was observed among *F. temperatum* strains. Additionally, the idiomorphs demonstrated differences in the probability of substitutions: transitional substitutions rates appeared to be higher for the MAT1-1 sequence, while transversional substitutions rates – for the MAT1-2. The coding regions of the MAT loci were comparatively less divergent.

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New plasmids of *Erwinia amylovora*

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Two new plasmids called pEA68 and pEA27 were found in *Erwinia amylovora* strains 692 and 651, respectively. Both strains were isolated from plants with fire blight symptoms in Poland: strain 692 from *Sorbus* in 1997 and strain 651 from apple in 2011. The plasmids were initially detected by RFLP analysis of plasmid DNA of strains 692 and 651. The sequence of the plasmids showed that they are circular.

Plasmid pEA68 consists of 68,761 bp with a G+C content of 60.37%. Annotation of the IncFII-Ia-type plasmid pEA68 revealed that it contains 79 predicted CDS, among which two operons (*tra*, *pil*) are associated with mobility. The relaxase MobA belongs to the MOB_{P13} family. Blast analysis of the sequence of pEA68 revealed that this plasmid is closely related to two plasmids that were found in different *E. amylovora* strains, namely plasmid pEA72 of *E. amylovora* strain ATCC 49946 (USA) and plasmid pEA78 of strain LA637 (Mexico).

Plasmid pEA27 is 29,462 bp in size with a 47.5% G+C content. The plasmid has a ColE1-type replicon (no *repA* gene), has a MOB_{P11} relaxase and contains a *trb-tra* system. It is member of a family of plasmids of similar size, which show major variations in the intergenic regions in the plasmids. Related members of this family are found in strains isolated worldwide.

Both plasmids do not possess genes associated with antibiotic resistance nor with virulence. The plasmids do not influence amylovoran production - the main factor of *E. amylovora* pathogenicity. Plasmid pEA68 has no effect on the virulence of *E. amylovora*. The role of plasmid pEA27 is still under study.

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Development of Nest Generation Sequencing Methods for Plant Virus Diagnostics in Grapevine and Tree Fruits

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Next generation sequencing (NGS) holds enormous potential to rapidly change the way plant viruses are detected and characterized. At the government grapevine and tree fruit diagnostic laboratory in Sidney, imported plant material is subjected to numerous tests to ascertain if the plant is free of harmful viruses. Molecular tests such as ELISA and PCR are routinely employed in addition to more traditional methods such as electron microscopy, herbaceous and woody bioassays. These traditional methods remain essential because they are able to detect new or poorly characterized viruses for which an adequate molecular assay is not available. Bioassays, however, have their own issues. Suitable indicators must be developed and the time frame from inoculation to symptom development can take several growing seasons. All the while suitable lands and growing conditions need to be maintained and the plants monitored throughout this period at considerable cost. NGS not only has a cost advantage compared to bioassays, it is also more rapid and potentially more sensitive and accurate. We are currently developing workflows and methods to bring NGS into the plant virus diagnostics laboratory.

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Session 5

Diseases of trees in forest and reaction sites

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Ophiostomatoid fungi associated with *Trypodendron domesticum* in Poland

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Ambrosia beetles and many phloem-feeding bark beetles are known to be associated with fungi, particularly species of the orders Ophiostomatales and Microascales. They are also named as the ophiostomatoid fungi. However, very little is known about fungal associates of wood-boring beetles that colonize deciduous trees in Europe. In Poland, the European hardwood ambrosia beetle (*Trypodendron domesticum*) is an economically important forest pest of trees and timber products. This insect inoculates the wood with symbiotic fungi which cause discoloration of wood tissue. The aim of this study was to investigate the species spectrum and abundance of ophiostomatoid fungi associated with *T. domesticum* in Poland. Beetles caught in pheromone traps and excised from the galleries during winter were collected in 2013 from the European beech (*Fagus sylvatica*) stands. Fungi were isolated directly from the beetles as well as from the sapwood of beetle-infested trees. Isolates were identified based on morphology and DNA sequence comparison for three gene regions (ITS, β-tubulin, EF-1α). In total 10 ophiostomatoid fungi were found to be associated with *T. domesticum* in Poland. *Leptographium* sp. and *Ophiostoma* sp. were most frequently isolated, and *O. arduennense*, *O. pluriannulatum* and *O. quercus* were also relatively common. The last mentioned three species, however, were isolated only from wintering beetles and insect galleries. Rare components of the ophiostomatoid mycobiota of *T. domesticum* included *Graphilbum fragrans*, *Graphium* sp., *Grosmannia* sp., *O.* cf. *fusiforme* and *O. minus*. *Ambrosiella ferruginea*, *Ophiostoma bacillisporum* and *O. torulosum*, previously reported as common fungal associates of *T. domesticum* were not found. Among identified species, at least four species, *Graphium* sp., *Grosmannia* sp., *Leptographium* sp. and *Ophiostoma* sp. appear to represent a new taxa, based morphological and DNA sequence comparisons.

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Pathogenicity of *Hymenoscyphus albidus* and *H. fraxineus* towards *Fraxinus excelsior* and *F. pennsylvanica*

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Pathogenicity tests with *Hymenoscyphus albidus* and *H. fraxineus* (=*H. pseudoalbidus*) on *Fraxinus excelsior* and *F. pennsylvanica* were conducted in late July 2012 in a nursery in the Stary Sącz Forest District (southern Poland). We used six different isolates of *H. fraxineus* from necrotic lesions on ash trees in southern Poland and three isolates of *H. albidus* from ash stands in Switzerland. Stems (n = 36 for each of the ash species) and rachises (n = 36 for each of the ash species) were inoculated by placing a colonized piece of wood on a small wound. For controls, sterile wood inocula were used. Reactions of host tissues were evaluated after 2 months on rachises and after 12 months on stems. Reisolations were made within 24 h after harvesting. Occurrence and extent of necrotic lesions adjacent to the inoculation site depended on the fungal species, ash species and inoculated organ. In the case of *H. albidus* necroses developed on 13.9% of the rachises in *F. excelsior* and on 30.6% of the rachises in *F. pennsylvanica*, with a maximum length of 1.4 cm including the inoculation wound. *H. albidus* did not cause necrosis in any of the inoculated stems, both in *F. excelsior* and *F. pennsylvanica*. *H. fraxineus* caused necroses in all inoculated rachises of both ash species. The mean length of the lesions was 8.8 cm (*F. excelsior*) and 2.7 cm (*F. pennsylvanica*). It also caused necroses on all inoculated stems of *F. excelsior* and their length averaged 18.0 cm. In *F. pennsylvanica* necroses occurred only on two stems and their mean length was 1.9 cm. There were no necroses on petioles and stems used as controls. *H. albidus* was reisolated only from few inoculated rachises and stems (5.6–16.7%), while *H. fraxineus* was observed in 16.7–66.7% of inoculated organs. None of the *Hymenoscyphus* species was isolated from the control.

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DNA isolation procedure – critical point of direct PWD diagnosis

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The Pinewood Nematode (PWN), *Bursaphelenchus xylophilus*, the etiological agent of the Pine Wilt Disease (PWD), is one of the worst plant pests of pines and coniferous trees in general.

It is on the A2 quarantine pest list of the EPPO (European and Mediterranean Plant Protection Organization) and the timely diagnostics plays a key role in its elimination. The determination of this plant pest based on its morphological features is very complex and requires an experienced nematologist, whereas the DNA based diagnostics is very fast and precise. This study deals with methods of DNA isolation directly from the wood samples, which would further increase the speed of the diagnostics. However, PCR inhibitors occurring in the wood of the coniferous trees make the isolation difficult. The used methods were tested with regards to the ability to untie these inhibitors and thus eliminate their influence on the purity of the DNA output. The isolations procedures were based on several principles: the ability of the polyvinylpyrrilidon (PVP) agent to bind the inhibitors during the classic isolation with CTAB (Cetyl trimethylammonium bromide) was used, the sensitivity of the commercial kit based on binding the DNA to the filter membrane was confirmed, the robotic isolation working on the principle of binding the DNA to magnetic particles was used and the fastest method was tested using FTA.

All these methods eliminated the PCR inhibitors from the DNA output reliably and are therefore suitable for the fast diagnostics of this plant pest.

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The Czech collection of phytopathogenic *Oomycetes*

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Phytopathogenic oomycetes – namely species from genus *Phytophthora* – belong to the most important pathogens of woody plants in Europe. The research of these pathogens has become very intensive, however many countries belonging to former eastern block have relatively poor tradition in this field. This problem has been more or less successfully eliminated yet at least in some countries. Among others, the collection and preservation of strains of these pathogens is very important as a source of valuable scientific material. The Czech Collection of phytopathogenic *Oomycetes* was established in 2006 and it has become one of the most important collections of these parasites in the region. More than six hundreds of strains of phytopathogenic oomycetes have been deposited up to date and more than one half of strains in public part of this collection serve as a source of comparative and research material for many scientists and specialists. There are deposited 21 taxa belonging to *Phytophthora* and 11 to *Pythium* respectively. In the collection are deposited many strains of invasive pathogens – for instance *P. alni*, *P. plurivora*, *P. ramorum*, *P. cinnamomi*, *P. cambivora* etc. and also the less important, but highly interesting species as *P. gallica*, *P. gregata*, *P. lacustris* and many others. The list of deposited species and catalogue are located at http://www.vukoz.cz/index.php/en/collections/collection-of-phytopathogenic-oomycetes-of-rilog.

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Diversity and pathogenicity of *Cylindrocarpon* species isolated from litter in the old-grown beech forests in Central Europe

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Cylindrocarpon species include numerous plant pathogens in agricultural ecosystems. However, very little is known about these fungi as potential pathogens of forest trees, especially in natural and semi-natural woodland in Europe. *Cylindrocarpon* species living in beech litter of the old-grown beech forests in Carpathians (Babia Góra National Park) and Alps (Dürrenstein, Rothwald) were studied. In addition, these fungi were also collected from one managed forest on Krakowsko-Częstochowska Highland (Zabierzowski Forest). The pathogenicity of the most common *Cylindrocarpon* species was evaluated through inoculations using sprouts of the European beech. Fungi were detected by trapping using beechnuts (experiment *in situ*) as well as pine seedlings (laboratory experiment). Isolation on MEA (malt extract agar) medium were made from decayed beechnuts as well as from necrotic parts of beech germinants and the roots of pine seedlings. Isolates were identified based on morphology and DNA sequence comparison for two gene regions (ITS, histone H3). In total, more than 700 isolates of *Cylindrocarpon* spp. representing 12 taxa were found to be associated with beech litter. There were *Ilyonectria crassa, I. panacis, I. pseudodestructans, I. rufa, Ilyonectria* sp. 1, *Ilyonectria* sp. 2, *Neonectria ramulariae s. stricto, Cylindrocarpon obtusisporum, Cylindrocarpon* sp. 1, *Cylindrocarpon* sp. 2, *Cylindrocarpon* sp. 3, *Cylindrocarpon* sp. 4. The frequency of *Cylindrocarpon* fungi was higher in beech litter in the old-growth beech forests compared with managed forest. *Cylindrocarpon* sp. 1 and *Cylindrocarpon* sp. 4 were most frequently isolated, and *I. rufa* was also relatively common in the laboratory experiment. All species tested possessed pathogenic ability to sprouts of the European beech.

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Interactions between callus cultures of *Pinus sylvestris* and fungi with different biotrophic properties (*Gremmeniella abietina*, *Anthostomella formosa*, *Phacidium lacerum*)

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The studies in dual *in vitro* cultures provided the results from three levels, i.e.: the cultures of non-embryogenic callus of *P. sylvestris* using biotechnological methods, the determination of fungal virulence based on its growth rate and changes in the phenotype of host plant tissues, as well as from the analysis of soluble proteins conducted by the SDS-PAGE method. The comparison of fungal species with different biotrophic properties was conducted, selecting those that induce characteristic defensive reactions. All genotypes of *P. sylvestris* callus, initiated by somatic embryogenesis (initiation frequency 5.5%), were used for establishing dual cultures. Fungal growth towards the plant callus tissue differed already at the cellular level. The results of fungal growth rate, supported by the Friedman's analysis of variance (ANOVA), arranged the fungi in terms of their colonization rate (the average rank values). *G. abietina* proved to be the slowest colonizer and *Ph. lacerum* – the fastest. *G. abietina* caused extensive necrosis of the callus, which was partially overgrown by this pathogen and died already in day 10. *A. formosa* did not cause evident symptoms of callus decay in the dual culture. Even after 10 days of dual culture the callus cells were greenish-colored and alive in c.a. 50%. The callus in dual culture with saprotroph *Ph. lacerum* remained alive until the end of the experiment and had white-creamy color with loose cell structure, i.e. it remained characteristic non-embryogenic callus. After the electrophoresis of proteins, additional bands of 25–35 kDa molecular weight were revealed in polyacrylamide gel only in the pathogen *G. abietina*, which indicated the presence of immunity proteins. It would mean that pathogenic fungi exhibit defensive reactions influenced by the stress factors, already at the embryonic level, by producing the PR-type proteins (pathogenesis related proteins). Moreover, the studies have shown that the callus is not equally responsive to the presence of different fungal isolates. In general, 1/3 of the examined isolates were characterized by greater virulence compared with the remaining ones. The presented experiments with callus tissues showed that dual cultures can be more useful as compared with other methods in proving the specificity of a given fungal species with respect to particular host plant tissue, including the resistance of different genotypes.

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Occurrence and characterization of *Phytophthora alni* sensu lato populations in Lithuania

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In Lithuania, *Phytophthora alni* sensu lato was for the first time isolated and identified from declining *Alnus glutinosa* in 1999; however, to date no detailed studies have been conducted on significance (magnitude of the caused damage) and distribution of the alder decline in our country. Moreover, no information is available which of the three species of *P. alni* complex (*P. alni* ssp. *alni*, *P. alni* ssp. *uniformis* or *P. alni* ssp. *multiformis*) is causing the damage, and genetic population structure of the occurring species is unknown.

There are several aims of this study: i) to determine the distribution and incidence of the alder decline in Lithuania; ii) to determine which species of the *P. alni* complex is/are responsible for the observed decline; and iii) to characterize genetic population structure of the sampled *P. alni* s.l. populations and to reconstruct the spread of different species of the *P. alni* complex. The aims would be achieved through extensive sampling and isolation (baiting) of *P. alni* s.l. from symptomatic *A. glutinosa* in forests and nurseries in various regions of Lithuania, morphological and molecular characterization of the obtained isolates and their microsatellite analysis to reveal genetic population structure. The first portion of symptomatic wood, possibly infested soil and water (for baiting) samples will be collected during spring-summer, 2014, aiming to obtain at least 50 isolates of *P. alni* s.l. for the pilot study. The target regions (ITS, nuclear genes RAS-Ypt and TRP1) will be amplified by PCR using primers specific to each of the three species of *P. alni* complex and sequenced. The obtained isolates will be further genotyped using specific microsatellite markers. Based on previous observations in Europe, we do not expect *P. alni* subsp. *multiformis* to be frequent in Lithuania, so this species will not be included in population genetic analyses.

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The occurrence of yellow spot needle on dwarf mountain pine (*Pinus mugo*) in Karkonosze Mts.

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The mountain dwarf pine (*Pinus mugo* Turra) is a native pine species in Europe, with a disjunctive areal covering several mountain ranges. In Karkonosze Mountains in Poland and the Czech Republic, this species climbs into the subalpine zone in 1250–1450 m above sea level. Very little attention has been paid to the needle's diseases of the mountain dwarf pine in this area so far. The valuation of healthiness of infected mountain's dwarf pine needles were conducted for three years (2011–2013) in few experimental places: Mt. Kopa, Śląskie Kamienie, Kocioł Smogorni, Mt. Sokolnik, Mt. Łabski Szczyt, Mt. Szrenica, Kocioł Małego and Wielkiego Stawu. Symptoms was determined and the percentage of infected needles on one shoot. For this purpose, at each selected four research facilities, which assessed symptoms of diseases data in percentage terms in 10 randomly selected shoots at a height of about 1.5 meters. In addition, assessed the health of needles based on the infected scale. Yellow spots on needles were found in subalpine zone of Karkonosze Mts. During the field research, it was found that most infected mountain's dwarf pine needle falls in July. Then observed the greatest severity of symptoms on the needles as yellow spots. In the first year of the study (2011) were yellow blotch symptoms at 90 - 94% of infected needles on the shoots. In the next year of the study had less shock in the same month of observation. In 2012 and 2013, signs were spotted yellow needles of 38% of infected needles to shoot in the area of Smogornia to 85% of infected needles on the objects in the region Śląskie Kamienie.

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Intensity of wood decay in Norway spruce caused by some isolates of white rot fungi

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Stump treatment with fungus *Phlebiopsis gigantea* has been widely used as the good method for the control of the conifer pathogen *Heterobasidion* spp. However, *P. gigantea* grows very well in wood of Scots pine stumps, but its growth in Norway spruce is lower. The aim of this work was to investigate the wood decay capacity of five isolates of *Pleurotus* sp., one of *Hypholoma* sp. and one – *Phlebiopsis gigantea* through the three- and six-months treatment, under laboratory conditions. Loss of dry mass of wood samples was found to be from 12.7% to 16.2% for three-months and from 20.7% to 33.6% for six-months decomposition of wood. The most intensive decomposition of wood samples was determined for the three-months treatment in the isolate of *Phlebiopsis gigantea* (16.2%) and for the six-month treatment in the isolate of *Hypholoma* sp. (33.6%).

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Botryosphaeriaceae on false cypress (*Chamaecyparis* spp.) in Serbia

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False cypress trees (*Chamaecyparis* spp.) are frequently planted ornamentals in public greens, parks and private gardens in the cities of Serbia. Various *Chamaecyparis* cultivars (e.g. „Columnaris", „Ellwodii", „Alumii") are also important conifers propagated in Serbian ornamental nurseries. In recent years, false cypress trees have exhibited top die-back and branch flagging accompanied by resin bleeding on the main stems or at the bases of dead shoots or branches. Diseased trees of all ages and seedlings were sampled from 2009–2014 and the most common fungi isolated from symptomatic samples were species of the family *Botryosphaeriaceae*. The aim of this study was to identify these fungi as part of a larger project to isolate and identify the *Botryosphaeriaceae* as potential pathogens of landscape trees in the Western Balkans. Isolates obtained were identified using anamorph morphology and comparisons of DNA sequence data for the internal transcribed spacer (ITS), translation elongation factor 1α (TEF 1- α), β-tubulin-2 (BT2), LSU and RPB2 gene regions. Six species of the *Botryosphaeriaceae* were identified, including *Diplodia mutila*, *D. seriata*, *Dothiorella sarmentorum*, *Do. coryli*, *Botryosphaeria dothidea* and *Neofusicoccum parvum*. The *Botryosphaeriaceae* isolated from *Chamaecyparis* spp. appeared to be generalists that also occur on various other woody plants in temperate and tropical climates. False cypress trees are highly regarded as attractive and popular ornamentals in Serbia and worldwide and this research contributes knowledge regarding on the diversity, distribution and ecology of the *Botryosphaeriaceae* as potential pathogens of *Chamaecyparis* spp.

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Damping-off of Scots pine seedlings in forest nurseries in Poland

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Damping-off is the most important disease of Scots pine (*Pinus sylvestris* L.) seedlings in Polish forest nurseries. The disease is caused mainly by *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp., *Alternaria* spp., and *Cylindrocarpon* spp. The range of pathogen species is very wide but usually the species most favoured by the environment cause the disease.

In this study the occurrence of damping-off pathogens was investigated from 2004 to 2009 in seven forest nurseries.

Soil samples and seedlings with visible damping-off symptoms were taken from each nursery and transported to the laboratory where the experiments were carried out. Soil samples were collected in early spring, before any plant protection products were used and always from places where Scots pine seeds were going to be sown. Soil taken from each nursery has been mixed and unified using method by Warcup (1950) modified by Mańka (1964). Soil was placed in one litre pots and kept at room temperature and watered with distilled water as needed. The isolates were obtained with baiting technique using Scots pine seeds.

The plants were heavily infested in two of the seven forest nurseries. In the five remaining nurseries damping-off occurred in a lesser extent.

As expected, *Fusarium* sp., *Rhizoctonia* sp., and *Pythium* sp. were among the most common occuring pathogens.

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The new pathogens decreasing decorative values of trees and shrubs in urban green areas in Poland

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New pathogens can often be found in Europe. While some of them spread slowly and do not cause major damage, others prove to be very invasive and cause epidemics in new areas. From 2005 to 2014 the spread and harmfulness of new pathogens, which had been described in different European countries, was assessed in urban green space. Every year there are epidemics of *Erysiphe azaleae* in collections, gardens and nurseries (U. Braun) U. Braun & S. Takamatsu. Mollis azaleas proved to be particularly vulnerable. In 2002 *E. flexuosa* was described on horse chestnut trees in Poland (Piątek 2002). As early as 2006 (Werner, 2007) the pathogen proved to be very invasive and now it causes epidemics. Depending on the year, it invades 49–60% of *Aesculus* x *carnea* trees and about 3–11% of *A. hippocastanum* trees. The powdery mildew of hornbeam trees (*E. carpinicola*) is relatively widespread. This pathogen usually infests hornbeams in hedges, where it forms few cleistothecia on leaves in late but warm autumn. *E. elevate* invades individual *Catalpa bignonioides* trees and it rarely forms cleistothecia. *Microsphaera syringae-japonicae* do not spread in Poland. The powdery mildew on *Syringa vulgaris* shrubs is caused by the native species of *M. lonicerae*. The powdery mildew was not observed on *S. josikaea* shrubs. The fungal pathogens which used to be found locally cause biological invasions in urban green space. Epidemics are caused by such pathogens as *Cumminisiella mirabilissima* (on *Mahonia aquifolium*), *Gymnosporangium confusum* (on hawthorns and common junipers), *Fabraea maculata* (on hawthorns), *M. palczewski* (on Siberian peashrubs) and *G. sabinae*. The epidemics caused by those pathogens expire gradually or they remain on *Caragana arborescens* shrubs in hedges, e.g. *M. palczewski*. *G. sabinae* is an exception. Every year more and more junipers are infested and unprotected pear trees die after a few years.

