2014 Potomac Division Meeting Abstracts

Abstracts presented at the APS Potomac Division meeting in Annapolis, Maryland, March 12–14, 2014. The abstracts are arranged alphabetically by the first author's name. Recommended format for citing division meeting abstracts, using the first abstract below as an example, is as follows: Baudoin, A. 2014. First confirmation of resistance to quinoxifen in grape powdery mildew in North America. (Abstr.) Phytopathology 104(Suppl. 3):S3.160. <http://dx.doi.org/10.1094/PHYTO-104-11-S3.160>

First confirmation of resistance to quinoxifen in grape powdery mildew in North America

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Powdery mildew (*Erysiphe necator*) is one of the most damaging diseases of grapes, and a key target of disease management spray programs. Among the available groups of fungicides, quinoxifen, although it has the drawback of only controlling powdery mildew and not other diseases, is nevertheless an attractive compound because of its high efficacy, few side effects, and because its mode of action lacks cross-resistance with other available compounds. We have surveyed *E. necator* in Virginia since 2005, and had not detected resistance to quinoxifen. However, in 2013, quinoxifen-resistant PM was detected in one commercial Virginia vineyard experiencing difficulties with powdery mildew control. When these isolates were inoculated onto leaves treated with quinoxifen at 3, 10, 30, and 100 µg/ml, growth was obtained at all rates, whereas sensitive isolates did not grow at 3 µg/ml. Percent spore germination was somewhat lower on treated leaves than on untreated leaves, but there was little inhibition of colony development or sporulation. No EC50 could be calculated as there was little rate dependence of effects. Small potted Chardonnay grape plants sprayed with 50 and 100 µg/ml and subsequently inoculated also sustained severe infection. Poor control of *E. necator* by quinoxifen in field trials in western New York in 2010 and 2011 was attributed to possible resistance; resistance was “hypothesized but not confirmed” (Wilcox and Riegel 2012, Plant Dis. Manag. Rep. 6:SMF044, SMF048, SMF049). The geographical distribution of quinoxifen resistance and the stability and competitiveness of resistant strains need further investigation.

Metagenomic insights into nitrogen and potassium impacts on microbial community structure in *Poa annua* putting green turfgrass

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Nitrogen (N) and potassium (K) fertility can have a significant impact on turfgrass diseases. Questions about whether these nutrients affect microbial community structure in the turfgrass rhizosphere remain unanswered. The objectives of this study were to evaluate the composition, diversity, and distribution of rhizosphere microorganisms in *Poa annua* (annual bluegrass) turf maintained as a putting green. Three 15.9 mm diameter × 50.8 mm long

soil cores were sampled from four replicated plots receiving: (1) an intermediate N rate (132 kg N ha⁻¹ yr⁻¹); (2) K (200 kg K₂O ha⁻¹ yr⁻¹); (3) N+K (1:1, N:K molar-adjusted ratio; 132 kg N ha⁻¹ yr⁻¹ and 200 kg K₂O ha⁻¹ yr⁻¹); (4) a low N rate, (100 kg N ha⁻¹ yr⁻¹); or (5) a high N rate, (200 kg N ha⁻¹ yr⁻¹) (total samples = 60). N and K were applied as urea [CO(NH₂)₂] and potassium chloride (KCl), respectively. Organism-specific DNA regions were PCR amplified from fungi (ITS), bacteria and archaea (rDNA 16S). Multiplexed next-generation sequencing on an Illumina platform generated 2.3 × 10⁷ reads (avg. 1.38 × 10⁵ seq/per sample; avg. length 331 bp). After barcode trimming, demultiplexing, and chimera removal, 7.2 × 10⁵ OTUs (97% identity) were identified using the open source QIIME pipeline in Python. Alpha diversity measures from rarified datasets showed that species diversity was highest in low and high N treatments, while diversity was lowest in plots receiving the intermediate rate of N. Detrended correspondence analysis performed using the Vegan module in R revealed significant differences in archaeal and bacterial communities from plots receiving intermediate N, K, or intermediate N+K treatments, compared to those receiving low or high rates of N. A similar finding was observed for the fungal community, indicating that differing management practices appear to be affecting the microbial community structure in this study.