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Diseases in *Brassicae* vegetables and possibility of their control

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Brassiceae are a very popular vegetable plants, grown as a commercial crop for local and export markets. In Poland grown about 55 000 ha of head cabbage, and about 800 000 ha other cruciferous plants. Black rot, clubroot and leaf spot diseases have become threat to the brassica crops. During 2013 and 2014 in Institute of horticulture work on eradicating those diseases were carried out.

Xanthomonas campestris cause the black rot in crucifers by darkening the vascular tissues. They can live in a soil for over a year and spread through any movement of water including rain, irrigation and surface water. In our study we used 5 natural and 2 chemical compounds. The results suggest that effectiveness of natural and chemical products is comparable and cause reduce the level of *X. campestris* infected plants in 55–70%. Clubroot cause by *Plasmodiophora brassicae* affect mainly head cabbage and oilseed rape. This pathogen attacks roots and causes significant yield losses. From 5 tested products only Altima 500 SC showed a significant decrease in clubroot disease. Catch crop cultivation gave acceptable results as well. In 2013 we tested 7 compounds on broccoli against *Alternaria* spp. Leaf spot symptoms caused by *Alternaria* spp. were not observe in the case of Score 250 EC, Signum 33,4 WG, Amistar 250 SC treatment. Fusarium yellows of cabbage is caused by soilborne fungus *Fusarium oxysporum f. sp. conglutinans*. Main symptoms of the disease are yellowing of leaves, defoliation of older plants and growth inhibition. The disease appeared in Poland recently and the main cause of it are climate changes. Currently, the most effective method of protecting crops against Fusarium yellows is cultivation a resistant plants.

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Evaluation of efficacy of control agents Myco-Sin VIN and VitiSan against brown rot (*Monilinia fructigena* Honey) on apple fruits

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During the storage periods 2012/2013 and 2013/2014 were carried out laboratory experiments with the effects of biopreparations Myco-Sin VIN and VitiSan against brown rot on apples. These products are registered for usage in the orchards with pome fruits in the Czech Republic. They might also be used in the orchards with the organic cultivation. The efficiency of comparison was evaluated to the untreated control. Thiram Granuflo fungicide was used as a chemical control with valid registration against brown rot on pome fruits. Tested variety of the apples in both years were Golden Delicious. Man-made infection by fungus M. fructigena (determined by PCR) was used for the tests and 24 hours after inoculation the friuts were wound by the scalpel's incision.

Evaluation of infestation percentage was categorized by the size of the lesions after the 5th and the 14th day when the experiment was established. Following values were determined during the storage period 2012/2013: the control variant on the 5th day was 24.7% infestation, on the 14th day the infestation was 73,5%; the treated variant with Myco -Sin VIN on the 5th day was 12% infestation, on the 14th day the infestation was 52,9%; the treated variant with VitiSan on the 5th day was 7.5% infestation, on the 14th day the infestation was 51.3%; the treated variant with Thiram Granuflo on the 5th day was 9.2% infestation, on the 14th day the infestation was 48.2%. The values obtained during the storage period 2013/2014: The control variant on the 5th day was 21.6% infestation, on the 14th day the infestation was 62% ; the treated variant with Myco-Sin VIN on the 5th day was 19.4% infestation, on the 14th day the infestation was 42.4%; the treated variant with VitiSan on the 5th day was 13.5% infestation, on the 14th day the infestation was 33.5%; the treated variant with Thiram Granuflo on the 5th day was 7.9% infestation, on the 14th day the infestation was 20.7%. In the treated variant with Myco-Sin VIN was observed at the beginning of testing apparent inhibition of the lesions' development (spots caused by pathogens were smaller and less colorful), but their quantity were not affected. In the evaluation at the end of the experiment, this inhibition has been overcome by the pathogen. The variant treated with VitiSan showed a lower level of infection as well as the variant treated with Thiram Granuflo. Based on the experiment's results it is recommended to use VitiSan against landfill diseases in organic production systems and also in the low-residue production systems (e.g. production of apples as a raw material for baby food).

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The effect of selected regulators of plant growth and development on *Trichoderma* spp. antagonistic fungi under *in vitro* conditions

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Trichoderma spp. fungi are powerful antagonists of parasitic soil fungi of the following genera: *Pythium, Verticillium, Gaeumannomyces, Sclerotinia, Rhizoctonia* and *Fusarium* inflicting plants with root-rots of seedlings, root rot and wilt which lead to plant withering. Some strains of *Trichoderma* promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance. Integrated plant protection systems strive to use antagonistic fungi together with chemical products. Microorganisms used in biopreparations will be successful in their protective roles in the environment on condition that they become insensitive to chemicals used in agriculture.

Investigations were carried out to study the effect regulators of plant growth and development (Gibrescol 10 MG – gibberillc acid 10%; Betokson Super 025 SL – petroleumacetic acid 2.5%; Ukorzeniacz Korzonek Z SD – indole acetic acid 0.2%, kaptane 1%) on a growth and biological activity of antagonistic fungi *Trichoderma harzianum* Rifai*, Trichoderma pseudokoningii* Rifai and *Trichoderma viride* Pers. ex Gray.

The work aimed to determine the effect of the regulators in various concentrations on the below-mentioned features of studied fungi: the linear growth rate and inhibition of mycelium development; morphology of mycelium and sporulation; spore germination and biological activity of *Trichoderma* fungi towards three soil pathogens, i.e. *Botrytis cinerea* Pers.*, Fusarium solani* Sacc. and *Rhizoctonia solani* Kühn. based on laboratory tests.

In vitro experiments revealed fungistatic properties of Ukorzeniacz Korzonek Z SD towards the *Trichoderma* spp. isolates. *T. harzianum* was the most sensitive on tested regulators.

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Morphological and histological effects of *Trichoderma* fungi on lettuce and peppers growth and development

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The aim of the study was the assessment of *Trichoderma* fungi influence on the structure and development of the root system and aboveground parts of the lettuce grown in plastic tents and pepper cultivated in field conditions. In experiments there were used *Trichoderma* strains: TR25, TR40, TR43, TR59, TR85, TR90, TR106, TR123. The morphology of plants was analysed with use of camera and stereoscopic microscope Olympus SZX16. The epidermal cells of leaves were isolated with the use of transparent scotch tape and stained with toluidyne blue for estimation of stomata morphology. The roots were fixed with CrAF (chromic acid, acetic acid, formalin), dehydrated in ethanol and prepared for light microscope Nicon Eclipse 80i. and scanning electron microscope JEOL JSM-6390LV. Some fragments were embedded in paraffin, cut and stained with safranine and fast green for tissue analysis and the other were desiccated with Critical Point Drying CO_2 and sputter – coated with gold for examination of root surfaces. Positive influence of the *Trichoderma* fungi on development and yield peppers and lettuce was shown. Strain TR90 affected the pepper plants, while lettuce responded best to the strains TR25 and TR106. Root systems of plants growing in supplementation with *Trichoderma* were characterized by increased mass and more developed root hair zone comparing with the control. Numerous bacteria and hyphae were observed only on the surface of plant roots of the objects TR. A greater number of flowers and fruit of pepper plants was observed with TR90 substrate than in the control. Lettuce heads, influenced with TR106 and TR25, were of increased weight and more compact than the control. The size of the stomata of pepper leaves was more diverse in plants with facilities TR than in control.

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Biological control of late blight of pepper caused by *Phytophthora capsici* using glucanolytic and ACC deaminase producing endophytic yeasts

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Seventeen endophytic yeast isolates were isolated from surface-disinfested green pepper roots and screened for the production of cell-wall degrading enzymes (glucanases) using mycelial (*Phytophthora capsici*) fragment agar. The eight most inhibitory isolates showed exceptional glucanolytic activity *in vitro* by producing β-1,3, β-1,4 and β-1,6-glucanases and caused lysis of *P. capsici* hyphae, the causal agent of late blight of green pepper in the United Arab Emirates (UAE). None of the eight isolates produced diffusible antifungal metabolites, volatile inhibitors or siderophores. These eight isolates were subsequently tested *in vitro* for their ability to produce 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, the immediate precursor of the stress hormone ethylene. Under greenhouse conditions, the endophytic eight glucanolytic yeast isolates capable of producing relatively high levels of ACC deaminase, significantly reduced disease incidence and severity of the late blight disease compared to the endophytic glucanolytic yeast isolates which did not produce ACC deaminase. The application of the ACC deaminase-producing isolates resulted in the reduction of the *in planta* levels of ACC and also increased photosynthetic pigment contents, carbon assimilation, and promoted pepper growth compared with pepper plants treated with the non-ACC deaminase producing glucanolytic isolates. In another greenhouse test, endophytic yeast isolates were recovered from inside the roots for up to 10 weeks after inoculation, indicating that the roots of healthy pepper may be a natural habitat for endophytic yeasts. This is the first successful use of endophytic yeasts as plant growth promoters and biocontrol agents against *Phytophthora* diseases. The results clearly show that these used endophytic yeasts could replace metalaxyl which is the currently recommended fungicide for *Phytophthora* diseases in the UAE. This study also demonstrated the ability of ACC deaminase-producing endophytic yeasts to promote plant growth under biotic stress consitions.

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Efficiency of Benefis and Polaris fungicide seed treatments on the complex of seed-borne and soil-borne pathogens of winter wheat in Northwest region of Russia

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The main causal agents of winter wheat root rot in the Northwest region of Russia are *Fusarium* spp., the share of *Pythium* spp. is significantly lower. Field trials were conducted in Leningrad region during 2012–2013 to evaluate the effect of fungicide seed treatments Benefis (50 g/l imazalil + 40 g/l metalaxyl + 30 g/l tebukonazole) at two rates (0.6 and 0.8 l/t) and Polaris (100 g/l prochloraz + 25 g/l imazalil + 15 g/l tebukonazole) at three rates (1.0, 1.2, 1.5 l/t) on seed molding, root rot and bunt smut of winter wheat cv. Inna. Biological efficiency of both rates of Benefis against seed-borne infection achieved about 30%. In autumn 2012 the biological effectiveness of Benefis against root rot reached 70,7–84,0%, at disease severity in untreated control 19%. Metalaxyl in this preparation allows to control the root rot caused by the *Pythium* spp. Biological efficiency of Polaris against seed-born infection at all rates exceeded 50%. Effect of Polaris on root rot was lower and achieved 60%. In spring 2013 the tendency of decrease in efficiency of treatments against root rot was noted. Both treatments gave a good protection of plants against bunt smut and reduced disease incident on 100%. Fungicide seed treatments allowed to get 19–30% increase of grain yield.

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Seed infection of cereals and efficacy of fungicides for seed treatment in Latvia

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The modern integrated technologies of cultivation of grain crops in Latvia include seed treatment as mandatory method to control seed-born diseases, as quality and health of seeds have an essential influence on formation of a high and stable yield. For precise choice of effective fungicide it is necessary to know causative agents of the important diseases, infestation level and intensity of the disease under different meteorological conditions. The aims of the investigation were to specify the causative agents of the most widespread disease, root rot of cereals, to identify particular *Fusarium* species in cereals and to establish the efficiency of fungicides used for seed dressing to control loose smut in barley, bunt in wheat, common root rot and snow mould in winter cereals.

In the phytopathological tests of the cereal seeds samples used in field trials of efficiency of new products for seed treatments, the healthy seeds free from any infection were not observed. Investigation of causative agents of common root rot showed that *Fusarium* species were the most widespread seed-borne pathogens identified in cereal. From key pathogens identified as causative agents of root rot in spring barley, 85.7% belonged to *Fusarium* species, among which *F. culmorum* and *F. equiseti* were prevaled. The presence of *F. culmorum, F. poae, F. graminearum, F. avenaceum* and *F. sporotrichioides* in the Latvian population of *Fusarium* species was associated with fusarium head blight in wheat. Seed infection of wheat and barley consisted 3–8% of *Fusarium* species and 1–5% of *Bipolaris sorokiniana*. Seed infection of rye by *Microdochium nivale* were 4–6%, infection level by *Fusarium culmorum* and *F. avenaceum* was not higher than 2–3%. Low level of *Fusarium* infection (1–2%) was observed in seed of winter triticale.

The efficiency of seed treatment to control common root rot in cereals reached 85–95%.

The efficiency of seed treatment to control snow mould in winter cereals achieved 70–75% under the optimal conditions for the development of the disease.

The high effect of different products for seed treatments to control bunt in winter wheat was shown in field trials (2010–2013) by artificial infection of seed by *Tilletia caries*.

The efficacy of new products for seed dressing to control *Ustilago nuda* f.sp. *hordei* was depended on dosage of active substance of fungicides used in the trial by high level of natural infection with loose smut in spring barley 'Tocada'.

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Effect of different fungicides on *Chalara fraxinea* and their potential for control of ash dieback

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Chalara fraxinea, anamorphic state of *Hymenoscyphus pseudoalbidus* causes ash dieback in many parts of Europe including the Czech Republic. Among others, the disease is responsible for extreme decline in ash production in nurseries, because attacked seedlings can die during one growing season. The use of effective fungicides could assure the production of healthy planting material.

Selected 26 fungicides with different active ingredients were assayed *in vitro* for their effect on mycelial growth of three isolates of the pathogen. Six concentrations were tested – 0.01, 0.1, 1, 10, 100 and 1000 μg of active ingredient (a.i.)/ml of medium. The colonies were measured after 10 and 20 days of incubation. Growth inhibition was calculated as the proportional decrease in radial growth to the control plates. The efficiency of the fungicides was estimated from the ED_{50} values (effective dose μg a.i./ml inhibiting radial mycelial growth by 50%) The most effective fungicides – e.g. Falcon 460 EC (a.i. spiroxamine, tebuconazole, triadimenol), Score 250 EC (a.i. difenoconazole) and Zato 50 WG (a.i. trifloxystrobin) showed the mean ED_{50} values under 0.05 μg a.i./ml.

Based on the results from the *in vitro* experiments, five fungicides were selected for *in planta* tests. The fungicides were tested on two year old ash seedlings in highly affected nursery. The first application of fungicides was carried out in the first half of july, the second one two weeks later. The effectiveness of fungicides was evaluated according to the amount of dead shoots in treated trees. The best results were obtained with Horizon 250 EW (a.i. tebuconazole).

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Antibacterial activity of edible mushroom, *Hericium erinaceus* (Bull.:Fr.) Pers. extracts on phytopathogenic bacteria

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The 10 edible mushroom species were screened for antimicrobial activity against the phytopathogenic bacteria. Of them, the culture extracts from *Hericium erinaceus* showed a clear inhibition zone against gram negative phytopathogenic bacteria, *Pectobacterium carotovorum* subsp. *carotovorum*, *Ralstonia solanacearum, Xanthomonas oryzae* pv. *oryzae, X. campestris* pv. *campestris, X. axonopodis* pv. *vesicatoria*. *X. axonopodis* pv. *citri, X. axonopodis pv. glycine* and mushroom pathogen, *Pseudomonas tolassi*. Futhermore, water extract from spent mushroom compost (SMS)of *H. erinaceus* was used to control tomato wilt disease caused by *R. solanacearum*. The active compounds were extracted from SMC of *H. erinaceus* with different solvents, and ethyl acetate and butyl alcohol extracts that showed antibacterial activity on the pathogens were subjected to purify the active compounds. These results suggest that SMC from edible mushroom, *H. erinaceus* have the potential to be developed into biocontrol agents for the control of phytopatogenic bacteria. Aknowlegement: this research is supported by research grant (Agenda project No. PJ009969) from Rural Department Administration, Suwon, South Korea, and BK plus project of National research foundation (NRF), South Korea.

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Know your enemy: Determination of the population structure of *Pyrenopeziza brassicae* for improved control of light leaf spot in brassicas

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Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is currently the major disease problem in oilseed rape production in the UK. Furthermore, *P. brassicae* can also be found on vegetable brassicas such as cabbage, cauliflower and Brussels sprouts. Effective control of light leaf spot is difficult to achieve. Chemical control is challenging as fungicides must be applied when the pathogen grows symptomless in plant tissue (Figueroa et al., 1994). Exploiting plant resistance against the pathogen could help control the disease but current commercial cultivars show poor light leaf spot resistance. The aim of the project is to identify the pathogen population structure, to determine if the same strains are able to infect oilseed rape and other brassicas, and to gain a better understanding of the plant-pathogen interactions. Therefore, isolates of *P. brassicae* are being collected from infected leaf (oilseed rape, vegetables) and bud tissue (Brussels sprouts). The isolates will be studied morphologically and molecularly using a combination of neutral marker to determine differences between the isolates. Certain isolates will be used for *in planta* screenings to discover differential interactions between isolates and potential host cultivars.

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Effect of repeated Ribavirin treatment on elimination of several viruses in Grapevine (*Vitis vinifera*) cultivated *in vitro*

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Four cultivars of Grapevine (*Vitis vinifera*) cultivated *in vitro* were repeatedly treated by Ribavirin for virus elimination. The cultivars used were Blauer Portugiese, Rheinriesling, Kerner, and Chardonnay. The plants were cultivated on Quoirin-Lepoivre medium with 10 or 20mg/l Ribavirin. The treatment duration was 4, 6 and 8 weeks, respectively. After the treatment, apical parts of the plants were transferred on fresh medium without Ribavirin for recovery (for 8 weeks), and then treated again. After each step, part of the plants were analysed by RT-PCR for presence of several viruses (*Grapevine leafroll-associated virus 1*, *Grapevine rupestris stem pitting-associated virus*, *Grapevine virus A*, *Grapevine Pinot Gris virus*, *Grapevine fanleaf virus*, and viruses of family *Tymoviridae*).

The experiments has not been finished yet, but the preliminary results show that duration of treatment has greater effect on virus elimination than Ribavirin concentration in the medium. After 6 and 8 weeks of treatment there were in average 30% successfully sanitated plants, in comparison with only 15% after 4 weeks of treatment. No significant difference was found between variants with 10 and 20 mg/l Ribavirin. Repeated treatment with Ribavirin further decreased the virus abundance.

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The effect of explant size on regeneration and elimination of viruses and *Hop latent viroid* from hop *(Humulus lupulus)*

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Healthy planting material of hop (*Humulus lupulus)* is very important for establishing commercial gardens of this perennial, vegetatively propagated crop. Using planting material free from viruses and *Hop latent viroid* (HLVd) guarantees a high yield and quality of hop raw material (with high content alpha acids which are very important for brewing industry as bittering agents in the process of beer production). The most common method of elimination of viruses (*Apple mosaic virus* – ApMV and *Hop mosaic virus* – HpMV) from hop plants is shoot tips *in vitro* culture combined with heat therapy. In this method, the viruses stop replicating but meristem continues to grow, therefore the viruses are absent in large part of meristem. This technique allows to eliminate viruses using larger explants (1–2 mm) which regenerate better than smaller ones. Unfortunately, this method is not effective in case of HLVd which replicates faster at relatively high temperatures and infects all tissues in the plant. Therefore, to obtain viroid free hop plants we couldn't use heat therapy. Meristem tips of about 0.1–0.2 mm (S) and 0.4–0.5 mm (L) were excised from suitable donor plants of two hop cultivars ('Magnum' and 'Magnat') and transferred to three different regeneration media (A, B, C). 'Magnum' showed the best regeneration capacity (81.0%) on medium A, whereas 'Magnat' on medium B (76.7%). It means that these two cultivars require different composition of medium. All regenerated plants were tested for the presence of viruses (ELISA) and HLVd (RT-PCR). Viruses were not detected in any of the regenerated plants but elimination of HLVd was less effective. Only 14.5% and 8.3% plantlets were free from HLVd for 'Magnum' and 'Magnat', respectively. All healthy plants were obtained from smaller explants (S).

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Effects of soil disinfection on health status, growth and yield of strawberry stock plants

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Two field trials comparing soil disinfection with different fumigants (metam sodium, dazomet, chloropicrin, chloropicrin + 1,3 D) and a steaming system exploiting the exothermic reaction between H_2O and CaO (Bioflash System™) were conducted in 2010–2012 to evaluate the effect of treatments on *Verticillium dahliae* inhabiting the soil, and on plant health, growth and yield of strawberry daughter plants. Chemical fumigants and active steam decreased the number of *V. dahliae* colonies in the soil, which corresponded to a reduced incidence of *Verticillium* wilt (efficacy about 80%). The use of chemical fumigants had a positive impact on the size of the mother plants. The surface area covered by plants grown on the treated plots was 1.1 to 1.7 times larger, than plants grown on non-fumigated control plots. The number of runners, as well as daughter plants, produced from plants grown on plots treated with all chemical fumigants, was significantly higher than in the non-fumigated control or in the steam treated plots. This disinfection significantly increased the marketable yield of the daughter plants, approximately 1.5–3 times higher in comparison to plants grown on control plots. Steam disinfection was the least effective treatment in this respect. The differences in marketable plants yield among the chemical fumigants significantly affected the net marginal return and the return on investment of the crop. The efficacy in controlling *Verticillium* wilt, even with low doses of metam sodium and dazomet and their influence on daughter plants yield and quality, is confirming the feasibility of these fumigants for strawberry nursery management.

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The influence of some preparations on the health status of garlic bulbs (*Allium sativum* L.)

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Field experiments were carried out in 2012 and 2013 on two cultivars of garlic: 'Arkus' and 'Garpek'. The following biological plant protection products were applied: Polyversum WP (*Pythium oligandrum*), Trifender WP (*Trichoderma asperellum*) and biotechnical products: Alginure (extract of *Ascophyllum nodosum*, *Laminaria* sp.) and Biosept Active (extract of grapefruit). As the standard fungicide Topsin M 500 SC (tiophanate methyl) was used. Unprotected plants presented control. The health status of the plants were studied during the vegetation, it was noted number of wilting and dying plants. After harvesting mycological analyzes were also carried out on infested bulbs. The most frequently fungi *Fusarium oxysporum* and *Penicillium* spp. were isolated from the infected bulbs. The most effective preparations for protection of garlic against *Fusarium* wilt were used preventively Topsin M 500 SC and Polyversum WP, they also limited the decay of roots. Preparations Topsin M 500 SC and Alginure efficiently inhibited the development of necrosis of bulbs. Preventive treatments with means Topsin M 500 SC and Biosept Active effective limited occurrence *Alternaria* spp. *Fusarium* spp. and *Penicillium* spp. on bulbs in comparison with plants from control combination.

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Strawberry *Botrytis cinerea* management using iMETOS®sm forecasting model

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One of the most important strawberry diseases in the world is grey mould, caused by *Botrytis cinerea*. The base of disease forecasting is the interaction of pathogen biological characteristics and meteorological conditions. The fungus has the competence to develop infection at the temperature from 2°C to 28°C (optimal 20°C) and leaf wetness periods above 80%, which lasts more than 4 hours. Traditional strawberries plant protection from grey mould strategy had become not so efficient and needed new solution. iMETOS®sm (Pessl Instruments, Austria) forecasting models allows more efficient, ecological and economical accepted control of strawberry diseases. The aim of investigation was to analyze strawberry grey mould management using iMETOS®sm. Forecasting model indicates the risk favorable periods of infection on the basis of the interaction between air temperature and leaf wetness duration. iMETOS®sm grey mould risk forecasting model has a potential to optimize the usage of fungicides, the first application of fungicides are more accurate. If the risk is higher than 60 points more than 3 days a spray against grey mould should be applied. Comparison of both systems showed that either disease management systems reduced the spread of grey mould in strawberries. The iMETOS®sm forecasting model is innovative plant protection implement which lets to improve the application time.

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Forecasting the spread of onions *Botrytis* spp. diseases

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Botrytis spp. infects more than 200 plants species in different climate areas of the world either in Lithuania and can reduce yield from 15% up to 50%, mostly onions diseases symptoms appear during the storage. Even seven *Botrytis* spp. have been relative with *Allium* crops diseases, but the main are *B. cinerea* which cause leaf spot and *B. squamosa* – leaf blight. To protect onions against *Botrytis* diseases a large part of fungicides are used for preventative applications. The research was carried out at the LRCAF Institute of Horticulture in 2012-2013. The aim was to evaluate iMETOS®sm (Pessl Instruments, Austria) disease forecasting models for integrated onion *Botrytis spp*. disease management system. Applications were made according traditional plant protection and forecasting models systems. Forecasting models lets indicate the optimal conditions for the disease development, application are made more accurate. Analysis of iMETOS®sm forecasting models records showed dissimilar *B. cinerea* and *B. squamosa* occurrence in both years of investigation. Compared both systems, traditional plant protection was more effective in 2012, and in 2013 according forecasting model. The iMETOS®sm forecasting model gives the opportunity to optimize the use of fungicides and sometimes reduce the number of applications, therefore allows more efficient and economically accepted control of onions.

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VIPS – an open source technology platform for implementation of IPM tools, aimed at international collaboration and local adaptations

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VIPS is a technology platform developed for international collaboration on IPM, where forecasting models can be easily distributed to users worldwide. The model output views are flexible and simple to incorporate in existing web sites or distribute on smart phones and tablets. Cooperation on development, implementation, testing and validation of forecasting models is made easy in VIPS, and the source code for the platform is released under an Open Source License. The VIPS-system is based on 13 years of experience with a web-based forecasting and information service for integrated management of pests and diseases in cereals, vegetables, and fruit crops in Norway. A totally reconstructed and internationally flexible version of VIPS will be tested in Sweden and Bosnia and Herzegovina in 2014. The system allows local adaptations, including language, incorporation of models and other services. Our aim is to create a technology platform for international collaboration on IPM.

The rapid worldwide adaptation of mobile telecom technology creates new opportunities for information flow and interactive forecasting of pests and diseases. We are seeking partners interested in cooperation on developing the system, for example through joint R & D projects that include implementation of forecasting models and development of applications.

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Biological control of Fusarium head blight in wheat

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Fusarium head blight (FHB) is a destructive disease of cereals causing significant economic losses worldwide. Apart from yield reduction, FHB results in accumulation of various mycotoxins such as deoxynivalenol (DON) in cereal grains, which is toxic to humans and animals. A shift towards increased incidence of the DON-producing species *F. graminearum* has been reported in different countries including Sweden. In organic farming the control of FHB is based on cultural practices and application of fungicides in conventional farming has shown conflicting results possibly due to variations in sensitivity of *Fusarium* species to chemicals. Development of biological control as an alternative environmentally friendly approach to combat FHB disease can be of great value to cereal production worldwide. In this study we aim to investigate the potential use of yeast and bacterial isolates and a commercial biocontrol product to control FHB and to minimize production of mycotoxins. A collection of microorganisms were obtained from diverse plant and soil samples. Two different climate chamber bioassays were developed to evaluate the antagonistic effect of isolates against *F. graminearum* infection in wheat and oat plants. The infection of coleoptile in seedlings and spikes in mature plants were evaluated for the effect of treatments. The disease severity was also determined as percentage of infected seeds. The preliminary results indicate presence of potential microorganisms capable of reducing FHB disease and DON content.

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Identification of the gene resistance to leaf rust (*Lr 50*) in wheat varieties differing in origin

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One of the most important diseases attacking wheat in Poland is leaf rust causing by *Puccinia recondita f.sp. tritici*. The most effective way to control this disease is to introduce the varieties resistance to the pathogen. Due to the possibility of breaking the resistance by new races of pathogen it is necessary to search for new sources of resistance and introduce new resistance gene like *Lr 50*. Identification of genes that determine the presence of disease resistance is possible by the use of molecular marker systems which increase effectiveness of selection. Use of SSR marker Xgdm87 linked to gene *Lr 50* could significantly decrease the cost of breeding new resistant varieties of wheat. The aim of the study was to verify the effectiveness of SSR marker Xgdm87 linked to a gene *Lr50*. In first step presence of gene Lr 50 was checking in three reference genotypes and next 18 Polish winter wheat were examined. Isolated DNA was amplified using primers specific SSR (GDM 87-L and 87-P GDM). After testing different conditions of polymerase chain reaction (PCR) we confirm the presence of the gene in reference genotypes: KS96WGRC36 and Tam 107 and in the 6 Polish varieties of wheat: Mobela, Korweta, Ostka Strzelecka, Nateja, Naridana, and Wydma. These Polish varieties could be the donor genotypes in breeding programs.

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Treatment of seed of winter wheat with *Clonostachys rosea*

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The effect of seed treatment of winter wheat (variety Potenzial) with *Clonostachys rosea* was tested in two-years field trials (conventional field) in order to verify the possibility of using this means of biological control under field conditions, in organic as well as conventional agriculture. Variants of the experiment: nontreated control, seed treated with *Clonostachys rosea* (a mixture of four strains of *C. rosea*, the biopesticide in wettable powder form, wet application) and, for comparison, seed treated with Celest Extra (fludioxonil + difenoconazole) was also evaluated. The emergence, overwintering, weight and length of plants were evaluated. The health of plants and yield were also observed. The trials followed the results of laboratory and glasshouse experiments, and we obtained results which confirmed the efficacy of tested isolates of *C. rosea* against a few seedborne pathogens of wheat. Depending on the specific experiment, we found for seed treatment with *C. rosea* either no influence on emergence or a positive one, and a positive influence on the length and weight of plants. We have not observed any difference among the varieties concerning plant health. The effect of *C. rosea* on plants was fully comparable with the effect of Celest Extra. These results indicate a real possibility of using biological seed treatment of winter wheat with *C. rosea*.

Results were obtained with supporting NAZV QI111C039.

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A novel strategy to reduce overwintering inoculums of *Monilinia laxa*

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Brown rot on cherry and plum, caused by *Monilinia laxa*, is an important disease, for which overwintered mummified fruit is believed to be a significant inoculum source for infection of flowers and fruit in the spring. Fungal strains from mummified fruit and green fruit the following season were sampled across several sites and genotyped with microsatellite markers. There were no significant differences between the two populations from mummified and green fruit, suggesting that mummified fruit was an important source of overwintering inoculum. Experiments were conducted to assess the potential of applying plant protection products in winter and/or early spring to suppress sporulation on mummified fruit. Products tested included Indar (a commercial fungicide), *Aureobasidium pullulans* Y126 (a candidate BCA), *Bacillus* sp. B91 (a candidate BCA) and Serenade (a commercial BCA), in addition to a tap water treated control. Indar and *A. pullulans* Y126 significantly reduced sporulation when applied once in winter. Overall, a single treatment in early spring was slightly more effective than the single treatment in winter. Application of all products in both winter and early spring led to significant reduction in sporulation. Indar had the highest efficacy, reducing the number of spores from ca. 9×10^5 to 5×10^3 per mummified plum when applied twice. Of the three BCAs applied on both occasions, *A. pullulans* Y126 had the highest efficacy, reducing the number of spores from 9×10^5 to 5×10^4 per mummified plum. There was an additive effect between the two applications in winter and early spring based on the Bliss independence test. These results suggest that reducing overwintering inoculum in dormant season is effective and should be part of an integrated management strategy for brown rot on stone fruit.

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Estimation of garlic leaf blight infection season and effective controlling time upon infection estimation

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Garlic is one of main spice crop in Korea and consumed as an important ingredient in *Kimdi* and other foodstuff and famous for cardiovascular benefits. In Korea, garlic cultivar is classified as two ecological cultivar, northern and southern type. Northern type can overwinter before sprout is not emerged from clove and on the other hand, southern type can overwinter after the sprout is emerged before winter season. Leaf blight is major disease in garlic and cause significant damage to plant leaf and in some case, plant is dried to death and bring a serious yield loss especially in northern type cultivar. In these days, concern on environmental impact caused by agrochemicals is rising, so the issue of reducing agrochemicals load in agricultural environment and sustaining agricultural productivity at the same time. To solve these problems, we have tried to find optimal spraying numbers and an appropriate timely agrochemicals spraying. To estimate spore infection season on leaf, plastic film tunnel house is fitted in garlic field and the plastic film is removed after each rainfall day from March to June. Infection rate is highest in mid-April rainfall and then reduced in late-April rainfall season, so spore infection time via rainfall is assumed as mid to late April. Control efficiency is also highest when *Propineb* is applied in mid-April season. When *Propineb* applied after typical symptoms are visible in early-May, the control efficiency is considerably lower than the before the typical symptoms occurred in mid- to late April season. The control efficiency for 3 times of *Propineb* spraying before garlic leaf blight appeared in mid-April is higher than spraying for 5 times in early-May season when symptom is developed in early stage. We can propose that a beforehand control measure is more effective than control number in garlic leaf blight and could help reducing environmental pollution in agricultural field.

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Suitable carriers for the preparation of formulations of biological products based on nematophagous fungi

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The nematophagous potential of *Arthrobotrys oligospora* was observed as a potential tool for regulation of nematode populations on fields. As a potential carrier we used 5 different nutritional ingredients: zeolite, kaolin, vermiculite, talc and perlite. The ability of fungal growth was observed at two mediums: corn meal agar and oat meal agar. We had also available 11 different strains of *A. oligospora* to find the best isolate possible to control nematodes. The suitability of the combination of all variables was evaluated by the measuring of the speed of fungal growth that was observed after 24, 48, 72, 120 and 144 hours of growth. The results showed that all of these nutrient ingredients are able to positively affect the growth in comparison with control. It also has showed, that all the ingredients support`s the increase of the plant more in different stages of its growth. It means that the requirements of nutrients are changing for all the strains every day and every strain has the different requirements. As the best combination of all variables it was determined the combination of zeolite, oat meal agar and the strains no. 5, and no. 6. The results of this research can help to protect the fields against nematodes by the nonchemical way.

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Fungicide resistance screening in Czech cucurbit powdery mildew populations

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A total of 150 cucurbit powdery mildew (CPM) isolates (78 *Golovinomyces orontii s.l.* /*Go*/, 72 *Podosphaera xanthii* /*Px*/) from the Czech Republic from 2007 to 2011, were screened for tolerance and/or resistance to the four frequently used fungicides (fenarimol /formulated as RUBIGAN 12 EC/, dinocap /KARATHANE LC/, thiophanate-methyl /TOPSIN M 70 WP/, azoxystrobin /ORTIVA/) and a control fungicide (benomyl /FUNDAZOL 50 WP/). Majority of screened CPM isolates (135) originated from infected leaves of *Cucurbita pepo* and *C. maxima*. Fungicide sensitivity was determined by a modified leaf-disc bioassay with five concentrations. Highly susceptible *Cucumis sativus* 'Stela F_1' was used for preparation of leaf discs. Efficacy of the tested fungicides towards screened CPM isolates varied significantly during the studied period and corresponded with the early published results. In the case of azoxystrobin, there has been available no reports about resistance/tolerance in Czech CPM populations since the year 2006. There were found differences in frequency of resistant/tolerant response of CPM strains against dinocap and azoxystrobin. Fenarimol was nearly 100% effective. Dinocap expressed the high level of effectiveness. Nevertheless, the strains of both CPM species with tolerance to the lowest tested dinocap-concentration, eventually concentration 1x higher, were found during the all studied period. In 2011, there were noted resistance to the lowest dinocap-concentration or tolerance to the recommended dinocap-concentration among *Px* strains. Benomyl and thiophanate-methyl were totally ineffective. Azoxystrobin showed decreased efficacy from 2007 to 2011. In 2010, frequency of CPM strains with resistance to all azoxystrobin-concentrations increased in both CPM species. In 2011, these strains even prevailed substantially.

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Antifungal activity of some plant extracts against *Alternaria alternata* (Fr.) Keissel in the blackcurrant crop (*Ribes nigrum* L.)

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Alcoholic extracts from different aromatic plant, manufactured by Hofigal S.A. were tested *in vitro* and *in vivo* for their biological activity against *Alternaria alternata* (Aa20 strain), isolated from blackcurrant (*Ribes nigrum* L.).

Satureja hortensis and *Valeriana officinalis* extracts (tested at 20% concentration) presented the highest fungitoxic activity *in vitro* (with an efficacy of 100% inhibition of fungal development compared to untreated control). Other extracts demonstrate a lower antifungal activity *in vitro*: extracts from *Rosmarinus officinalis* (E = 88.6%), *Tagetes patula* (E = 78.5%), *Mentha piperita* (E = 77.1%) and *Allium sativum* (E = 72.8%), all tested at 20% concentration. Among these, the extracts of *Rosmarinus officinalis*, *Tagetes patula* and *Mentha piperita* showed efficacies between 72.8 to 64.3%, tested at 10 and 5% concs. A moderate activity against *Alternaria alternata* (E = 31.4% – 57.1%) has been noticed for the extracts from *Allium sativum* (tested at 10% conc.), *Achillea millefolium* (tested at all concs.) and *Hyssopus officinalis* (tested at 20% conc.). The lowest activity against *Alternaria alternata*, respectively no effect compared to untreated control, was noted when extracts from *Artemisia dracunculus 'sativa'*, at all concs., and *Hyssopus officinalis* at 10–5% concs, are used. The extracts of *Valeriana officinalis* and *Saturaja hortensis* proved to be efficient also in protecting blackcurrant plant against *Alternaria* disease. Compared with the untreated control, where a 9.05% average disease prevalence index was record, the disease prevalence index on blackcurrant treated with *V. officinalis* and *S. hortensis* was 0.85% and, respectively, 1.4%. These extracts, highly efficient *in vivo* against *Alternaria alternata*, can be recommended as an environmental-friendly alternative, especially on organic horticulture, for the protection of blackcurrant crop against blight disease.

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Net blotch occurrence and control in spring barley

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Net blotch (*Pyrenophora teres)* is a major disease of spring barley in Lithuania. The management strategy of the disease is focussed on growing resistant varieties, using pathogen-free seeds, applying effective seed treatment fungicides and foliar sprays, burying of crop residues, following crop rotation. Although varietal resistance is an important tool in Integrated Pest Management (IPM), resistance to diseases is generally not the main factor in farmers' choice. The susceptibility to net blotch of the most popular 23 varieties was tested during 2014 at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. The varieties Tocada, Rosalina, Kangoo and Luokė showed high susceptibility to net blotch from early growth stages while the varieties KWS Orphelia, Azit, Honey and Noja DS were the least damaged by the disease at grain filling stage. An important IPM tool for net blotch control and an environmentally safer practice than foliar application is seed treatment. Investigations on the efficacy of seed treatment fungicides against seed borne diseases show good results from year to year. The differences between the efficacies of seed treatment products and difference from the untreated control were clearly seen in 2014 until the flag leaf stage- in the best treatments net blotch severity was about 50% less compared to the untreated. However, application of fungicides is inevitable in barley-growing technology because susceptible to net blotch varieties grown often. With the aim of providing barley growers with robust independent information on fungicide performance in barley, in 2013–2014 different fungicides were tested in 2 varieties – Tipple and Quench. Products containing strobilurines and SDHI showed strong efficacy against net blotch, while propiconazole gave only slight control. The highest yields were obtained with a fungicide Viverda at full dose (2.5 l ha^{-1}) in the variety Tipple and with a fungicide Adexar (2.0 l ha^{-1}) in the variety Quench.

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Improvement of Polish hop cultivars by elimination of viruses and *Hop latent viroid* using *in vitro* cultures

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Hop mosaic virus (HpMV), *Apple mosaic virus* (ApMV) and *Hop latent viroid* (HLVd) are commonly detected in plants of all hop cultivars commercially produced in Poland. These pathogens can be responsible for reduction of yield and changes in the composition of secondary metabolites, although hop plants are usually without symptoms. In order to increase quality and productivity of hops the programme of production of virus and viroid free planting materials was established in the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy. Elimination of viruses and viroid was done by *in vitro* culture of isolated meristem tips 0,1–0,2 mm long. Regenerated plantlets with developed shoots and roots were tested for the presence of viruses and viroid using ELISA and RT-PCR methods respectively. Meristem culture have been successful in eliminating both viruses as well as HLVd, although its efficiency in case of viruses was much better. All plantlets regenerated from meristems were free of the HpMV and ApMV, whereas only from 1,6% to 15,7% of them, depending of hop cultivar, were free of HLVd. Healthy plantlets were multiply *in vitro* and then transferred to the greenhouse and tested again for viruses and viroid infections. Only plants with negative tests results were released for further propagation. These mother plants were propagated by single-node cuttings under controlled conditions in the greenhouse. Since 2013 healthy mother plants of all hop cultivars commercially produced in Poland were obtained. More than 10 000 virus and viroid free plants for planting were produced and released to hop growers.

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Production and health control of virus free hop

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The hop plant, *Humulus lupulus* L., is a dioecious perennial species, and only female cones are used for beer brewing. Hops are used to impart bitterness, flavour and preservation properties to modern beers. Hop as a perennial and vegetative-propagated crop is endangered by viruses and viroids. The most important viruses are: Apple mosaic virus (ApMV) from the genus *Ilarvirus*, Hop mosaic virus (HpMV) and Hop latent virus (HLV) from the genus *Carlavirus* as well as Arabis mosaic virus (ArMV) from the genus *Nepovirus*. Hop latent viroid (HLVd) from the group *Pospiviroid* is the most frequent viroid found out in hops.

They cause economical losses showing in lower yield and alpha bitter acid contents. These substances are very important for beer production. So as to be able to cope with this problem a recovery process has been started in Czech Republic. Meristem tips cultures are used to obtain virus free basic material. Production of planting material is carried out in accordance with EPPO guidelines (PM 4/16(1)). Assessment of health state is carried out at the level of *in vitro* cultures, technical and space isolation, high quality mother plants and propagated material in glasshouses. Health state of plants in hop gardens, which are applied for admitting process of exploitation of planting material and material in rootstock nurseries, are tested on virus presence. Long-range observation of healthy status in hop gardens planted with recovered hop plants is an inseparable part of our recovery program.

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Selection system for beneficial microorganisms following *Trichoderma* example

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Application of active microorganisms is considered as one of complementary methods in integrated farming system. Despite of various commercial preparations containing bacteria and fungi, presented on the market, new and more effective products are demanded. That requires efficient selection of microorganisms with satisfactory activity in crop production. In this study a simple and complementary screening system for *Trichoderma* fungi was developed. The *Trichoderma* strains were isolated form growing media, soil or rhizosphere of plants in main cropping systems. More than one hundred isolates were screened with system named A.M.O.R., where antagonism (A), mycoparasitism (M), ability to induce resistance (O) and plant growth promotion (R) were the main features of *Trichoderma* examined for further selection of appropriate strains. To study these abilities a set of complementary tests was established for each step of the selection. The results of the following tests confirmed activity of the fungi observed in former tests, and simultaneously offered additional information. Such system limited the necessity to repeat the experiments and reduced number of assays and labor time. The activities of *Trichoderma* were evaluated according to established indexes. The summary index number informed about properties of each isolate. The strains with the highest score were chosen for field experiments and positive results were obtained in cultivation of several vegetable crops treated with selected *Trichoderma*.

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Decision Support Systems (DSS) in winter barley control against powdery mildew (*Blumeria graminis* f. sp. *hordei*)

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One of the crucial elements of integrated plant control are different decision support systems (DSS). The main aim of DSS is clear indicating optimal time of chemical treatment. Different programs of DSS allow to reduce a number of chemical treatments while an efficacy of the treatment is satisfactory. The assumptions of DSS provide consumers and environment protection and improve the level of plants control. The DSS program has to answer to basic questions: When the chemical treatment should be done? Is the treatment economically well-founded? Which chemical product should be used? Some DSS require automatically submitted meteorological data day by day, directly from the meteorological station to the computer. The DSS NegFry for signaling late blight (*Phytophthora infestans*) can an example. There are also DSS programs mainly operating on the base of mathematical models (without day by day meteorological data automatically submitted to the computer), namely Epipre, MetPole, Kentucky Decision Guide, PC- Plant Protection. Programs for control main disease occurring on cereals.

The aim of the research was usefulness and effectiveness of three DSS programs (Epipre, Met-Pole, Kentucky Decision Guide) on winter barley plantations in Wielkopolska region.