***Septoria lepidii*, causes destructive leaf spot of whitetop (*Lepidium draba*) and is a potential biological control of this weed**

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Whitetop (hoary cress, *Lepidium draba* L., family Brassicaceae) is native to western Asia and Eastern Europe. It is an invasive species in North America, and a target of biological control efforts. Diseased leaves of *L. draba*, bearing leaf spots and pycnidia, were collected in Greece, Russia, Tunisia, and Turkey. Diseased plant material was brought back to the ARS, FDWSRU containment greenhouse facility at Fort Detrick. *Septoria lepidii* Desm. was consistently isolated from diseased leaf material. Even though the disease appears to be wide-spread, successful reproduction of the disease and fulfillment of Koch's postulates has not been reported in the literature, despite suggestions that *S. lepidii* is a potential biological control agent of this weed. Pea brine agar plates were inoculated with culture plugs of a Turkish isolate (# 01-614), left on a bench top at 20°C for four days, and placed in a 15°C dew chamber with no light for 40 days. Pycnidia developed in cultures after this incubation period. Thickened (3.62 µm wide) conidia produced in these pycnidia were germinable and infective; thinner (1.93 µm wide) conidia were not. Different geographical accessions of *L. draba* were sprayed until runoff with a spore suspension of 1 × 10⁶ conidia (thick and thin mixture) ml⁻¹. Inoculated plants were given three 16-hr dark dew periods at 25°C with 8 hour daylight intervals between dew treatments, and then placed in a 25°C greenhouse. Symptom expression began 18 days post-inoculation, and sporulating pycnidia developed four weeks after inoculation. The fungus was re-isolated from conidia oozing out of these pycnidia. Inoculations of two related species, *L. latifolium* and *L. perfoliatum*, did not result in disease, suggesting potential host-specificity of *S. lepidii*.

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The rust fungus *Puccinia punctiformis*, a systemic root-parasitic killer of Canada thistle (*Cirsium arvense*)

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Canada thistle (*Cirsium arvense*, CT) is one of the worst weeds in temperate areas of the world. The rust fungus *Puccinia punctiformis* causes systemic disease on CT, is specific to CT, is found in all regions where CT is found, and is a potential biological control agent for this weed. In the spring, shoots systemically diseased with the rust emerge from overwintering diseased roots. The systemically diseased shoots produce aeciospores which spread to leaves of nearby shoots, and these infections produce uredinia that transition to telia in late summer and early fall. Leaves bearing telia abscise in mid to late fall and are deposited on newly-emerging rosettes where teliospores germinate and basidiospores infect roots of these rosettes. The fungus overwinters in the roots and results in permanent systemic infection; all of the shoots on infected root systems eventually die. Newly-emerged rosettes of CT, in 13 fields in four countries, were inoculated in the fall with ground leaves bearing telia of the rust that were collected in mid-summer in each country. In the spring following inoculation systemically diseased shoots emerged in all inoculated fields. Spread of the fungus by aeciospores, urediniospores, teliospores, and mycelia in roots leads to the epiphytotic of systemic disease. ELISA tests to detect the fungus in roots showed that asymptomatic infection of roots can be widespread. Thistle density declines averaging 43.3% in nine fields were observed 18 months after inoculations and declines of 46–100% were observed in four sites by 30 months after inoculations.

Differential response of Japanese stiltgrass accessions to isolates of *Bipolaris microstegii* and *B. drechsleri*

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Japanese stiltgrass (*Microstegium vimineum*, JSG) is an invasive species of natural areas that is changing forest ecosystems in the Eastern United States. Recently there have been reports of significant damage from a leaf spotting disease at several locations, caused either by *Bipolaris microstegii* or *B. drechsleri*. Comparison of disease severity (% diseased plant tissue) from the two *Bipolaris* species was made on several accessions of JSG in two replicated experiments with 6-wk-old JSG inoculated to runoff with 10^5 conidia/ml and dew for 16-hr at 25°C. Disease (%) data were collected 4 days after inoculations and analyzed after square root transformation. Disease response from ten isolates (four of *B. microstegii*, two of *B. drechsleri*, and four of *Bipolaris* sp.) on the Indian Springs Road (ISR), Frederick, MD accession of JSG ranged from 10 to 15% for three isolates of *B. microstegii* and two *Bipolaris* sp. isolates. Significantly less ($P = 0.05$) disease (1 and 2.4%) resulted from inoculation by *B. drechsleri*, and there were intermediate responses (4 to 6%) associated with ISR inoculated with the remaining three isolates, including one of *B. microstegii*. The second experiment measured disease response on different JSG accessions inoculated either with *B. microstegii* or *B. drechsleri*. Disease was significantly greater on ISR JSG (9.8%, $P = 0.05$) from *B. microstegii*; disease was lower (2.0 to 3.3%) after inoculations with *B. drechsleri* on JSG from ISR, Indiana, and three other Maryland locations; and disease was lowest (0.4 to 1.7%) after inoculation of the Indiana and three non-ISR Maryland accessions with *B. microstegii*. Differential responses between *B. microstegii* and *B. drechsleri* on selected plant accessions, suggest need for clarification of host-pathogen interactions for JSG and two leaf-spotting diseases.