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The effects of fungicides and biotechnological control agents on winter wheat infection by *Mycosphaerella graminicola* and the biochemical properties of grain

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A field experiment was carried out in 2010–2012 in Tomaszkowo near Olsztyn (NE Poland). Winter wheat (*Triticum aestivum* L., cv. Bogatka) was sown in 25 m^2 plots. The experiment had a randomized block design. During the growing season, benzimidazole, azole, morpholine and strobilurin fungicides were applied twice or three times at the stem elongation (first node) stage (BBCH 31), at the heading stage (BBCH 55) and at the watery ripe stage (BBCH 71). Winter wheat was also treated with a plant growth regulator, a plant growth promoter and a resistance inducer. The aim of this study was to estimate the severity of infection by *Mycosphaerella graminicola* on the flag and penultimate leaves of winter wheat, and to analyze the content and composition of protein fractions in kernels. Heavy rainfalls in May 2010 contributed to Septoria tritici blotch epidemics caused by *M. graminicola*, and fungicide treatment was ineffective in protecting the flag and penultimate leaves of winter wheat. In 2011 and 2012, the average efficacy of the second treatment with azole and strobilurin fungicides reached 79.6%. A positive correlation was observed between the intensity of Septoria tritici blotch symptoms on flag leaves and the content of albumins and globulins, and the concentrations of gliadin fractions in wheat grain. All protective treatments significantly decreased the albumin and globulin content of wheat grain in comparison with control samples. The highest total gluten content of grain was noted in winter wheat plants treated with fungicides (fenpropimorph, pyraclostrobin, epoxiconazole); the highest gliadin and glutenin levels were determined in treatments with tiophanate-methyl and tebuconazole, respectively.

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Sensitivity *in vitro* of *Colletotrichum truncatum* to essential oils

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Anthracnose caused by *Colletotrichum truncatum* is a serious disease problem for the soybean crop in the North and Center-West regions of Brazil. Seeking an alternative technique to control the disease, this study aimed to identify essential oils to inhibit the *in vitro* development of that fungus. The experiment, performed in duplicate, was carried out in a completely randomized design in a factorial scheme 8 × 4 [8 essential oils (neem, lemongrass, citronella, rosemary, eucalyptus, cloves, ginger and basil) × 4 concentrations (0.25%; 0.5%, 0.75% and 1.0%)] and two control treatments, with 3 replications. The oils were added to PDA medium at predetermined concentrations; one of the control treatments consisted of only PDA, and another, of PDA + fungicide. A disc of 0.5 cm diameter of PDA medium with the fungus was placed in the center of each petri dish containing the treatments. The plates were maintained at 22°C and 12 hour-photoperiod. To evaluate the inhibitory effect of the oils, mycelial growth (diameter of colony) and sporulation (number of spores/mL) for each treatment were determined. In both experiments, fungicide and essential oils of lemongrass, citronella, eucalyptus, clove and basil inhibited mycelial growth and sporulation completely, in all concentrations. High sporulation was observed in PDA + EO of neem and ginger in two trials, showing that these oils didn't have a good inhibitory effect. According to the results, it can be concluded that essential oils of lemongrass, citronella, eucalyptus, clove and basil have a potential use for the alternative control of *C. truncatum*.

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Evaluation of different plants as hosts for *Ditylenchus dipsaci* isolated from garlic

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Stem and bulb nematode *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 could cause serious problems in some temperate climate regions, e.g. in countries of the Central Europe. The biological race phenomenon of this species has been studied for many years. Long-term study of the spread of *D. dipsaci* garlic race in the Czech Republic did not explain the survival of this important pest of garlic. The aim of this study was to verify the host range of the garlic race of *D. dipsaci* isolated from infected *Allium sativum* L. planted in field conditions. In total, 548 experimental plants from 51 different species and varieties and from 19 botanical families were grown, infested and evaluated. One variant included 7–11 plants. No artificial path has been made for facilitation of nematode penetration into the plants. The group of cultivated plants included 27 variants; the group of ornamental plants included 15 variants. Four medicinal, aromatic and spice plants and 5 weed species were also evaluated. Eight weeks after inoculation, the experimental plants were tested for presence of the nematodes. It was discovered that the garlic biological race of *D. dipsaci* successfully survives in some plants species, which were formerly categorized into the group of non-host plants, namely cucumber, garden lettuce, rutabaga, bread wheat and two-roved barley. Otherwise, no compatible relationship was confirmed in case of the tomato as the host plant. Some of the tested weed species could also be suitable plants for survival of this parasite. Plant protection against *D. dipsaci* is not an easy task. New EU legislation follows reducing application of hazardous pesticides. If some field has already been infested, to choose suitable crop rotation is the only possible way to control this nematode

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Usefulness of some preparations for potato protection against early blight (*Alternaria* spp.) *in vitro* and field experiments

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An objective of executed research was valuation of influence of multiple spraying plants with substances: Biochikol 020 PC (a.s. chitosan), Grevit 200 SL (a.s. grapefruit extract), Prev-AM (a.s. orange oil) and valuation of those substances in limiting of linear growth of *Alternaria alternata* and *A. solani* mycelium *in vitro*. Acrobat MZ 69 WG (a.s. dimetomorf + mankozeb) was used as standard substance.

Results obtained from three year field tests show that in first (2009) and third (2011) year of research potato paralysis by *Alternaria* spp was significantly lower in all combinations protected by tested substances in comparison to control. In second year of research (2010) paralysis of potato leafs was less significant only in combinations protected by Grevit 200 SL and fungicide Acrobat MZ 69 WG. The reason of lower effectiveness of natural substances in reducing sickness development could be different weather conditions during potatoes vegetation time. Among tested natural substances it has been observed that as well during first as during second and third year of research leafs sprayed with substance based on grapefruit extract have shown clear signs trends in inhibition of *Alternaria spp*.development.

In vitro fungus activity expressed as percentage of mycelium growth reduction was depending on the substance, its concentration and tested fungus. The most effectively mycelium growth of both pathogens was inhibited by substance based on grapefruit extract. The weakest in reducing linear growth of mycelium of both *Alternaria alternata* and *A. solani* was substance based on chitosan.

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Influence of mycorrhized vaccines on health conditions of chosen plants

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The objective of executed research was valuation of influence which used mycorrhized vaccines have on growth of northern highbush blueberry (*Vaccinium corymbosum* L.). Field test was conducted in 4 combinations in 3 repetitions (subsoil without pathogen and mycorrhize, subsoil with *Alternaria alternata*, subsoil with mycorrhize and subsoil with *Alternaria alternata* and mycorrhiza).

Shoots growth and plant health condition was annually measured and valuated. Results obtained from three year field tests show that pathogen *Alternaria alternata* introduced into subsoil had inhibitory influence on growth of examined plants as compared to control. Mycorrhized vaccine introduced into infected subsoil influenced positively improvement of plants vegetation; both elongation and condition. In combination with mycorrhized vaccine in presence of *Alternaria alternataa* was stated bigger growth as compared to control. The best influence of myccorrhized fungi was visible on plants with pathogen-free subsoil. Presence of mycorrhized fungi was causing increased annual plant growth.

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Strong evidence for the transmission of *Tomato torrado virus* through the seeds of *Physalis floridana*

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During the last decade, *Tomato torrado virus* (ToTV) was recognized and identified on tomato plants. The occurrence and spread of the virus was reported in many countries of Europe as well as Central America and Australia. The virus very effectively is transmitted by whiteflies and grafting. There is no information on the transmission of ToTV by seeds. The establishment of ToTV over large geographical areas araises? the question of how ToTV managed to spread over such long distances. The goal of this research was to evaluate whether ToTV can be transmitted through *Physalis floridana* seeds.

In 2013 at summer period, *P. floridana* seedlings which were used for seed production were mechanically infected with the virus and kept in a greenhouse at temperatures ranging from 20°C to 25°C, and a 14 h photoperiod. Seeds were harvested on October and before sowing were kept in the refrigerator. In early spring of 2014, seeds treated with a 10% solution of K_3PO_4 were sown individually in small pots filled with soil. During 4 weeks after germination, the symptoms appearance on the young plants was observed. The presence of the virus in symptomatic seedlings or plants were analyzed by immunocapture real-time RT-PCR (IC/real-time RT-PCR) and reinoculation on *Nicotiana benthamiana*.

Among the 2520 tested seeds 33 plants with symptoms were observed. All of 33 symptomatic gave positive results in IC/real-time RT-PCR and 31 plant were positive in reinoculation on *N. benthamiana*. Identification of ToTV in *N. benthamina* was done by IC/real-time PCR. Sequencing of positively detected samples revealed a 99% nucleotide sequence identity in comparison with the sequence of ToTV used in the study. The rate of ToTV seed transmission in *P. floridana* based on the number of symptomatic plants was 1.31%. This is the first demonstration that ToTV is transmitted through *P. floridana* seeds.

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Pathogenic fungi against creeping thistle (*Cirsium arvense* (L.) Scop.) and possibilities of their application in control of this weed

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The creeping thistle is a common weed in many countries. In Poland it is a problem at the plantations of vegetables, cereals and other crops. It is also found in meadows and pastures, where it causes not only lower yield but it also reduces the value of grass and hay. It is difficult to remove the weed from such places. The aim of the study was to search for the fungal pathogens of *Cirsium arvense* in natural meadow agrocenoses, to assess their pathogenicity in experimental infections and to select the most effective isolates which could be used to prepare a mycoherbicide which would limit the creeping thistle population. The plants growing in natural meadow agrocenoses infested with *C. arvense* in the Warta Landscape Park between Dolany and Ląd (Greater Poland Voivodeship, Poland) were inspected and their health was assessed. The health of creeping thistles was assessed in 2010, 2011 and 2012. 21 fungal species of 14 genera were acquired (*Absidia*, *Acremonium*, *Alternaria*, *Boeremia*, *Botrytis*, *Colletrichum*, *Epicoccum*, *Fusarium*, *Glomerella*, *Lewia*, *Phomopsis*, *Sclerotinia*, *Sordaria*, *Verticillium*). The isolates of the species which were most numerously isolated from the infested plants were selected for further research. The experimental infections conducted in the laboratory and in the field revealed that the selected isolates of *Alternaria citri* and *Epicoccum nigrum* were distinguished by the greatest pathogenicity to *C. arvense*. However, natural secondary infections were observed only on the plants inoculated with *A. citri*. Among the isolates of *Sclerotinia sclerotiorum* which were isolated from creeping thistles and crops isolate 5c, which was isolated from creeping thistles, proved to be the best. The experimental infections proved that the *S. sclerotiorum* isolates under study exhibited diversified pathogenicity both to *C. arvense* and to other crops which may be potentially threatened by *S. sclerotiorum*. Among the *S. sclerotiorum* isolates under investigation isolate 5c proved to be the most useful. The health of the weeds inoculated with a suspension of the spores of the fungi under study was assessed in the meadow agrocenosis. The assessment revealed that the selected isolates of *A. citri* and *S. sclerotiorum* may be useful for the biological control of *C. arvense* population.

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The effect of nutrients on *Aphanomyces* root rot in pea

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Root rot in pea (*Pisum sativum* L.) and other legumes, caused by *Aphanomyces euteiches* sp. *pisi*, is responsible for the loss of about 10% yield on a global scale. Neither control nor suppression of *Aphanomyces* root rot by economical methods is currently available. In individual fields most plants may be damaged when the conditions are favorable for the disease development, so alternative ways to reduce *Aphanomyces* root rot are needed. Nutrients influence on plants, pathogens and soil – all elements of soil-borne disease interaction and are important for growth and development of plants and microorganisms, as well as plant resistance/tolerance to disease. Moreover they can affect soil properties, e.g. pH, electrical conductivity. However, it looks that nutrients potential have not been fully investigated yet. The objectives of our study were to test the effect of chosen nutrients: B, P and Cu on *Aphanomyces* root rot on pea. Three soils naturally infested with *A.euteiches* with different disease severity indices were chosen for the experiment. Positive tendency in reduction of disease severity was observed on plants treated with boron. Slight toxic effect of B was observed on plants, but it did not influence on fresh and dry weight of plants. Moreover on roots of B-treated pea plants greater numbers of large nodules were observed. The explanation of this phenomenon could be that boron plays a key role in the structure and integrity of cell walls and plasma membrane of root cells, strengthening of cell walls and protecting plants against pathogen attack and movement within host plant. Approximately 80% of all commercial fields in Sweden are B-deficient, what result in increasing permeability of plant cell walls, losing of nutrients through root exudation and attracting pathogens. Our conclusion is that B reduces pea root rot and may become an element of disease management strategy.

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The repeatability of field soil sampling and qPCR analysis of soil-borne pathogens in different soils

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A real-time quantitative PCR method for rapid detection and accurate quantifying of *Plasmodiophora brassicae* in soil samples is now available for Swedish farmers. This method allows detecting as little as 500 spores g^{-1} soil. However to get reliable results of qPCR analysis, the soil sample representative for the whole field has to be taken and proper DNA-extraction and purification has to be made. In the case of soil-borne pathogens, the patchiness on the field was observed, what makes the proper sampling very difficult. Moreover, it was observed that soil composition may influence on the DNA-detection level with qPCR. We did repeated sampling (4 pools, each consisting 10 randomly distributed cores collected within 3-m radius) at three points close to each other on the commercial cabbage field outside Lidköping (Sweden), to test the repeatability of such soil sampling. The results indicate that the level of pathogen in samples collected around the same point is similar. Although, quite big differences in DNA level were observed between the points. These results confirmed that sampling in just one point is not enough to get the results reflected the infestation level of the whole field. We also test the influence of soil chemical properties (pH, clay, sand and organic matter) on limit of detection of *P. brassicae*. Non-infested field soils were inoculated with *P. brassicae* spores, series of dilution were made and then DNA was extracted and analyzed with qPCR. It was observed that qPCR results for soil with high clay content were the most repeatable and closer to the expected amount of pathogen in the samples. In the case of soil with high organic matter content, strong inhibition of reaction and results inconsistency, especially in samples with lower infestation level, was observed. It is probably caused by disturbing of polymerase activity by humic substances.

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Pathogenicity of binucleate *Rhizoctonia* to cereals

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The aim of this study was to evaluate pathogenicity of binucleate *Rhizoctonia* (BNR) strains towards four winter cereals seedlings (barley, rye, triticale, wheat) in laboratory conditions. Twenty-eight of BNR strains, belonging to seven anastomosis groups (AG-A, AG-Bo, AG-C, AG-E, AG-I, AG-K, AG-D) obtained from soil, stems and roots of barley, rye, triticale, wheat, bentgras and zoysia grass collected in different countries, mainly in Poland, were used. Pathogenicity of the *Rhizoctonia* strains was investigated on Petri dishes in four replications. Pieces of PDA colonized by the tested strains were put on filter paper soaked with distilled water and placed into Petri dish. Twenty seeds of wheat were placed into each dish. Each strain was tested on 80 grains of each cereal. Infection on roots, coleoptiles and leaves was assessed after 14 days of incubation. The degree of the intensity of pathogenicity was determined applying the 0–4° scale. Air-dried matter of plants was also estimated. The analysis of variance showed differentiation between strains for infected roots, coleoptiles and leaves for all tested plant species. All AG-D strains were highly virulent to barley, rye, triticale and wheat. Most symptoms developed on coleoptiles, but also leaves and roots were infested. Among the other anastomosis groups the AG-Bo and AG-E caused less intense symptoms on plants. Tested *Rhizoctonia* strains belonging to the AG-A, AG-C, AG-I, AG-K were not pathogenic to cereal seedlings. Respective isolates also had an effect of on the air-dried weight of seedlings; however, the differences were not significant. The dry weight of seedlings depended considerably on the infection of cereals. The greater the infection resulted the lower the air-dried weight. There was observed no considerable dependences between the geographic origin of the isolates, the host plant and the infection of seedlings of the cereals researched.

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Molecular methods for discrimination of European isolates of *Soil-borne wheat mosaic virus* (SBWMV)

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Soil-borne wheat mosaic virus (SBWMV) belonging to the genus *Furovirus*, family *Virgaviridae* was first detected in central part of the USA, in 1923. The virus is transmitted by plasmodiophorid *Polymyxa graminis* Led. and it is a well known pathogen causing mosaic, stunting and reduced yield of winter wheat and other cereal species. In Europe the virus presence was confirmed in Germany (2002, named SBWMV-De1) and in Poland (2010, named SBWMV-Pol1). Molecular analyses of RNA2 fragment encoding CP gene indicated that nucleotide sequence of Polish and German isolates was identical and closely related with American US-Nebraska isolate of SBWMV. In 2013 another isolates named SBW-SHI3 and SBW-SHI4 were detected in the north of Germany. Using molecular methods, the virus was identified as a new for Europe, New York-type isolate of SBWMV. Plants infected with all presented SBWMV isolates reacted positively in ELISA with commercial antiserum (Loewe), and that serological method do not discriminate the viral strains. The aim of this work was development of rapid and reliable molecular methods for discrimination of distinct European isolates of SBWMV.

The virus typing was conducted by both RT-PCR-RFLP and Real-Time IC-RT-PCR methods. An 587 bp RT-PCR fragment was successfully amplified with the primers pair SWrflp-F(AAAGGTTACACTGGTTACAA) and SWrflp(GCAATTATGAGTCGATTCTG), and then was used for specific digestion by *Spe*I and *Bst*UI endonucleases, *EcoRI* was used as a negative control. Obtained restriction profiles specifically discriminate between the Nebraska-type and New-York- type isolates of SBWMV. Both studied SBWMV isolates were clearly distinguished by Real-Time IC-RT-PCR with SWhrm-F (GACGGTGACGAGATTTTCTG) and SWhrm-R1 (AAATCTTTCGTACGCACAAA) primers pair. The results for studied isolates were analyzed by the derivative plot of the melting curve, and were presented as two separate peaks with different T_m.

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Winter wheat root rot forecast in the condition of the Republic of Belarus

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Plant debris is one of the main sources of inoculum of *Fusarium* fungi causing winter wheat root rot in Belarus. Asexual conidia production on debris requires moist conditions so relative humidity is a key factor affecting production and dispersal of this kind of inoculum. Autumn field conditions characterized by relative humidity less than 80% are favourable for the production of *Fusarium graminearum* and *F. avenaceum* ascospores that act as additional inoculum source of root rot. In the conditions of hydrothermic coefficient excess, soil conditions for winter wheat growth become unfavourable what leads to root rot severity increasing. In 2010–2013 relationships between the previous year autumn weather condition and root rot severity of winter wheat were examined. It is determined that disease severity at tillering depends on average relative humidity and hydrothermic coefficient from August to October of previous year ($p < 0.01$, $R^2 = 0.99$). The linear equation is , where Y is the root rot severity at GS 25, X_1 is average relative humidity for the period August–October and X_2 is hydrothermic coefficient for the same period. Forecast accuracy in 2014 depending on winter wheat variety has made 73.9–98.7%. Temperature and relative humidity are the key factors for prediction root rot severity at booting (growth stage 32) ($p < 0.02$, $R^2 = 0.99$). The linear equation is , where Y_1 is the root rot severity at GS 32, X_3 is the average temperature for October and X_4 is relative humidity for the same period. Forecast accuracy in 2014 depending on winter wheat variety has made 51.1–70.6%.

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Identification and quantification of *Fusaria* in air samples from Poland in 2011–2013

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Infection of wheat by fungi from *Fusarium* genus, especially *F. avenaceum*, *F. graminearum* and *F. culmorum,* leads to Fusarium head blight – a disease which causes loss of crop quantity and quality due to mycotoxin production. The majority of infections begin at wheat flowering period, when fungal spores are released to air, especially in favourable weather conditions. To investigate diversity and occurrence of *Fusaria* in air we collected 3780 spore samples on wheat fields located in four geographical and climatic regions in Poland. The monitoring was done around wheat flowering period using MicroBio MB1 volumetric spore traps. At first, fungal strains were preliminarily identified using microscopic analysis and then the identification of species was done using PCR-RFLP, CAPS and dCAPS techniques. Spore numbers and species composition varied between analysed regions, years and months of study. *F. avenaceum* was the most prevalent species while the occurrence of *F. sporotrichioides*, *F. acuminatum*, *F. culmorum* and *F. graminearum* was at intermediate level. The spores of other species, such as *F. tricinctum, F. cerealis*, *F. poae, F. equiseti, F. proliferatum* were much less frequent. The highest and lowest numbers of *Fusarium* spp. spores were observed in Charbielin (south-west of Poland), respectively in 2011 (566 isolates) and 2013 (157 isolates). Considering all studied years, the highest summary number of spores was observed in Krasne (south-east of Poland, 1044 isolates), and the lowest in Choryń (central-west, 794 isolates). The spore count of *Fusarium* spp. in air was the highest in 2011 (1443 samples), and in 2012 and 2013 this parameter was lower (respectively 1144 and 1193 samples).

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Ecological conditions of occurrence of *Phylloporia ribis* (Schumach.) Ryvarden on *Euonymus europaeus* L. and the influence of chosen substrates on its growth

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Phylloporia ribis is a fungus parasitizing mainly on euonymus and currant. The presence of its fruiting bodies was detected on 95% of the examined euonymus bushes growing in proximity of Kraków. The high frequency of occurrence indicates high susceptibility of euonymus to infections as well as significant expansiveness of this pathogen. *P. ribis* was responsible for cancerous wounds in basal part of the stem, reaching 0.5 meter in height and located mainly on the northern and northeastern side.

The growth of *P. ribis* cultures on a medium containing sawdust from euonymus and currant did not differ significantly but it was 28% higher in comparison to the cultures growing without sawdust. However, decay of blocks of euonymus and currant wood was relatively weak and averaged 2,6 and 1,8% respectively after three months. So slow decay of wood might be the reason of the slow, long-term development of disease on euonymus infected by this fungus. The more intense decay of euonymus wood in comparison to currant wood may indicate differentiation in nutritional preference or the occurrence of variations within the species. Further research on ecology of the fungus might prove useful, among other things, because of pharmacological properties of the extracts of its fruiting bodies used in transplantology.

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Fine mapping and development of Single Nucleotide Polymorphism markers for clubroot resistance locus in *Brassica rapa*

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Clubroot disease is one of the most economically important diseases affecting Brassica crops worldwide including oilseed *Brassica napus*, vegetable brassicas such as *B. rapa* and *B. oleracea*. The genetic basis of clubroot resistance has been well studied and mapping of resistance loci have been reported in these species. Previously, we reported the mapping of clubroot resistance, *CRb* locus, located in A3 chromosome of *B. rapa* using $F_{2/3}$ mapping populations derived from resistance and susceptible parental crossing and tightly linked sequence characterized amplified region (SCAR) markers. In this study, fine mapping of *CRb* locus was done using 1500 F_2 population.

Genetic mapping using gene specific markers, comparative mapping with *Arabidopsis thaliana*, and whole genome re-sequencing of resistant and susceptible parental lines were used to identify potential resistance genes. We identified several candidate genes around *CRb* locus and parental nucleotide polymorphism were used to design SNP primers and validated by mapping.

Twenty genes were found as putative candidate genes. Out of these, one gene represented co-segregating with resistance phenotype. We developed few SNP markers based on parental differences observed in that gene.

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Comparative mapping of *Raphanus sativus* genome using *Brassica* markers and quantitative trait loci analysis for the Fusarium wilt resistance trait

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Fusarium wilt (FW), caused by the soil-borne fungal pathogen *Fusarium oxysporum* is a serious disease in cruciferous plants, including the radish, *Raphanus sativus*. To identify quantitative trait loci (QTL) conferring resistance to FW, we constructed a genetic map of *R. sativus* using an F_2 mapping population derived by crossing the inbred lines '835', susceptible line, and 'B2', resistant line. A total of 220 markers distributed in 9 linkage groups were mapped in the *Raphanus* genome, covering a distance of 1041.5 cM with an average distance between adjacent markers of 4.7 cM. Comparative analysis of the *R. sativus* genome with that of *Arabidopsis thaliana* and *Brassica rapa* revealed 21 and 22 conserved syntenic regions, respectively. QTL mapping detected a total of 8 loci conferring FW resistance that were distributed on 4 LGs, namely, 2, 3, 6, and 7 of the *Raphanus* genome. Of the detected QTL, 3 QTLs (2 on LG3 and 1 on LG7) were constitutively detected throughout the 2-years experiment. QTL analysis of LG3, flanked by ACMP0609 and cnu_mBRPGM0085, showed a comparatively higher logarithm of the odds (LOD) value and percentage of phenotypic variation. Synteny analysis using the linked markers to this QTL showed homology to *A. thaliana* chromosome 3, which contains disease-resistance gene clusters, suggesting conservation of resistance genes between them.

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Inheritance of PVY resistance in subsequent generations of transgenic tobacco lines

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Growing of resistant cultivars is the most effective method of plant protection against viral diseases. Different sources of resistance are used for breeding new tobacco cultivars resistant to *Potato virus Y* (PVY). One of them are transgenic lines coming from transformation of cultivars MN 944 and AC Gayed. It is important to determine the effectiveness of transgenes in protection from the virus and also whether they are stably inherited.

We tested two transgenic breeding lines: MN 944 LMV (containing gene of lettuce mosaic virus coat protein) and AC Gayed ROKY2 (containing antisense gene of PVY replicase), which were previously transformed using *Agrobacterium tumefaciens* and leaf disk culture. The subsequent generations of transgenic plants obtained by self-pollination were studied to determine the rate of plants containing transgenes. The individuals, which showed to be transgenic, were also tested for resistance applying artificial PVY inoculation under greenhouse conditions. We reported segregation of plants in respect of presence of the transgenes and increasing rate of transgenic plants was observed in subsequent generations. However, the high frequency of transgenes not always correlated with increased resistance of plants. In case of line MN 944 LMV increasing level of resistance up to generation T_4 was reported but in T_5 we observed the resistance decreased and many plants showed to be susceptible in spite of the presence of transgene LMV. In turn, within line AC Gayed ROKY2 the resistance of plants was generally high but unstably inherited. The results showed changeable effectiveness of transgenes and indicated the need for further analyses including studies on expression of transgenes.

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Effectiveness of combining resistance to *Potato Virus Y* and *Chalara elegans* in tobacco doubled haploids

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Black root rot (BRR) caused by soil born fungal pathogen *Chalara elegans* and brown veinal necrosis induced by PVY (*Potato virus Y*) are the most severe diseases of tobacco (*Nicotiana tabacum* L.). The most effective way to minimize *Chalara elegans* and PVY damage to tobacco would be to grow resistant cultivars. The breeding WGL line carrying resistance to *Ch. elegans* as well as to isolates of the $PVY^{N}W$ group was crossed with the BPA line that exhibits tolerance to highly virulent PVY^{N} isolates. The anther culture technique was used to recover haploids from F_1 hybrids combining resistance to both pathogens. The regenerated plants were analyzed using DNA flow cytometry. Ploidy analysis showed that 112 of the 115 genotypes were haploids while the remaining 3 were spontaneous doubled haploids. Haploid genotypes were first cloned and then simultaneously screened for PVY and *Ch. elegans* resistance in greenhouse tests. Biological inoculation with the $PVY^{N}W$ isolate was done. From a total of 78 haploids, symptomless and ELISA negative plants accounted for 34.6%, twenty nine (37.2%) were tolerant while twenty two (28.2%) of them were susceptible. The presence of *Ch. elegans* spores was detected by the microscopic evaluation of roots. Forty haploid plants (51.3%) had no symptoms of infection and were regarded as resistant. Based on PVY and BRR greenhouse tests, genotypes combining resistance/tolerance to both diseases were chosen and cultured *in vitro* to generate doubled haploids (DH). Doubled haploids were tested using PVY^{N} isolate that shows the ability to overcome the main sources of resistance, that can be found in the cultivated tobacco. A total of 18.2% of DH were resistant (symptomless and ELISA negative), 18.2% were tolerant and 63.6% became infected and developed veinal necrosis. Altogether, 4 doubled haploids showed multiple resistance to PVY^{N} and *Ch. elegans*.

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Vegetation type and air humidity determine the extent of ash dieback

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In 2011 a study of ash dieback distribution and importance was performed in Lusatian Mountains PLA with use of 80 permanent plots divided in five groups according to vegetation type. The extent of ash dieback (rate of the crown withering) and different stand factors were investigated in each plot. It was found out, that pathogen *Hymenoscyphus pseudoalbidus* was presented in 94% of the investigated plots and the crown damage reached 10.3% in average (more than 4100 m^3/ha after recalculation). Vegetation type groups statistically differed in the extent of damage ($p < 0.05$) and they were separated into two homogenous groups. The riparian stands and ash-alder alluvial forests were significantly more damaged than the solitaires, scattered plantings in open landscape and forest stands on slopes. In 2012 air humidity was measured with using of automatic sensors on 50 plots (10 sensors in each vegetation type). It was found out, that the average air humidity statistically differed among vegetation types ($p < 0.001$) and corresponded to the pattern of the disease impact. The highest air humidity was identified in the riparian stands and in ash-alder mixed alluvial forests. The significant regression of extent of damage to summer air humidity was found out ($r = 0.36$; $p = 0.012$). Moreover, a negative regression of extent of ash dieback on terrain slope of plots was identified ($r = –0.28$; $p < 0.05$).

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Resistance screening of *Alnus glutinosa* and *Fraxinus excelsior* to invasive pathogens *Phytophthora alni* and *Chalara fraxinea*

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Black alder (*Alnus glutinosa*) and European ash (*Fraxinus excelsior*) are important native woody plants in the Czech Republic. Lately, their stands are devastated by alien invasive pathogens *Phytophthora alni* subsp. *alni* (PAA) and *Hymenoscyphus pseudoalbidus* (anamorph: *Chalara fraxinea*), respectively. One of the main environment-friendly approaches to finding a solution to this problem is to identify naturally resistant genotypes and to use them for restoration plantings and in resistance breeding programmes. The aim of the work was to identify the potential variability in resistance to the mentioned pathogens of both taxa using a series of artificial inoculation experiments. For this reason ninety black alder genotypes from different regions of the Czech Republic, seven ash genotypes of different health status and two isolates of the PAA and *C. fraxinea* were selected for use in *in vitro* infectivity trials, respectively. Subsequently the resistance/susceptibility levels of ten of the best-performing black alder genotypes and one with a high level of susceptibility were tested again in a second round of tests using five other PAA isolates. Host susceptibility varied significantly and widely among individuals of both taxa. Significant variation in host tree resistance and the isolate pathogenicity was observed. The observed differences in the black alder populations were also found to be dependent on the particular isolate used, altitude and geographical origin of the genotypes and in the European ash was found to be dependent on the particular isolate used and health status. All the best-performing genotypes identified in these trials were subsequently propagated and are conserved. Observed findings suggest the considerable potential for selection of resistant individuals of both taxa to the mentioned pathogens in natural populations that could be used in resistance breeding.

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Study on the susceptibility of Redtop (*Agrostis gigantea* L.) on the fungal pathogens

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The subject of the conducted research was to assess the potential threat of Redtop grass cultivated for seeds in different row spacing to infection by the most dangerous pathogens. The level of infection of Stefka, Gosta, Mieta and Paula cultivars planted at the following row spacing: 24 and 48 cm was estimated in the field conditions. Occurrence of disease symptoms was analyzed before seed harvesting. Leaf spots (*Drechslera* spp., *Bipolaris* spp.) and rusts (*Puccinia* spp.) were observed on Redtop grass in the years of study. There were significant differences in susceptibility of studied cultivars to infection by pathogens. The most susceptible cultivar to infection by leaf spots and rust was Stefka (disease indexes: 57.7% and 63.3% respectively). The least susceptible cultivar to infection by leaf spots was Mieta (disease index: 35.0%) and Gosta showed the lowest level of infection by rust fungi (disease index: 29.5%). There was not observed any effect of row spacing on the occurrence of leaf spots and rusts during the vegetation periods of the studied years.

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Animal proteins as a source of resistance inducers to *Leptosphaeria maculans* in oilseed rape

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The phenomenon of induced resistance has been known for decades; however, its practical exploitation is still limited due to its lesser effect in comparison with traditional pesticides. Recent European legislation on pesticide limitations motivates scientists to search for new efficient, cheap and environmentally friendly compounds, which could be used as a pesticide substitute. A number of elicitors derived from pathogens and various chemical compounds have been shown to improve plant resistance in a number of species, however their potential to be broadly utilized is limited by phytotoxicity, high price, low stability, etc.

Here we demonstrate that hydrolyzates of animal protein wastes produced in leather processing efficiently induce plant resistance to pathogen. The pretreatment of oilseed rape (*Brassica napus*) cotyledons with the hydrolysed collagen and keratin induced resistance to ascomycete *Leptosphaeria maculans*, the causal agent of stem canker of oilseed rape. The area of lesions caused by the pathogen dramatically decreased in treated plants. The application of the hydrolysates induced defence responses regulated by the plant hormone salicylic acid (SA) and ethylene, which was demonstrated by the expression of SA- and ethylene-dependent genes (*PR-1*, *WRKY70* and *HEL*). We suppose that efficiency of *B. napus* protection is depended on the size of peptides produced by protein hydrolysis. Our results indicate that collagen and keratin waste could serve as a source of compounds inducing resistance in plants.

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Enhanced resistance to bacterial blight diseases in transgenic rice plants overexpressing antimicrobial peptides

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The antimicrobial peptide possesses defence system to virus, fungi and bacteria. To study antibiotic in plant, antimicrobial peptides were obtained by PCR analysis by primers designed from antimicrobial peptides (Gene bank accession no. NM-004345), cloned in pET28 expression vector and the vector transformed into *E. coli*. And this gene was inserted into Ti-plasmid VB2 vector, which contained the pGD1 promoter. The expression construction was transformed into *Agrobacterium* EHA105 and then plant tissues of rice (*Oryza sativa*). Seeds from transgenic plants (T0) were germinated on selective media containing spectinomycin 50 mg/L. Selected plants and wild type were analyzed by PCR and RT-PCR with pGD1 promoter region and transgene specific primer set. All transgenic plants showed expression pattern of similar levels. We showed that the chromobody is effective in binding GFP- and *antimicrobial peptide* gene in tobacco leaf. Most interestingly, this can be applied to interfere with the function of GFP fusion protein and to mislocalize (trap) GFP fusions to the plant cytoplasm in order to alter the phenotype mediated by the targeted proteins. Bacterial blight disease was enhanced resistance in transgenic lines. These results showed that antibiotic peptides might show a broad-spectrum antimicrobial activity.

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Effect of *Neotyphodium lolii* endophyte on production of Pathogenesis-Related Proteins in perennial ryegrass (*Lolium perenne* L.) infected by *Fusarium poae*

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Perennial ryegrass is a very important grass in Polish farming. Diseases caused by pathogenic fungi are frequent problem in its cultivation. Their harmfulness can be limited thanks to natural symbiotic systems between *L. perenne* and *N. lolii*. The exact mechanism of higher resistance of endophyte infected plant is yet not fully understood, but it can be assumed that Pathogenesis-related proteins play an important role in this process. Chitinases and β-1,3-glucanases are enzymes, which are related to the plant resistance to pathogens. They usually occur together, as their integrated action can cause more damage in fungi cell wall, than any of them could cause alone. The aim of the research was to determine the impact of *N. lolii* on induction of specific defense mechanism, including production of pathogenesis-related proteins: chitinases and β-1,3-glucanases. The plants inhabited by endophyte were much more resistant to infection by *F. poae*. Presence of the endophyte has affected the amount of chitinases in perennial ryegrass. It was significantly higher in E+ plants and dependant on the time after infection. Upward tendency was being observed since first to third day after inoculation. No significant influence of *N. lolii* on β-1,3-glucanases production and general protein content was observed.

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Can PDV influence the presence of PPV in peach tree GF305?

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A set of one hundred peach tree rootstocks was inoculated with *Plum pox virus* (PPV) and *Prune dwarf virus* (PDV) in five different manners: 20 trees only PPV; 20 trees only PDV; 20 trees PPV and PDV at the same time; 20 trees first PPV, then PDV; and 20 trees first PDV and then PDV. During six vegetation periods (2009–2014) the DAS-ELISA analysis and reverse transcription PCR were performed to detect the viruses present in the trees. The positive samples were then tested using RT-qPCR for the quantification of the virus. During the years, the presence of the PPV seems to be decreasing, especially in the presence of PDV. In some cases, the PPV was not detect at all, even though during the first years of the experiment the trees were tested positively. This was observed especially in the trees infected at the same time with PPV and PDV.

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Variation in susceptibility of winter barley cultivars to *Rhizoctonia cerealis* (sharp eyespot) and *R. solani*

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In the field study period from 2007 to 2013, at Chrząstowo (53°09′ N; 17°35′ E), the incidence and severity of sharp eyespot were studied on 25 of winter barley cultivars. At the BBCH 75–77 random samples were taken. The percentage of stems with symptoms of sharp eyespot was evaluated. The degree of the intensity of sharp eyespot was determined, applying the 0–4° scale. The evaluation of the plants' health status was supplemented by mycological analysis and PCR assay using the specific SCAR primers. Susceptibility of 34 cultivars of barley to *R. cerealis* (AG-D subgroup I) and *R. solani* (AG-5) was studied under laboratory conditions. In the laboratory test pieces of PDA colonized by the test isolates were put on filter paper soaked with distilled water and placed into Petri dish. Infection on roots, coleoptiles and leaves was assessed after 14 days for *R. cerealis* and after 10 days for *R. solani*. There was much variation in incidence and severity of sharp eyespot between years. The mean percentage of diseased stems on 25 cultivars was 1.3–30.5 (–51.0), and the mean disease index was 0.3–10.2 (–22.5), with the lowest and highest values in 2008 and 2007, respectively. The cultivars with least intense disease were Karakan, Reflexion, Epoque and Traminer. The cultivar with most intense disease was Gregor. Culture analysis on PDA medium and PCR assay confirmed that *R. cerealis* was the main causal organism; *R. solani* was detected less frequently. There was a wide variation in the susceptibility of barley cultivars to both *Rhizoctonia* species. Cultivar Corbie showed low, while cv. Nicoletta, Metaxa and Eureka – high susceptibility to *R. cerealis*. Cultivars Lomerit and Bombay had low, and cv. Seduction and Amarena had high susceptibility to *R. solani*. No cultivar was resistant to *R. cerealis* and *R. solani*.

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Belarusian potato varieties as a source of PVY resistance genes

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Potato virus Y (PVY) is one of the most essential pathogen for potato farming in all the Europe. Cultivation of resistant varieties is considered to be the best mean for plant protection and preventing virus spreading. One hundred varieties and tetraploid *S. tuberosum* hybrids that used as the parental lines in breeding programs in Belarus were studied on the presence of PCR markers for genes *Ryadg* and *Ryf-sto* of high resistance (immunity) to PVY. The marker $RYSC3_{321}$ of gene Ry_{adg} (originated from *S. andigenum* ssp *tuberosum*) was revealed in 22 varieties and 15 hybrids (in 36% of studied genotypes). The PCR-RFLP marker $GP122/EcoRV_{564}$ of gene $Ry_{f\text{-}sto}$ (introgressed to cultivated potato from *S. stoloniferum*) was much rare: it was present in only two varieties (Vetraz and Ragneda) and five hybrids. The Belarusian variety Vetraz and two hybrids carried both of the genes. Generally, the resistance genes were present in some studied potato varieties in simplex allelic form (according to segregating analysis). Nevertheless, we have found that the Belarusian variety Universal was triplex for Ry_{adg}. This assures lack of segregation for PVY resistance in hybrid populations resulted from crosses with it.

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Molecular marker for selection of *Rph7* gene and effective *Mla* alleles in malting barley

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Barley is one of the most economically important crops around the world. Diseases caused by fungal pathogens significantly reduce yield. The most important fungal diseases are leaf rust caused by *Puccinia hordei,* and powdery mildew caused by *Blumeria graminis* f. sp. *hordei*. The most economical and ecologically friendly way to avoid losses caused by these diseases is growing resistant varieties. Concerning practical breeding for powdery mildew and leaf rust resistance, simple, cheap and robust selection methods are required. Marker assisted selection (MAS) is of great potential to fulfil this demand. We present development of a robust duplex marker for simultaneous selection of *Rph7*, and presence of one of the following *Mla* alleles: *Mla16*, *Mla19*, *Mla20*, *Mla21*, *Mla27* or *Mla28*. Ecotilling marker was converted to more breeder-friendly CAPS, and added simultaneous selection for *Rph7* gene in a duplex reaction. Cleavage by *RsaI* endonuclease was shown to be robust enough, and specific for the resistant phenotype, so it is possible to use it for marker assisted selection of effective *Mla* alleles. PCR and cleavage of its products by *RsaI* endonuclease can be successfully run in duplex for effective *Mla* alleles and *Rph7* gene brings along the possibility to select the tracked genes more effectively. This duplex marker can be used in pyramiding strategies in common barley breeding programs.

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Pathogenicity of *Leptosphaeria maculans* isolates obtained from *Brassica napus* (oilseed rape) cultivars with the *Rlm7* resistance gene

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Isolates obtained from winter oilseed rape cultivars with the resistance gene *Rlm7* were examined for their pathogenicity by inoculation onto cotyledons or true leaves of the susceptible cultivar Drakkar (no *R* gene) and cultivars with the resistance gene *Rlm7*e (Excel, Roxet, Hearty and line 01-23-2-1). After assessment of lesions, cotyledons and true leaves were detached 17 and 21 dpi, respectively, and incubated in darkness under high humidity to assess pycnidial development and conidial production. All the isolates tested on Drakkar produced typical large/grey lesions (susceptible phenotype) on both cotyledons and true leaves; large numbers of pycnidia with conidial masses were produced within and outside the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation. All the isolates tested on the four *Rlm7* cultivars produced small lesions surrounded by dark margins (resistant phenotype) on cotyledons with no difference in lesion area between isolates. However, there were differences between isolates on true leaves of Roxet but not on true leaves of Excel, Hearty and 01-23-2-1. Most of the isolates produced small numbers of immature pycnidia on the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation of the four cultivars with the *Rlm7* gene. However, these pycnidia were not able to produce conidia. After further incubation, when cotyledons and true leaves senesced, mature pyncidia developed outside the lesions and produced conidia.

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Lolitrem B content in perennial ryegrass (*Lolium perenne L.*) infected with selected *Neotyphodium lolii* isolates as affected by temperature of plants growth

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Grasses often are infected by nonpathogenic fungi belonging to the *Neotyphodium* genus. Presence of the symbiont in plants tissue may increase the host resistance to biotic and abiotic stress. This effect is stimulated by a vide range of chemicals e.g. second metabolites that are accumulated in plants tissue. These compounds possess antagonistic activity to herbivores and high concentrations of the toxins can lead to animals toxicoses. The content of the toxins varies through the season, which suggests a high effect of external factors on its production. The objective of our research was to evaluate the effect of the temperature on Lolitrem B concentration. The lolitrem B content in perennial ryegrass infected with *Neotyphodium lolii* endophyte was analysed. Four perennial ryegrass/endophyte associations: Nl10, Nl22, Nl24 and Nl104 were used. Three ranges of temperature: 14–15°C, 19–20°C and 24–25°C were applied. Experiment was conducted in pot conditions. Lolitrem B was detected with HPLC method. Significant effect of temperature on lolitrem B production was observed. The higher temperature of plants growth significantly increased the content of the alkaloid. Associations Nl10 and Nl22 produced the highest amounts of the toxin. The influence of the host and endophyte genotypes on lolitrem B content was noted. The lowest level of toxin was detected in perennial ryegrass ecotype infected with isolate Nl24. This isolate, after detail research, could be used as biological control agent in grass protection against stress factors.

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Protective effect of *Neotyphodium uncinatum* on meadow fescue (*Festuca pratensis* Huds.) attacked by pathogens

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The objective of our research was to assess the beneficial impact of the *Neotyphodium uncinatum* endophyte on its natural host – meadow fescue, measured by susceptibility of the host plants to infection by pathogens and a content of the toxic alkaloid – ergovaline in field conditions. The research involved 'Justa' meadow fescue. Studied factors were as follows: endophyte infection (E+ and E–) and system of use (for pasture and for cut). The infestation of 'Justa' meadow fescue by the endophyte, *N. uncinatum,* significantly protected the plants from infection with fungi causing leaf spot. The endophyte, however, did not affect the development of powdery mildew and rust fungi. 'Justa' meadow fescue showed a relatively high content of ergovaline when grown in the field. The level of the toxin in the season varies a lot, which suggests a high effect of external factors on its production. Due to the toxin production, the animal feed made from infested plants can pose a threat to animals when longer administered. *Neotyphodium uncinatum* isolates from 'Justa' meadow fescue cannot be used as a biological control agents to improve the growth and resistance of other cultivars due to ability to ergovaline production.

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Comparison of available sources of PVY resistance in tobacco using virus isolates from central Europe

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Potato virus Y (PVY) is one of the most important pathogen of tobacco leading to significant losses of the crop, decrease of its quality and increase of the nitrate content in tobacco leaves. PVY is transmitted by aphids in a non-persistent manner. The use of insecticides to control spread of the virus have been ineffective, therefore, a lot of effort has been put into producing resistant tobacco cultivars. There are few sources of PVY resistance in tobacco acquired through large scale breeding programmes (e.g. cultivar VSCR), X-ray induced mutagenesis (cultivars VAM and TN86) and resistance transferred from wild species such as *Nicotiana africana* (BPA breeding line). Here, we compare major sources of PVY resistance in inoculation tests using ten PVY isolates differing with their virulence and collected in Central Europe (Poland and Germany). None of the tested sources of resistance showed a complete resistance against all ten isolates. However, BPA line infected with all isolates did not develop vein necrosis despite chlorotic spots observed on the leaves and PVY detected in the tissues using ELISA tests (PVY tolerance).

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Susceptibility of German winter barley varieties against fusarium head blight

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Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe) and *Fusarium culmorum* (W.G. Smith Sacc.) belongs to the most damaging diseases in cereal crops. *F. graminearum* and *F. culmorum* were described to produce Deoxynivalenol (DON), but also ability to produce DON derivates and zearalenone has been recognized. The aim of contribution was to estimate winter barley varieties for head blight resistance to *Fusarium culmorum* and to allocate cultivars to resistance types.

The susceptibility of 35 six and two rowed winter barley cultivars against fusarium head blight was evaluated in field experience 2011–2013 using the spray inoculation with a conidia suspension of *Fusarium culmorum*. In each plot the incidence and severity as percentage of the fusarium infection was visual assessed. Fusarium head blight (FHB) and AUDPC were calculated using both parameters. Barley kernels were harvested and analyzed for presence of Deoxynivalenol (DON) using the ELISA-testkit.

In the resistance trials the six rowed cultivars showed a triple so high fusarium head blight than the two rowed cultivars. The most of two rowed cv. (e.g. Duet, Passion) and some six rowed cv. (e.g. Merylin, Naomie) had a significantly low susceptibility. Analysing the toxin data sets; there was a mean of 6.0 mg/kg DON. For cv. Merilyn, Passion and Campanile with low values of disease symptoms a low DON contamination was detected. High susceptible were the six rowed cv. Candesse, Laverda and Lomerit. Correlation among FHB and DON was high with a coefficient of $r^2 = 0.77^{**}$.

The two rowed cultivars can assigned to resistance type II. The severity was rather low and for the most varieties a spreading of *Fusarium culmorum* were not assessed.

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Development of method for evaluation of pear cultivar resistance to scab and *Venturia pyrina* virulence *in-vitro*

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The pear scab caused by *Venturia pyrina* Aderh. is an economically important disease worldwide, especially in organic orchards. The disease is becoming more important in European countries by breaking the cultivar resistance, however, the breeding programs for pear scab-resistant varieties are still under development. Evaluation of pear cultivar resistance to scab or pathogen's virulence usually is performed by inoculating plants in the greenhouse or monitoring of the disease development in the orchards under natural infection conditions. Both methods have some restrictions, such as screening of resistance reactions of larger numbers of plant genotypes with specific isolates or number of isolates is limited, quantification and characterization of the pathogen in the field is not possible. Attempts were made to develop a method for initial screening of pear genotype resistance to scab fungus *V. pyrina* and for evaluation of the pathogen virulence in-vitro by inoculating detached leaves and immature fruits. Two ways of inoculation were tested. Spore suspension of the mixed field population was used as a positive control. Eight pear cultivars and seven *V. pyrina* isolates originated from different host genotypes and locations in Latvia were included in the experiments. The cultivars with variable susceptibility to the scab were selected based on field observation. Microscopic and macroscopic tissue reactions were monitored during the experiments. Both susceptible and resistant reactions were observed in different combinations of the host genotype and the pathogen isolate. The typical resistance reactions, such as pinpoint lesions, necrosis or chlorosis without the sporulation were seen in some genotype/isolate combinations. Variability among *V. pyrina* isolates was also detected.

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Study of transcriptome changes in tomato plants after application of elicitin oligandrin and β-aminobutyric acid (BABA)

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Compared to well characterise defence reaction in tobacco plants the key components of tomato plants defence system have not been well understood up today. Even though both plants are closely related from taxonomically point of view, defence mechanisms seems to be very distant. In the study the effect of β-aminobutyric acid (BABA), considered as a priming agent, and elicitin like protein oligandrin on transcripts accumulation of 45 tomato genes was determined. All measured genes were selected on the basis of previous studies and covered basic classes of pathogenesis related proteins (defensins, germins, β-1,3-glucanases, β-1,4-glucanases, heveins, chitinases, osmotins, systemin and PR-1 proteins). Moreover, two tomato species differ in resistant, highly resistant species *Solanum habrochaithes* and susceptible species *Solanum lycopersicum* cv. Amateur, were used. The changes in transcripts levels were measured two days after inoculation by RT-qPCR using SYBR green chemistry and relative quantification by ΔΔCt method.

Both cultivars showed many up-regulated genes after oligandrin treatment. On the other hand, BABA treatment elicited up-regulation of genes only in *S. lycopersicum* species and almost no in *S. habrochaithes* species. In advance, in *S. lycopersicum* species the up-regulated genes after BABA and oligandrin treatment are almost completely different. It could be suggested that observed differences are caused by different signal pathway of oligadrin and BABA. While in case of elicitins salicylic acid seems to be a basic signal molecule, in BABA treatment ethylene plays a crucial role. To clear-up the role of individual signalling molecules in tomato defence reaction, nowadays we are performing the phytoprotection tests with different types of pathogens on both studied tomato species.

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Effector-triggered defence against apoplastic fungal pathogens

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The plant immune system was originally defined to include pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). These immune responses control interactions with haustoria-forming pathogens, but the terms PTI and ETI do not adequately explain interactions with apoplastic fungal pathogens. Haustorial pathogens generally deliver their effectors into the host cell, whereas apoplastic fungal pathogens deliver effectors into the apoplast to target extracellular or membrane proteins. To better explain interactions between apoplastic fungal pathogens and their host plants, this resistance is referred to as effector-triggered defence (ETD). This response is mediated by *R* genes encoding cell surface-localized receptor-like proteins (RLPs) that engage the receptor-like kinase SOBIR1. In contrast, ETI depends on cytosolic nucleotide binding site (NBS) leucine-rich repeat (LRR) receptors that directly or indirectly recognise pathogen effectors targeted into host cells. Unlike ETI, which is usually associated with fast, hypersensitive host cell death, ETD often triggers host cell death only after a period of endophytic pathogen growth. This resistance does not generally eliminate apoplastic pathogens because pathogens like *Pyrenopeziza brassicae* and *Zymoseptoria tritici* are able to complete their sexual life cycle on the resistant host.

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Transgenic plums – hope of resistance to *Plum pox virus*?

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Transgenic plum (*Prunus domestica*) cv. "Honey Sweet", clone C5 is highly resistant to *Plum pox virus* (PPV). The resistance is based on the post transcriptional gene silencing (PTGS) and is conferred by siRNAs. The highly resistant clone C5 was grafted onto the rootstock St. Julien, which was inoculated by PPV-Rec infected graft (P. domestica cv. Emma Leppermann) in the field condition (from 2003 – till now). Therefore, the tree was under continuous infection pressure from susceptible scion of inoculated plum cv. Emma Leppermann. The quantitative analysis of virus titre was assessed by RT-qPCR in both Emma Leppermann and C5 scions. In the non-resistant virus source cv. Emma Leppermann the viral titre grew exponentially over the years, but in the C5 scion the virus was almost undetectable. Hence, silencing signal confers the stable resistance phenotype in C5 even under extreme virus pressure caused by PPV infected susceptible graft, which is unlikely, occurred in a natural growing conditions of stone fruit trees.

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Effect of *Colletotrichum acutatum* on the yield of selected strawberry cultivars

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In 2013 the experiment was conducted in plastic tunnel with eight cultivars of strawberry: 'Alfa Centauri', 'Camarosa', 'Darselect', 'Elsanta', 'Florence', 'Honeoye', 'Selva' and 'Senga Sengana'. The plants were inoculated with a mixture of *Colletotrichum acutatum* isolates at a concentration of 5×10^6 conidia/ml. The isolates, obtained from strawberry plants were cultured in the dark on the PDA medium. The plants in the control combination were sprayed with the sterilized water. In June the yield of fruit was estimated (five harvests), separately for healthy fruits, for fruits with disease symptoms and for misshapen fruits. In all combinations the yield of healthy fruits was higher for control plants than for those inoculated with the pathogen. The biggest reduction of healthy fruit yield was noticed for cv. 'Camarosa' (by >50%). Also cvs. 'Alfa Centauri' and 'Elsanta' reacted negatively to *C. acutatum*, their yield decreased by 30% and 19%, respectively. The lowest reduction of healthy fruit yield was observed for cvs. 'Darselect' and 'Senga Sengana' (by 1.9%). After the harvest the plants were collected for mycological analysis. *C. acutatum* was obtained from plants of all cultivars and was a predominating species (62% of all colonies). Other isolated fungi were: *Botrytis cinerea, Cylindrocarpon destructans, Fusarium oxysporum, Penicillium purpurogenum* and *Pestalotia truncata*.

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RNA-Seq analysis of BABA-induced resistance to *Phytophthora parasitica* in tomato emphasizes a hyper-responsive plant status

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The non-protein amino acid BABA (β-aminobutyric acid) induces resistance in tomato towards downy mildew and root knot diseases, caused by an oomycete and a nematode, respectively (Cohen et al., 1994; Oka et al., 1999). The aim of our work is to decipher gene networks that can explain induced resistance of tomato to pests, in order to further evaluate molecules and products that might be used for sustainable crop protection. BABA is considered here as a positive control since the molecule is unadapted for field applications due to its perceptible phytotoxic effects. After direct foliar spraying, BABA (10 mM) induces a strong resistance to *Phytophthora parasitica* infection, reducing disease symptoms by 90%. To monitor the BABA-induced changes in transcript accumulation, we collected tomato leaf samples from 6 different plants, and in 3 independent replicates at 24 h after treatment. Transcript profiling was performed with NGS (Solid™), reads were mapped to the tomato genome (www.solgenomics.net), and sequencing depth was adjusted to near 100-fold the genome size. We found that more than 1,300 genes were up-, and almost 200 genes were down-regulated upon BABA treatment ($padj < 0.01$). A majority of up-regulated genes code proteins involved in signal perception (membrane-bound and intracellular receptors), the regulation of transcription (ERF, WRKY, and MYB transcription factors), and the execution of defense (PR-proteins). BABA also triggers a noteworthy up-regulation of genes encoding tomato defense proteases that are targeted by Avr2 (Rooney et al., 2005) and *P. infestans* effectors (Song et al., 2009). We also show that the coordinated regulation of enzymes from the secondary metabolism is fully supported by analytical results. Our data let us conclude that BABA responses are mainly governed by ethylene and that this molecule promotes pathogens perception.

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Biostimulants and *Pythium ultimum* in cut chrysanthemum

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Soil borne pathogens may cause major damage to chrysanthemum (*Chrysanthemum morifolium* Ramat.) in greenhouse horticulture. This research investigated sustainable alternatives to chemical crop protection against *Pythium ultimum*. A greenhouse field experiment was conducted using a randomized block design including eighteen treatments divided over three blocks. The treatments *Streptomyces griseoviridis,* potassium phosphite, *Bacillus* sp. and calcium provided a significant protection against *P. ultimum* in chrysanthemum cv. Grand Pink. Subsequently, a bio-assay was conducted to optimize the treatment with *Streptomyces*. A double dosage of *Streptomyces* increased the mean fresh weight of chrysanthemum with twenty-three gram. An addition of calcium did not affect this; however, an addition of dicyandiamide reduced fresh weight comparable to that of the untreated control.

In addition, six soils were sampled at different chrysanthemum growing greenhouses. Bio-assays with *Chrysanthemum morifolium* cv. Grand Pink were used to estimate the level of soil disease suppression. Simultaneously, a rapid germination test was conducted together with determination of the available carbon fraction in soils. Results obtained from two latter tests were in agreement with bio-assay results. Thus, both can be considered as a good, rapid and inexpensive alternative to measure soil disease suppression towards *P. ultimum*. According to cluster analysis, the higher level of suppression of the soils were positively correlated to EC, sodium, clay fraction, CEC, calcium, silt, and negatively to sodium soil occupation and sand fraction. The results are discussed with respect to the soil suppressiveness model developed by our institute.

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Methods of increasing the resistance of field cucumber to the bacterium *Pseudomonas syringae* pv. *lachrymans*

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The aim of the research was: to test the susceptibility of six cultivars of cucumber (*Cucumis sativus* L.) and two of its cultivars sown from encrusted seeds to the bacterium, an increase in the resistance of a susceptible cultivar to the pathogen and an analysis of the causes of a diverse resistance of plants. Encrusted: 'Śremski' is more resistant than 'Aladyn', but from six: 'Fortuna Snow' is susceptible, but 'Cezar' – the most resistant to this pathogen. With the use of scanning electron microscopy there was observed the morphology of leaves of both the cultivars. A susceptible cultivar has more stomata (which correlated with a stronger development of the disease) than the resistant one. Seedlings of cucumber 'Fortuna Snow' (at the stage of three leaves and after seven days) were sprayed with solutions of: jasmonic acid methyl ester – Me-JA (0.001, 0.0025, 0.005, 0.01)%, β-aminobutyric acid – BABA (5, 10, 20) mM, vitamin C (0.05, 0.5, 1.0, 2.5) mM, Talius 200 EC (7.5 and $8.9 \cdot 10^{-5}$, $1.7 \cdot 10^{-4}$, $5 \cdot 10^{-3}$)%. After the next 4 days they were treated with the suspension of bacteria. On the 30th day after the inoculation of 'Fortuna Snow' leaves with the pathogen the highest protection of plants (72%) was obtained after the application of a 0.01% of Me-JA solution. There was found no toxic action of the mentioned solutions on plants nor antimicrobial properties, but *in vitro* H_2O_2 (10 and 20 mM) had an antibacterial action. *In vivo* a high accumulation of lignins and H_2O_2 more than 3.3'-diaminobenzidine peroxidase correlated with the natural resistance of cucumber at 24 h after the inoculation of leaves with the pathogen. Lignins, which are a main indicator of the induction of plant resistance occurred in a susceptible cultivar treated with 0.01% solution of Me-JA.

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The induction of a resistance of the greenhouse tomato to *Pepino mosaic virus*

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The aim of the study was the examination of a degree of the resistance of four cultivars of the greenhouse tomato (*Lycopersicon esculentum* Mill.) to *Pepino mosaic virus* and the increase of their resistance. Naturally susceptible to PepMV tomato cultivar was 'Robin', but the most resistant – 'Pelikan'. For the immunisation, the following concentrations of inducers: benzo-thiadiazole – BTH (0.03, 0.25, 0.5) mM, β-aminobutyric acid – BABA (5, 10, 20) mM, the Indian chitosan: 0.006% in 0.003% acetic acid, 0.006% in 0.003% acetic acid with 0.03 mM BTH, 0.05% in 0.025% acetic acid, 0.05% in 0.025% acetic acid with 0.25 mM BTH, 0.1% in 0.05% acetic acid and 0.1% in 0.05% acetic acid with 0.5 mM BTH were used. All cultivars of the tomato were treated with milk. Upper sides of leaf blades of each plant at the stage of eight developed leaves were sprayed with different solutions twice at an interval of seven days. Four days later two leaves (the 3rd and 4th) were inoculated mechanically with PepMV. The percentage of an infected surface of fruits was calculated after 3.5 months from the inoculation of leaves with the virus. The most effective in increasing the resistance were different solutions for each cultivar. The highest protection of fruits was obtained after the treatment of leaves with chitosan: 0.006% in 0.003% acetic acid with 0.03 mM BTH – 93% ('Corindo'), 0.006% in 0.003% acetic acid – 88% ('Faraon'), 0.05% in 0.025% acetic acid – 85% ('Pelikan') and only with milk – 82% ('Robin'). All mentioned solutions were not phytotoxic.

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Global transcriptome and metabolome profiles of *Arabidopsis thaliana* nonhost resistance to *Leptosphaeria maculans*

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Nonhost resistance is a widespread phenomenon exhibited by most plant species that are able to resist pathogens, which successfully infect host plants. *Leptosphaeria maculans* is an important fungal pathogen, causing blackleg disease in canola. *Arabidopsis thaliana* is a nonhost of the blackleg pathogen. Plants of *A. thaliana* accession Col-0 were inoculated with *L. maculans* isolate SC006 and mock-inoculated with water as a control. Three biological replicates of leaf samples were harvested from 10 plants per sample at 3 days post-inoculation. Global transcriptome and metabolome profiles were determined on Illumina Hiseq 2500 and LTQ Orbitrap Mass Spectrometry, respectively. Over 140 million of short reads from the six samples were assembled into an *A. thaliana* reference genome package (TAIR 10) and identified differentially expressed genes (DEGs) using Lasergene Genomics Suite 11.2.1. Differential gene expression at 95% confidence was found for 5876 genes: 2211 up-regulated and 3665 down-regulated, which is equivalent to almost 20% of the *A. thaliana* expressed genes in this experiment. Among the DEGs, 308 genes are involved in immune system processes. In accordance, a strong discrimination was observed between the metabolomes of the inoculated and control plants. After data deconvolution from a large number of metabolites that were discovered as biomarkers ($P < 0.05$), 328 metabolites involved in plant response to biotic stress were identified. Pathogen invasion caused a general disturbance of the plant's primary and secondary metabolism, up-regulating among others, a-linolenic acid metabolism and flavone and flavonol biosynthesis. The integration of transcriptomics with metabolomics data will provide a global overview of *Arabidopsis* metabolism regulation in response to *L. maculans* within a systems biology approach.

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Transcriptome analysis of defense related mechanisms in *Brassica napus* against the fungal pathogen *Leptosphaeria maculans*

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Leptosphaeria maculans, the causal agent of blackleg on canola, is among the major concerns of fungal pathogens on *Brassica napus* (canola) production worldwide. The utilization of genetic resistance is the most economical and environmentally friendly strategy to manage this disease. Global changes of gene expression following *L. maculans* challenge in both resistant and susceptible *B. napus* accessions were assayed by RNA sequencing to investigate key factors involved in disease defense mechanisms. Several selected marker genes were used to select time points for RNA-seq: *PR-1*, *BnWRKY75,* and lignin metabolism related genes. Quantitative real time PCR (qPCR) was employed to examine the expression pattern of each gene prior to RNA-seq. Expression of all marker genes increased in both resistant and susceptible accessions. Expression of *PR-1* reached high level after 3 days post inoculation (dpi) in both resistant and susceptible accessions, and kept stable in the resistant accession after 5dpi. High level expression of *BnWRKY75* was detected in resistant accession on 3dpi, but on 7 dpi in susceptible accession. The relative expression of lignin metabolism related genes increased following fungal infection. Two time points (3dpi and 7dpi) were further selected for RNA-seq based on marker genes expression. The results revealed high degree of differential gene expression post infection between resistant and susceptible accessions. Variation of gene expression patterns relative to several time points (3 dpi and 7 dpi) was also detected. Function of differentially expressed genes was then characterised. The main focus of this study was genes involved in the blackleg resistance pathways and plant defence mechanisms.

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Effects of different fungicides on development of phoma stem canker and oilseed rape yield

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Phoma stem canker, a disease of oilseed rape (*Brassica napus*) caused by pathogens *Leptosphaeria maculans* and *L. biglobosa*, is an economically important disease causing annual yield losses of approximately £1000M worldwide (Fitt et al., 2008). Both pathogens follow a monocylic disease cycle causing leaf spotting in autumn/winter and stem cankers in spring/summer. Most severe cankers decrease transportation of water and nutrients to the developing seeds, resulting in reduced yield (Eckert et al., 2009). The use of fungicides is an important tool for controlling phoma stem canker in the UK. The triazole fungicides currently dominate the market, although reduced sensitivity in some plant pathogen populations is an emergent concern (Carter et al., 2013). This current study aims to examine the efficacy of a novel fungicide when compared with flusilazole, a commonly used fungicide at the time of assessment. It also aims to investigate whether *L. biglobosa* is less sensitive to triazole fungicides in comparison to *L. maculans*. Field trials were established in Boxworth (Cambridgeshire) in the 2012/2013 and 2013/2014 cropping seasons. Four fungicides were applied: penthiopyrad, picoxystrobin, flusilazole and a novel fungicide. Spray timings were divided into three sprays T1, T2 and T3, with T1 being applied in autumn when phoma leaf spotting incidence was ≥ 10% per plant and T2 sprayed four weeks later. T3 was applied in spring against Sclerotinia sclerotiorum. Phoma leaf spotting incidence, stem canker severity and yield were recorded. *In vitro* sensitivity testing was done on one *L. maculans* and one *L. biglobosa* isolate. Flusilazole, at various concentrations, was applied to PDA medium, which was later inoculated with a mycelium plug. Flusilazole showed no noteworthy advantage over the novel fungicide in both the canker severity scoring or yield results. A significant difference in growth inhibition was observed between *L. biglobosa* and *L. maculans* isolates ($P \leq 0.05$). Field trials indicate that the novel fungicide is as equally effective as the more commonly used triazole fungicides and could replace flusilazole for commercial use. Fungicide sensitivity testing showed that *L. biglobosa* does have an increased level of resistance to triazole chemistry when compared with *L. maculans*. This interaction confirms previous studies that have also determined a reduced sensitivity to triazole fungicides in *L. biglobosa* populations (Eckert et al., 2009; Huang et al., 2011).

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Comparative analysis of Immunophilins in *Leptosphaeria maculans* and *Saccharomyces cerevisiae*

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Cyclophilins (CYPs) and FK506-binding proteins (FKBPs) are abundant ubiquitous proteins belonging to the peptidyl-prolyl cis/trans isomerase (PPIase) family . They are collectively referred to as Immunophilins (IMMs). They have been implicated in both biotic and abiotic stresses. Phytopathogenic fungus *Leptosphaeria maculans* damages oilseed rape crops worldwide by causing blackleg disease. IMM genes in *L. maculans* were identified and classified. 12 CYPs and 5 FKBPs were identified in total. These numbers are more or less similar to other phytopathogenic fungi. Domain architecture analysis revealed the presence of conserved catalytic domain cyclophilin-like domain (CLD) in CYPs and FKBP_C in case of FKBPs. Interestingly, IMMs in *L. maculans* were found to be sub-grouped into single domain (SD) and multidomains (MD). They were mainly found to be localized in cytoplasm, nucleus, and mitochondria. Homologous and orthologous gene pairs were also determined by comparing IMMs from *L. maculans* with model organism *Saccharomyces cerevisiae*. Notably, we observed that IMMs in *L. maculans* posses shorter introns as compared to exons. Moreover, CYPs contain low number of exons on contrary to FKBPs. However, two CYPs were determined to be intronless. Secondary structure feature revealed the presence of typical eight β strands and two α helices fold architecture. Conserved motifs were also elucidated and represented in form of sequence LOGO. Gene ontology analysis predicted their significant role in protein folding and PPIase activity.

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Fungicide spray time greatly affects the incidence of stem canker and seed yield of winter oilseed rape

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Among the diseases that affect oilseed rape, one of the most damaging is stem canker or blackleg caused by *Leptosphaeria maculans* and *L. biglobosa*. The main source of plant infection are ascospores originating from pseudothecia, produced on stubble from the previous vegetative season. Monitoring of spore dispersal is of great importance for disease risk assessment. The aim of this work was to determine the effect of the autumn and early spring spraying time on the incidence and severity of stem canker in relation to the presence of spores in the air. The experiments were done over five seasons: 2008/2009–2012/2013. Depending of the vegetative season the fields with winter oilseed rape cultivar PR46W10 (Pioneer Hi-Bred) were placed in 3–5 sites located in different regions of Poland. Fungicide treatments were done at weekly intervals from late September to mid-November, using Capitan 250 EW, containing 250 g of flusilasole per 1 L of the fungicide.

We have demonstrated that time of fungicide application had a strong, statistically significant influence on the effectiveness of protection of oilseed rape against stem canker. The percentage of healthy plants was significantly different between assessment dates, years and locations. Each time the lowest percentage of healthy plants was observed on plants untreated with fungicides. A spray was most effective, when applied up to 14 days after the detection of the highest concentration of *Leptosphaeria* ascospores in air samples. An application of fungicide at this time reduced the incidence of phoma leaf spotting by 12.5–24.7%, stem canker symptoms by 11.3–33.0% and decreased yield loss by 2.7–5.0 dt ha^{-1}. We conclude that knowledge about the concentration of pathogen inoculum in the air allows more efficient protection of oilseed rape against stem canker.

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Resistance of interspecific hybrids within the genus *Brassica* to blackleg (*Leptosphaeria maculans*) in glasshouse and field conditions

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Leptosphaeria maculans is the most ubiquitous fungal pathogen of *Brassica* crops and causes the devastating stem canker disease of oilseed rape worldwide. Strategies for stem canker disease management include cultural practices such as crop rotation, isolation of the crops from infected stubble of the previous growing season crops, and stubble management. Some control is achieved through the use of fungicides but, at present, disease control relies mainly on the use of disease-resistant cultivars. That's why the incorporation of *L. maculans* resistance into *Brassica* lines with desirable agronomic and quality traits is the major objective in breeding programs. Screening of plant susceptibility/resistance to *L. maculans* was done for 130 hybrids between *Brassica* species and 5 parental genotypes. The hybrids represented F3–F6 generations of crossings between *Brassica napus* (*male sterile line*) *and B. carinata*, *B. juncea*, *B. campestris ssp. pekinensis* and *B. campestris ssp. trilocularis*. In glasshouse screening was conducted using a cotyledon test based on inoculation of two-week old seedlings with spore suspension of four *L. maculans* isolates (10^7 conidia ml^{-1}). The plants were grown in a controlled environment chamber, maintained at alternating periods of 16 h light at 20–22°C and 8 h of darkness at 16–18°C. Droplets of spore suspension were placed on each half of a cotyledon – 4 inoculation points per plant – previously wounded by puncturing with a thin needle. Host response was scored using 0–6 scale, where 1–3 score was recorded as a resistant reaction and 4–6 score corresponded to susceptible reaction. The field experiment was carried out in a randomized complete block design with 3 replicates. Disease incidence was assessed in summer, two week before harvest, on 50 plants per replicate plot, according to scale 0–9. The tested genotypes differed with their reaction to the pathogen, allowing to select some genotypes for further studies.

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Development of sexual stage of blackleg disease in oilseed rape in Bulgaria

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Experiment focused on *Leptosphaeria maculans/biglobosa* sexual stage development was set up in growing season 2013/2014 in Plovdiv (Central South Bulgaria). Field as well as laboratory monitoring on infected canola debris were performed. In the autumn only picnidia were formed on the canola stalks, but no sexual fruiting bodies were found as well as infected nearby seedlings. First pseudotheica were recorded on 7.2.14 (observations interval – 7 days), afterwards squash mounts microscoping was done on weekly basis with classification of asci according to 4 grades scale described by Toscano-Underwood et al. (2003). Results were as follows: 25th Feb. – 50% A and 50% B class, 4th Mar. – 73% A, 24 B, 2% C, 19th Mar. – 2% A, 31% C and for the first time 67% D, on 24.3, 2.04, 11.04 – data ranged between 7–9% C and 90–92% D class. On 17.4 only class D asci were present, approx. 5% of them were already empty (class E). Moist chamber test for 4 hrs at T = 18°C was done on 11.3.14 and it confirmed physiologically mature and readily discharged ascospores in 3 out of 4 separate samples (canola stalks). Within 14 days first black leg symptoms appeared in the field. Rain events (>1mm) were only 19 from 1.7.13–1.2.14, 8 out of them were recorded till 30.10.13 when temperature has still been about 22°C (upper threshold in some forecasting models). Winter was relatively mild but still days bellow 0°C, which should be unfavorable in respect of pseudothecia development, took place. Interestingly first pseudothecia appeared just after snowfall (26.1–3.2.14), which was the first serious event of the kind during this winter. Results indicate that climate and pathogen progress could be taken into consideration before fungicides treatment are recommended. Such approach is in accordance with IPM and contrasts with present calendar based sprays most Bulgarian farmers perform every autumn.

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Clubroot resistance management in European oilseed rape crops

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Clubroot has developed into a major concern for European oilseed rape growers (*Brassica napus* L.). The release of the clubroot resistant winter oilseed rape cultivar 'Mendel' has been a mile stone in clubroot control in oilseed rape and the efficacy of this resistance source is of key relevance not only in Europe. 'Mendel' is on the European seed market since 2001 and despite its race-specific resistance this cultivar and its successors "Mendelson" and "Mentor", which share the same resistance, still give control in most cropping areas. Virulent pathotypes can be found occasionally and the number of virulent incidences on 'Mendel' is slowly increasing. Breeding attempts are undertaken to broaden the genetic basis of clubroot resistance in oilseed rape. Major clubroot resistance loci have been identified in the *Brassica rapa* A genome on chromosomes A03 and A08 (Werner et al. (2008), TAG 116, 363–372). These loci have been transferred from *B. rapa* to *B. napus* and *B. napus* differential lines representing either of these have been developed. Clubroot reactions of the differential hosts with *Plasmodiophora brassicae* isolates from European oilseed rape crops will be presented and the value of the different resistance loci for resistance breeding will be discussed in view of resistance management concepts. The combination of resistant cultivars with other IPM measures should be pursued to achieve healthy crops and to prevent the erosion of clubroot resistance efficacy.

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New possibilities to control the *Plasmodiophora brassicae* in *Brassicae* plants

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In 2010–2014 about 100 fields from different parts of Poland were examined for presence of *P. brassicae* in soil. This pathogen causes clubroot – severe problem on the *Brassicae* plants in Poland. In cabbage and oilseed rape yield losses of 10 to 50% have been reported. Long lived resting spores making the pathogen difficult to control and eradicate. Methods based on DNA technologies make it possible to specifically estimate infestation level of pathogen in soil. Average number of spores per g of soil in 25% of tested samples exceeded 700 000 spores per g of soil. On so heavily infected fields, brassica crops should not grow. One of the natural control methods of soilborne pathogens – especially *P. brassicae* – may be achieved by use catch crops. This kind of plants stimulate the germination of pathogen resting spores, resulting in decreasing or limiting expression of disease symptoms. Quantitative real time PCR allows to determine the level of spores before and after the catch crops treatment and define the effectiveness of this method. The effects of selected catch crops on the development of clubroot on brassica plants were tested in 1 m^2 size microplots in 2012 and 2013. During 2014 three field trials witch selected catch crops were conducted. All experiments showed more than 50% decreased of clubroot in field after catch crops cultivation compared to the control.

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Metabolic interactions between *Plasmodiophora brassicae* and *Arabidopsis thaliana*

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Clubroot (*Plasmodiophora brassicae*) is a serious agricultural problem affecting Brassica crop production worldwide. It also infects the model plant *Arabidopsis thaliana*. During infection, this biotrophic pathogen manipulates the development and metabolism of its host leading to the development of galls in the root and hypocotyl. In turn, its own development is strongly influenced by the host. The aim of this study is to investigate the metabolism of clubroot-infected plants using a combination of transcriptomic and metabolomic approaches. We have used direct injection mass spectrometry to obtain a metabolic fingerprint of when changes in the metabolome occur. We have also examined changes in carbohydrate and nitrogen metabolism during *P. brassicae* infection in *A. thaliana* plants. Microarray analysis showed that host genes associated with sugar transport and metabolism were induced during gall formation. In addition alterations in host nitrate transport and metabolism were also evident. We have examined the impact of inactivating host sucrose synthase and cytoplasmic invertase on infection using *sus1/2/3/4* and *cinv1/cinv2* mutants respectively. Infected cells of these mutants were notably smaller than that of infected wild type plants, particularly in the centre of the hypocotyls. A similar result was observed in mutants that altered either host sugar or nitrate transporter activity. We hypothesize that these changes in host metabolism restrict nutrient transfer to the pathogen and thus inhibits its development. Complementary studies are in progress investigating changes in pathogen metabolism using RNA-seq.

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Studies of clubroot (*Plasmodiophora brassicae* Wor.) on oilseed rape in the Czech Republic

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Clubroot disease, caused by *Plasmodiophora brassicae* (Wor.), has been spreading on winter rape (*Brassica napus* L.) in the Czech Republic over the past three years. Research on *P. brassicae* in the Czech Republic is therefore important for the development of effective strategies to manage clubroot under Czech environmental conditions. Experiments with clubroot resistant cultivars of winter rape were carried out in the field and greenhouse. In the greenhouse, six clubroot resistant cultivars were grown in infested soil collected from various fields in the Czech Republic, and assessed for disease severity. The soil samples also were tested for the presence and amount of *P. brassicae* inoculum by conventional and quantitative PCR analysis. In the field experiment, seven clubroot resistant cultivars were grown and disease development was monitored monthly. Yields were measured at the end of the cropping season. Finally, a set of 17 *P. brassicae* field isolates from across the Czech Republic were assessed for pathotype designation on the differential hosts of Williams, Somé et al., and the European Clubroot Differential set. Collectively, the information obtained on the effectiveness of host resistance and pathogenic diversity of *P. brassicae* populations from the Czech Republic may help to more effectively manage clubroot in this country.

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Plasmodiophora brassicae – the important pathogen of crucifers in Latvia

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The sowing area of winter oilseed rape, has been rapidly increasing during the last ten years in Latvia, therefore oilseed rape diseases have become an important risk factor. Club rot, caused by *Plasmodiophora brassicae* is important disease of different cabbages in Latvia. Damaged oilseed rape was observed only in 2006, and occurrence of this disease has increased during last years. It might be possible that weeds – shepherd's-purse (*Capsella bursa-pastoris*), yellow rocketcress (*Barbarea arcuata*) and others are the important infection source of disease under conditions of Latvia.

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Genotypic and phenotypic correlations in *Plasmodiophora brassicae* isolates and their potential use in marker assisted identification of pathotypes

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As revealed out by using differential host tests *Plasmodiophora brassicae* field isolates in Europe display great pathogenic variation. However, little is known about the genetic and molecular basis of pathogenicity. The objective of this study was to differentiate *Plasmodiophora* field isolates from different regions in Germany according to genotype and virulence phenotype, and to detect genetic polymorphisms directly related to pathotype classification. In total, 56 isolates of *Plasmodiophora* were collected from regions that differ in oilseed rape cropping history, oilseed rape acreage and incidence of clubroot. Using AFLP analyses every isolate displayed an unique genotype pattern and the mean gene diversity of 0.27 indicated that *P. brassicae* is a genetically diverse species. Regarding the differential host test, out of 8 *Brassica* lines tested, six reacted differentially to the isolates. Three isolates were virulent against 'Mendelson' and originated from the same region. Principal component analysis (PCA) grouped the AFLP genotypes as well as the virulence phenotypes into the same two clusters based on the geographic origin. Mantel test showed that the genotype and phenotype pattern were significantly correlated. Hypotheses about association of genotypes and virulence phenotypes with different spatial scales were tested with generalized linear model (GLM): The region, reflecting the cropping history, had a significant effect on genotypes and virulence phenotypes. We propose that geographic differentiation results from low levels of gene flow due to the limited dispersal of this soil-borne pathogen and from localized selection pressure as unifying force. Random forest identified DNA fragments related to pathotype classification. Further research has to clarify if these DNA fragments can be used for marker assisted identification of pathotypes. Markers for virulent pathotypes against 'Mendelson' could be an important tool in the monitoring for these pathotypes provided by the breeding company of 'Mendelson'.

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Clubroot disease of oilseed rape: epidemics and strategies for improving resistance management

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The soil-borne pathogen, *Plasmodiophora brassicae*, has gained increasing importance as the causal agent of clubroot disease on oilseed rape (*Brassica napus*) in Germany. Previous studies have described that the field population of *P. brassicae* is highly variable in pathogenicity. Therefore, an improved knowledge of the pathotype of the fungal population is needed to ensure a better use of available resistant OSR cultivars. Infected root samples from 35 OSR fields in different states in Germany were gathered in 2013. The pathotype classification of each isolate was identified on the differential hosts of Somé (INRA set) and the European Clubroot Differential (ECD) set. The isolates were classified to 13 groups on ECD set and 5 pathotypes on the host of Somé, respectively. Most of populations were highly virulent on the *B. napus* hosts, among them four isolates were virulent on resistant OSR cultivar Mendel.

In a parallel study, a field experiment was conducted to evaluate the effect of timing and amount of fertilizer application (Calciumcyanamid) for suppression of *P. brassicae* pathogenicity. Visual disease assessments showed well variation among the treatments in symptom development. Changing the time of application had significant effect on control efficiency. Control was most effective when OSR plants treated by a single application with 300 kg/ha fertilizer at growth stage 11–12. The clubroot disease severity index was 6.1–24.4% for the treated plants, while it was 51.1–77.8% in untreated controls. Results showed that the application of Calciumcyanamid has a well potential for the control of *P. brassicae* in OSR plants.

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Application of Fluorescence in Situ Hybridization (FISH) for the studies of *Brassica* hybrids with known resistance to clubroot

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Brassica napus L. is a leading oilseed crop in many parts of the world. The big increase of rapeseed acreage is causing problems connected with yielding and cultivation of this species. Therefore, the search of forms with improved traits is highly desirable. From that point of view interspecific crossing is a valuable tool for widening the variability of useful traits e.g. seed quality and resistance to some diseases such as clubroot caused by *Plasmodiophora brassicae*. In this study FISH and BAC-FISH analysis of *Brassica* were applied to study interspecific hybrids. The attention was focused on (i) an analysis of ribosomal DNA (rDNA) loci number and location in individuals of F_3–F_6 generations, which resulted from the crosses between *B. napus* and *B. campestris* ssp. *pekinensis* as well as *B. campestris* ssp. *trilocularis*, (ii) determination of the parental genomes using FISH with C-genome specific BAC-based probes (BAC-FISH) and (iii) an assignment of known *Brassica* chromosomal markers to their corresponding genomes in studied forms. The resistance of described plant genotypes to *P. brassicae* was studied using a biotest performed in controlled environment conditions. The seeds of hybrids and their parental lines of *B. napus* germinated for 5 days on petri dishes, then the small seedlings were transplanted to soil substrate and inoculated with spore suspensions of different races of *P. brassicae*. Susceptibility/resistance of particular lines was assessed 8 weeks after plant inoculation and interpreted based on the results of cytogenetic studies. Among *Brassica* interspecific hybrids, different numbers of both types of rDNA sequences were observed, indicating the genome re-organization. The use of *B. oleracea* BAC clone revealed the chromosome re-arrangements between A- and C-genomes in the synthetic *B. napus* forms, which can be a rapid response to formation of the allotetraploid *B. napus* genome.

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Tracking development of clubroot in long–term fertility experiments using qPCR

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Clubroot (*Plasmodiophora brassicae)* is recognised as a serious soil-borne disease in Brassica crops associated with appreciable yield losses. The disease is disseminated world-wide and,outbreaks of clubroot are in recent years reported frequently in winter oilseed rape districts in south Sweden. In five long-term soil fertility experiment established in 1957 a 4-year course-rotation with spring oil seed Brassica has been performed in south Sweden and 6-year Brassica course rotation was established at three sites in 1969 in central Sweden. The experimental plan includes two crop rotations withspring oil seed rape (OSR) and one without OSR in combinations with different levels of NPK (Nitrogen/Phosphorus/Potassium) fertilizers. The 4-year rotation included spring oil seed rape, winter wheat, sugar beets and spring barley. During 2003–2010, after approximately, twelve OSR-crops club root caused total damage in spring oil seed rape at three of the sites in south Sweden, where as clubroot was not detected at one of the sites. Soil samples were drawn each 4th year in the crop rotation from the start of the experiments. The level of *P. brassicae* has been estimated with our qPCR-method. The level of *P.brassicae* DNA increased in the soil at the end of 1990´s and early 2000´s followed by great outbreaks of club-root in spring OSR. The level of infestation has thereafter declined significantly. The results indicate that the qPCR-method can be used to detect and demonstrate the site-specific development of *P. brassicae*.

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Discovery of SNP markers tightly linked to clubroot resistance gene *Rpb1* through RNA-seq

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A clubroot resistance gene *Rpb1* was previously mapped to *Brassica rapa* chromosome A03 in a pak choy cultivar "Flower Nabana" based on resistance to pathotype 3 of *Plasmodiophora brassicae*. Pathotype 3 is the most prevalent pathotype on canola in Canada. Differentially expressed genes associated with *Rpb1* were identified through RNA-seq. Also, we conducted genetic mapping of resistance against pathotypes 2, 5 and 6 of *P. brassicae* and found that resistance to these pathotypes co-segregated with *Rpb1*, indicating that *Rpb1* may confer a broad spectrum of resistance against *P. brassicae*. To discover SNP markers tightly linked to *Rpb1*, we assembled approximately 800 million Illumina Hiseq short reads from resistant and susceptible bulks into *B. rapa* reference genome v1.5 using Lasergene Genomics Suite. A total of 1,418,780 SNPs including 237,240 indels (16.7%) were identified in 10 *B. rapa* chromosomes. Based on the *B. rapa* reference genome information, there are four TIR-NBS-LRR class genes annotated in the *Rpb1* region. We analyzed 11 SNP markers identified in these TIR-NBS-LRR genes using Kompetitive Alelle Specific PCR. All co-segregated with 12 recombinants obtained from a segregating population consisting of 1600 plants, indicating these gene-specific SNP markers are tightly linked to *Rpb1*. Three susceptible canola (*B. napus*) lines were tested with these SNP markers and 9 of them were polymorphic between the resistant donor and the canola lines, indicating they can be used for marker-assisted selection for introgression of the resistance gene *Rpb1* into canola.

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The fungal endophyte *Acremonium alternatum* primes *Arabidopsis thaliana* against clubroot (*Plasmodiophora brassicae*)

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Beneficial root-inhabiting microbes are known to prime their host plants in order to establish a functioning mutualistic relationship. Until now, a large part of how these interactions work is not well understood. In most cases endophytes colonise the plants without causing any symptoms of disease. At the same time they increase the stress tolerance of their host and enhance resistance against soil and foliar pathogens. One example of this is the soil borne biocontrol agent *Acremonium alternatum*.

It controls other Ascomycetes and showed a promising effect against the clubroot pathogen *Plasmodiophora brassicae* in *Brassica rapa* and *Arabidopsis* in earlier experiments from our group. To reveal the molecular mechanism behind this we carried out a microarray analysis 72 hours after treatment on root samples of *Arabidopsis*.

Several early resistance genes were differentially expressed under the influence of the endophyte. Among them were jasmonate responsive transcription factors of the WRKY and MYB family and the ethylene responsive ERF14, indicating that *Arabidopsis* was primed by the fungus to withstand further pathogen attacks.

Additionally, the brassinosteroid receptor BAK1 was upregulated in clubroot infected plants, confirming the onset of systemic acquired resistance (SAR) as early as three days after inoculation. This is in accordance with our RT-qPCR data from later time points where the SAR marker gene PR1 was significantly upregulated in pathogen infected *Arabidopsis* roots.

We assessed disease symptoms on the roots four weeks after treatment and found smaller galls in clubroot infected plants that were coinoculated with *Acremonium*.

Currently we validate the biocontrol potential of *Acremonium alternatum* on *Brassica napus*, a good host of clubroot and economically important crop worldwide.

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Towards proper methodology of detection and quantification of *Plasmodiophora brassicae* occurring in soils of Poland

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Plasmodiophora brassicae, the cause of clubroot, is a very serious problem preventing from successful and profitable cultivation of oilseed rape in Poland. The pathogen was found in all main growing areas of oilseed rape (*Brassica napus*). The aim of this work was to detect and quantify the amount of the pathogen in different soil types, frequently used for the cultivation of oilseed rape in Poland. We have selected 30 soil samples originating from Great Poland (Wielkopolska), Lower Silesia (Dolny Śląsk), Upper Silesia (Górny Śląsk), Little Poland (Małopolska) and Opole (Opolszczyzna) regions, of pH varying from 4.5 to 7.0. Additonally 4 other substrates were used: muddy soil, podsol, sand and perlite. The substrates were tested for the presence of clubroot using a soil biotest and RealTime PCR with TaqMan chemistry (qPCR). Only the soils below the level of *P. brassicae* detectable by the qPCR method, what always coincided with the negative result of the biotest, were subjected to further analyses. Viable club of *P. brassicae* was homogenised, the spores released and adjusted to 1×10^5, 1×10^4, 5×10^3 and 1×10^3 spores per 1 g of dry soil. Such soil was mixed and dried at 40°C overnight and then studied for the presence of *P. brassicae* using the same qPCR procedure. The detection was positive in all samples, regardless of the concentration of the pathogen. The detection was also successful from spores of five main races of *P. brassicae* encountered in Poland. The sequencing of the ITS1-5.8S-ITS2 fragment revealed small variation between these races, with only one substitution, that was not corresponding to the allocation of races. The results of this experiment show, that the successful detection of *P. brassicae* belonging to the most common races in Poland is possible, regardless of the soil type, with the exception of very low concentrations of the pathogen in soils with pH below 5.0.

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Association of sharp eyespot (*Rhizoctonia cerealis*) with colonization of winter wheat grain by other fungi

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Four commercial crops of winter wheat were grown in successive years (harvested in 2006–2009) at each of four sites, Chrząstowo, Minikowo, Mochełek and Sobiejuchy, located in north-central Poland. Colonization of grain by fungi was studied on samples taken from shoots with symptoms of sharp eyespot caused by *Rhizoctonia cerealis* (severe infection), and healthy controls. One hundred grains, selected randomly from each sample were surface disinfected in sodium hypochlorite and placed on PDA medium in Petri dishes. In 2007–2009 additional 100 grains from the same disease categories were placed on PDA without surface disinfection. All cultures were identified according to their morphology and PCR assay. Fungi recorded from non-disinfected grains were considered to have grown, at least partly, from the surface of the grains, and those recorded from surface disinfected grains were considered to have grown from inside of grain. Occurrence of sharp eyespot was associated with increased colonization of grain by fungi. On average there were 2.4–4.0 and 2.7–4.4 fungal isolates per grain from healthy and diseased plants, respectively, and 2.4–4.4 and 3.3–4.1 fungal isolates per grain from grain surface (non-disinfected) and interior (surface-disinfected), respectively. *Alternaria alternata* and *Epicoccum nigrum* were the most common fungi. Both fungi occurred significantly more often on healthy plants only at Minikowo. *Arthrinium phaeospermum*, *Aspergillus niger*, *Botrytis cinerea*, *E. nigrum*, *Fusarium culmorum*, *Gibberella zeae*, *Khuskia oryzae*, *Microdochium bolleyi*, *Mucor mucedo*, *Penicillium granulatum* and *Trichoderma viride* occurred more often on the grain surface, and *A. alternata*, *Cladosporium herbarum* and *Cochliobolus sativus* more often inside grain. *Cladosporium herbarum* and *Gibberella tricincta* tended to occur more often in/on grain from healthy plants, and *Fusarium poae*, *K. oryzae*, *M. bolleyi* and *T. viride* on grain from diseased plants. The latter fungi seemed to be secondary colonizers which take advantage of the weakened tissue.

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Fusarium Head Blight (*Fusarium* spp.) and fungi colonizing the grain of spring wheat cultivars grown in the Żuławy region

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Despite numerous studies, Fusarium Head Blight (FHB) of wheat is still remains a serious problem. Standard program of wheat protection against fungal diseases, does not always result in reduction of the development of *Fusarium* spp. on the ears. Agricultural practices are not sufficient effective. The biggest hope for solving this problem is resistant breeding. Experiments conducted in 2011-2013 were located in Lisewo (Żulawy region, 54°06′ N; 18°50′ E), an area with an increased risk of *Fusarium* infection.

The aim of the studies was to determine the prevalence of symptoms of FHB on different cultivars as well as seed colonization by fungi. The evaluation was performed on 200 randomly selected ears (50 ears in 4 replications). There was noted considerable variation in the severity of the disease each year. In 2011 symptoms were found in 0.5 to 11.5% of ears. The higher symptoms intensity of FHB was observed in 2012 (7.0–14.0%) and 2013 (6.5–25.5%). On average, for all years, most symptoms were found on cv. Tybalt (19.8%). Significantly lower infestation was on cultivars 'Łagwa', 'Arabella', 'Hewilla', 'Izera', 'Ostka Smolicka' and 'Candela' (8.0–10.0%). Mycological analysis also showed large variation of grain settlement by *Fusarium* spp. The highest number of these fungi was isolated from 'Tybalt' – 36%, 'Katoda' – 28.5%, 'Trappe' – 28%, 'Radocha' – 26%, and 'KWS Torridon' – 23.5%. The group of cultivars with a lower percentage of kernels colonized by *Fusarium* spp were: 'Isar' – 13%, 'Arabella' – 15%, 'Łagwa' – 15.5%. The most frequently isolated species were: *F. poae* – 7.2% followed by *F. avenaceum* – 5.3%, *F. culmorum* – 5.0%, and occasionally also *F. tricinctum*, *F. graminearum*, *F. sporotrichioides* and *F. equiseti*. Other fungal species frequently isolated were *Alternaria alternata*, *Epicoccum nigrum* and *Arthrinium phaeospermum*.

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Fungi colonizing maize (*Zea mays*) kernels from direct sowing and tillage in monoculture and crop rotation

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Fungi, especially pathogenic ones occurring on the grain not only reduce the yield but also deteriorate its quality. The highest threat on maize grain are fungi from genus *Fusarium* and *Penicillium* producing mycotoxins. In recent years there are used simplified cultivation techniques, including direct sowing system. Such cultivation may under certain environmental conditions affect the higher survival of fungi and may be a source of infection for the subsequent crops.

The aim of research conducted in 2009–2011 in Grabów (51°21′ N; 21°40′ E) and Baborówko (52°35′ N; 16°38′ E) was comparison of fungi occurring on the grain of maize grown in crop rotation and monoculture in which there was applied: direct sowing and ploughing. Grain was disinfected for 2.5 min in a 1% solution of NaOCl and then washed three times in sterile water, placed on PDA medium. Obtained fungal cultures were identified with mycological keys and randomly verified with PCR assay. In each combination 3 × 100 kernels were tested. Results were subjected to statistical calculations.

Colonization of maize grain by fungi varied depending on method of cultivation, location and year of the study. In all years each combination was settled the most by *Fusarium* spp. There was found a significant effect of tillage on grain colonization by these fungi. The least favourable was direct sowing in monoculture. In Grabów an average settlement of grain harvested from direct sowing in monoculture was 32.3%, monoculture with ploughing – 15.9%, and for crop rotation – 5.6%. These differences were statistically significant. In Baborówko colonization of grain by *Fusarium* spp. was less differentiated: 8.6%, 12.1% and 2.1% respectively. Species isolated most often were *F. graminearum*, *F. solani*, while *F. poae*, *F. avenaceum*, *F. culmorum* and *F. sporotrichioides* occured rarely. There were also isolated *Cladosporium herbarum*, *Nigrospora oryzae* and *Penicillium* spp.

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Sydowia polyspora may reduce emergence of noble fir seed

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The fungus *Sydowia polyspora* is associated with two serious diseases on noble fir (*Abies procerea*) and other fir (*Abies*) species: Current season needle necrosis (CSNN) and *Sclerophoma* shoot dieback. Both diseases are commonly observed in forest nurseries, and Christmas tree and bough plantations. CSNN causes chlorotic spots and bands on new needles shortly after elongation. The spots turn necrotic during summer and may expand and cause death of entire needles and subsequently heavy needle cast. There are great losses due to CSNN on different fir species both in Europe and USA. The *Sclerophoma* stage has often been found on dead shoots of both fir and spruce (*Picea* spp.) in Norway. During recent years we have detected the fungus on seeds from different species within seven conifer genera (*Abies, Larix, Picea, Pinus, Pseudotsuga, Thuja,* and *Tsuga*.), including noble fir seeds. Prior to that, the fungus had only been reported to be seed borne on Scots pine (*Pinus sylvestris*). Possible influence of *S. polyspora* on the emergence of noble fir seed was studied in an inoculation experiment in a growth chamber. Before sowing, the seed was stratified for three weeks in Petri dishes with moist filter paper at 4°C in darkness. The seeds were exposed to the following treatments with *S. polyspora*: i) a spore suspension was sprayed on the seeds before stratification, ii) stratified seeds were soaked in spore suspension overnight during the last day before sowing, and iii) a spore suspension was sprayed on the seeds immediately before sowing. Three replicates of 100 seeds of each treatment in addition to non-inoculated seeds were sown in a soil-sand mix in plastic trays. Emergence recorded six weeks after sowing was significantly lower for seed that had received fungal inoculum before stratification and seed soaked in spore suspension before sowing, compared to the non-inoculated seeds and the seeds spray-inoculated just prior to sowing. These findings clearly indicate that the presence of *S. polyspora* on seeds of *Abies* may strongly reduce their emergence ability.

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Comparison of methods for detecting fungi in lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.) seeds

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Seed associated fungi are often responsible for seedling damping-off and plant diseases in the field. However, there are no standard methods for identification of many seed-borne pathogens. The aim of the experiment was to compare various incubation agar tests to find the best media for detection *Botrytis* spp. on lettuce and onion seeds. The experiment was performed on two samples of lettuce (cv. Królowa Majowych) and onion (cv. Octawia and cv. Sochaczewska) seeds. The following media were tested: potato dextrose agar (PDA, 39 g PDA · 1 l^{-1} distilled water + 100 ppm Streptomycin sulfate), reduced PDA (RPDA, 10 g agar and 12 g PDA · 1 l^{-1} distilled water + 100 ppm Streptomycin sulfate), PDA and RPDA with an addition of ascorbic acid (100 ppm), PDA and RPDA with an addition of mannitol (osmotic potential –0.9 MPa), PDA and RPDA with an addition of Bengal rose (50 ppm), PDA and RPDA with an addition of ascorbic acid and mannitol, PDA and RPDA with an addition of ascorbic acid and Bengal rose, PDA and RPDA with an addition of mannitol and Bengal rose, PDA and RPDA with an addition of ascorbic acid, mannitol and Bengal rose. For each sample 200 hundred seeds (10 seeds per plate) were tested on each media. The seeds were incubated for 8 days at 20°C, under the alternating cycle of NUV light and darkness (12 h of light and 12 h of darkness). The fungi: *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium* spp. *Epicoccum purpurascens*, *Fusarium* spp., *Penicillium* spp. and *Rhizopus nigricans* were frequently identified on lettuce seeds, whereas: *A. alternata*, *Botrytis allii*, *B. cinerea*, *Cladosporium* spp., *Penicillium* spp., *R. nigricans* and *Stemphylium botryosum* prevailed on onion seeds. Generally, an addition of mannitol and/or ascorbic acid to the PDA and RPDA media favoured detection of *B. cinerea* on lettuce and onion seeds. The best media for the detection of *B. allii* on onion seeds were RPDA and RPDA with an addition of mannitol. The addition of Bengal rose to the media limited detection of *Penicillium* spp. and *R. nigricans* in all examined samples.

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Real-time PCR as a tool to verify the different effect of elicitor application against *F. culmorum* in rye and wheat

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Fusarium culmorum is one of the most common cereal pathogen in Poland that decreases yield quantity and quality and produces mycotoxins. Elicitors that induce plant defense may be helpful in reducing its development. The efficiency of 10 elicitors obtained from cell walls of *F. culmorum* strains with different properties (plant growth promoting DEMFc2, deleterious DEMFc5 and pathogenic DEMFc37) as well as rye and wheat roots to induce resistance of these plants against fusariosis was analysed. Sowing material and heads were inoculated with elicitors, and during anthesis plants were infected with macroconidia suspension of DEMFc37. Real-time PCR assay with SYBR Green was carried out (LightCycler 480 II, Roche) to assess the level of grain infection by *F. culmorum*. Variable effect of the elicitors was found. Some of them, in combinations where the grain before sowing were inoculated, reduced the level of infection even more than 50 times. Application on heads returned no positive results. Reaction of cereals to some elicitors was different. In the case of rye unfavorable effect of two elicitors applied on sowing material was observed. Generally it was found that the inoculation of sowing material with elicitors was clearly more effective in reducing the infection of harvested grain by *F. culmorum* than inoculation of heads in the anthesis.

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The influence of *Trichoderma* as seed treatment on growth parameters and healthiness of vegetable seedlings

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Traditionally, conventional fungicides have been used to reduce seed infection by pathogens. However, the number of chemical coatings is more and more limited. Thus, there is a demand for alternative preparations for seed treatment. Several strains of *Trichoderma* fungi were selected as antagonistic to pathogens. They were also recognized as efficient plant growth promoters. These fungi were examined in container experiments for their effect on seed germination and seedlings growth in potting medium infested with pathogenic *Pythium* sp. The application was studied using seeds of three different vegetable species: carrot, cucumber and onion. The seeds were immersed in a suspensions of *Trichoderma* spores preparations on organic carriers (density of spores 10^4, 10^5, 10^6 cfu/ml), and then dried overnight. Control treatments included: non inoculated seeds, chemical coating with thiram, seeds treated with commercial product Trianum (*Trichoderma* strain T22). The seeds were sown into containers, filled with potting medium mixed with *Pythium* and kept in a greenhouse. The percent of germinated seeds and seedlings biomass were estimated after five weeks. Coating of carrot seeds with *Trichoderma* spores significantly increased germination and seedling biomass in medium infested with damping-off pathogen. The efficacy of selected strains of *Trichoderma* was higher than chemical and commercial treatments. Good results were also obtained with cucumber seeds. However, *Trichoderma* treatments were less effective in seed protection than chemical preparation. In the case of onion the positive effect of *Trichoderma* on seed germination was not observed.

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Effect of treatment with essential oils on soybean seeds infected with *Colletotrichum truncatum*

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The use of pathogen-free seeds and seed treatment is among the main techniques for controlling anthracnose of soybeans, a major disease in the Brazilian Savanna . The objective of this study was to evaluate the treatment effect with essential oils (EO) on the sanitary quality of soybean seeds infected with *Colletotrichum truncatum*, causal agent of anthracnose. The experiment was carried out in a completely randomized design with the following treatments: a) lemongrass EO, 0.25%; b) lemongrass EO, 0.50% ; c) clove EO, 0.25%; d) clove EO, 0.50%; e) basil EO, 0.25%; f) basil EO, 0.5%; g) inoculated seeds without EO; and h) non-inoculated seeds without EO. Three replications for each treatment were used. Soybean artificially inoculated seeds and non-inoculated ones were mixed to compose a lot of seeds with 20% of infection. The oils were added in water + Tween 80, at each predetermined concentration, and seeds were immersed in the solution for treatment for 5 minutes. Seeds of "g" e "h" treatments were immersed in distilled water + Tween 80 without EOs. Blotter test was carried out to evaluate the sanitary seed quality and results were expressed as incidence (%) of seeds with the fungus. Lemongrass EO provided the best results in controlling the fungus. Neither basil EO, in both concentrations, nor clove EO at 0.25% controlled the fungus present in the seeds, showing an incidence similar to the inoculated, non-treated seeds. Thus, it can be concluded that lemongrass EO has a potential use in the treatment of soybean seeds to control the fungus *C. truncatum*.

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The effect of Biosept Active and storage on health, germination and vigour of carrot (*Daucus carota* L.) seeds

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The grapefruit seed extract shows antimicrobial and antioxidant activities, because it contains flavonoids and ascorbic acid. The aim of the study was to investigate the effect of Biosept Active (a.i. grapefruit extract 33%) and storage on health, germination and vigour of carrot seeds. Seeds were treated with 1.0% aqueous solution of Biosept Active for 30 min and afterwards were dried at 20°C and 45% RH for 48 h. Controls were untreated seeds. Untreated and treated seeds were stored in moisture-proof plastic containers at 4°C and 20°C. Seed quality parameters were evaluated at 20°C before and after 3 and 5 month storage. The deep-freeze blotter test was applied for mycological analysis. The percentage of seeds infested with individual fungi was determined. Moreover, the total percentage of germinating seeds, germination at Ist and IInd count, the numbers of abnormal diseased seedlings and dead seeds were evaluated. Seed vigour was determined by means of the speed of germination (T_{10} and T_{50}). The incidence of *Alternaria radicina* on treated seeds after 3 and 5 month storage was lower than before storage. Treated seeds were infested with this pathogen to a lower extent than untreated ones after 3 month storage at 4°C and 5 month storage at both temperatures. Storing untreated and treated seeds for 3 months at 20°C and for 5 months at 4°C and 20°C decreased their infestation with *Fusarium* spp. Germination at Ist and IInd count of treated seeds improved after 3 and 5 month storage at 4°C. Generally, treated seeds showed better vigour than control seeds both before and after storage.

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Occurrence of FHB (*Fusarium* spp.), colonization of grain by fungi and mycotoxin content depending on the program of chemical wheat protection

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Fusarium Head Blight occurrence not only reduces yield but also deteriorates quality of the grain because of it contamination with mycotoxins. Two fungicidal treatments, normally used during cropping season at BBCH 29-59, does not effectively protect plants against FHB. Therefore, under conditions favourable for infection, there should be applied third treatment at flowering stage (BBCH 61-69).

The aim of experiments conducted in 2011–2013 at ESCT in Radostowo (53°59′ N ; 18°43′ E) was to determine the effectiveness of various plant protection programs against FHB of wheat. In the BBCH 29-59 there was used standard protection. At BBCH 65 there were used various combinations of active substances, doses as well as their mixtures. 2–3 days later ears were inoculated with a spore suspension of *F. culmorum* (DON and NIV chemotype) at a concentration of 1×10^6 ml^{-1}. Control plots (K1) were inoculated with fungus but not treated and (K2) inoculated, in which no treatment was performed at flowering stage. In stage BBCH 73-75 there was assessed severity of FHB symptoms using 0–5° scale, while after harvest – colonization of grain by fungi. Intensity of FHB in particular years varied. Maximum value was noted in 2012. K1 combination showed 43% of ears with symptoms, while K2 – 41.5%. All combinations used effectively reduced severity of fusariosis (from 4.0 up to 13.5%).

A high percentage of kernels settled by *F. culmorum* in combination K1 (90%) demonstrates both high pathogenicity of isolates and favourable weather conditions for infection. Thus, the results can be considered reliable. Among other fungi being isolated, *Alternaria alternata* and *Epicoccum nigrum* dominated, but they occurred less numerously in combinations where grain was highly settled by *Fusarium*. In kernels from control combinations there was 848 ppb DON in K1; 670 in K2; while in others 89–411 ppb.

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The health status of organic seeds

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The land area under organic management has been steadily increasing over the recent years in Lithuania. The proportion of the agricultural land area that has been fully converted to organic is about 7% (about 160 thousand hectares). The crop production farms dominate. The main problem for organic farms is lack of certified seed material because currently there are 3–5 active seed growers who provide seeds for organic farmers, therefore the main seed material is own grown. In crop production farms cereals (winter wheat, rye, triticale and spring barley and oats) and legumes (peas, beans and lupines) prevail. Seed borne diseases can cause serious problems in the production of these crops, especially in organic seed production where seed borne diseases can greatly influence the production in terms of both quality and quantity. The current certification practice of commercial seeds does not involve analyses of seed infection. To establish health status of organic seeds, samples were taken from organic farms during 2012–2014 and analysed in Plant Pathology and Protection Department of Institute of Agriculture. *Fusarium* spp. dominated on cereal seeds: infection level on winter wheat seeds ranged from 4.5 to 77.0%, on winter rye – 9.0–61.0%, on winter triticale – 9.0–42.5%, on spring barley – 5.0–82.0%, on oats – 27.5–95.0%. *Cochliobolus sativus* was identified in all samples of spring barley seeds, the percentage of infected seeds varied from 6.0 to 100%. High infection of *Penicillium* spp. in some samples of cereals and peas is a signal of unsuitable seed storage conditions. Our study suggested that many of the seed samples had high levels of infection and were not suitable for organic production; therefore efforts must be concentrated on the development of robust seed production system for organic agriculture.

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Influence of some natural preparations on nutritional values of sweet pepper fruits

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The purpose of the experiment was to estimate the effect of selected natural preparations on nutritional values of sweet pepper fruits. The effect of preparations on total content of sugars (fructose, sucrose, glucose), proteins and dry weight was investigated. In 2010–2013 pepper plants were sprayed (6 treatments) with Bioczos Plynny (garlic pulp), Biosept 33SL (grapefruit extract) and Bioalgeen S90 Plus (sea algae). The plants without treatments and plants protected with Amistar 250SC (azoxystrobine) were the control combinations. The lowest content of sucrose was in fruits protected with Bioczos Plynny (0,507 g · 100 g^{-1}) and the highest in the fruits sprayed with Bioalgeen (0,837 g · 100 g^{-1}). The highest content of glucose and fructose was in the pepper fruits protected with Bioalgeen and the lowest in combination with Biosept. Mean content of sugars from experimental combination did not differ from the controls. The content of proteins in fruits reaged from 0,9% (Biosept) to 1,07% (control). Statistical analysis did not show differences between experimental combinations. Mean contents of dry weight of fruits was lower for fruits protected with Biosept and Bioczos Płynny but did not differ statistically from the control. Biotechnical preparations did not have any influence on the nutritional values of sweet pepper fruits.

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