***Botrytis* characterization and phylogenetic analysis: Understanding the causal agents of chocolate spot of faba bean (*Vicia faba*)**

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In collaboration with the Foundation for the Promotion and Investigation of Andean Products (PROINPA), this research seeks to identify plant-associated microbes (PAMs) that can increase plant nutrition and yield, while suppressing disease in broad bean (*Vicia faba*), an important nitrogen fixing crop for the high Andes. One of the most significant diseases that affects broad bean is chocolate spot caused by *Botrytis fabae* and *B. cinerea*. In order to characterize the causal agents of chocolate spot *Botrytis* isolates were collected from diseased faba plants in Pennsylvania field trials in 2 subsequent years at the Russell Larson Research Center in Spring Mills. Of these isolates 16 were selected based on differential culture morphology, ability to cause disease and production of sclerotia and/or conidia in culture. Sequences of three nuclear protein-coding genes were generated: Glyceraldehyde-3-

phosphate dehydrogenase (G3PDH), Heat-shock Protein 60 (HSP60), and DNA-dependent RNA polymerase subunit II (RPB2). In addition the nuclear ribosomal internal transcribed spacer (ITS) was sequenced. The 3 genes were chosen because ITS provides limited phylogenetic information for the *Botrytis* genus. These genes were selected because they are evolutionarily conserved and resolve adequately at the species level. Individual trees and combined trees of these genes were created. These trees consistently show grouping of the Pennsylvania isolates into different clades. The first group consists of 11 isolates that may potentially be a new species. The second group consists of 5 isolates related to *Botryotinia fuckeliana* (sexual stage of *B. cinerea*) and *Botrytis fabae*. These results will help determine which *Botrytis* isolates should be used to study the ability of PAMs to reduce disease through Induced Systemic Resistance (ISR).

Seeking enhanced guidelines for describing novel ‘*Candidatus* Phytoplasma’ Taxa: ‘*Ca. Phytoplasma pruni*’ vs ‘*Ca. Phytoplasma pruni*’-related strains in North American grapevine yellows

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Current guidelines for describing novel ‘*Candidatus* Phytoplasma’ species rest heavily on distinctions afforded by analysis of 16S rRNA gene sequences. According to the guidelines, a phytoplasma strain may be described as a novel species if the strain shares <97.5% 16S rRNA gene sequence identity with any previously described species. A single, unique 16S rRNA gene sequence >1200 bases in length should be specified for the reference strain, and species-unique, nucleotide sequence segments of the 16S rRNA gene must be designated. Significantly, the guidelines specify that “Strains in which even minimal differences in the 16S rRNA gene sequence from the reference strain are detected do not ‘belong’ to the *Candidatus* species, but are ‘related’ to it.” Our recent studies of North American grapevine yellows (NAGY) underscore the distinction between strains of a ‘*Ca. Phytoplasma*’ species and ‘*Ca. Phytoplasma*’ species-related strains. NAGY disease has sometimes been ascribed to infection of *Vitis vinifera* L. by X-disease phytoplasma, but the accuracy of this attribution is open to question. We found that group 16SrIII NAGY phytoplasma strains were closely related to, but distinct from, strains of ‘*Ca. Phytoplasma pruni*’, cause of *Prunus* X-disease, based on deduced 3-dimensional structure of SecY proteins, and SNPs in 16S rRNA, *secY*, and ribosomal protein (rp) genes. Importantly, the NAGYIII strains differed from ‘*Ca. Phytoplasma pruni*’ in regions of the 16S rRNA gene corresponding to three segments described as species-unique for ‘*Ca. Phytoplasma pruni*’. Although *Prunus* X-disease and grapevine NAGYIII disease are apparently caused by different phytoplasmas, we neither refer to NAGYIII strains as strains of ‘*Ca. Phytoplasma pruni*’, nor suggest at this time that NAGYIII strains represent a novel species. Instead, we tentatively refer to the NAGYIII strains as ‘*Ca. Phytoplasma pruni*’-related strains, recognizing that the time to seek revisions in the guidelines may be at hand.

Identification and characterization of nucleotide binding site-encoding resistance genes in the bioenergy plant switchgrass (*Panicum virgatum* L.)

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Switchgrass has received attention for its potential use as a second generation biofuel feedstock. Sustainable switchgrass biomass production could be negatively impacted by the epidemics of various disease problems, such as switchgrass rust. Therefore, the molecular mechanisms underlying tolerance to various diseases must be elucidated. The majority of plant disease resistance (R) genes belong to a large family of nucleotide binding - leucine rich repeat (NB-LRR) genes that can be classified into two major groups: Toll-IL-1 receptor (TIR)-NB-LRR and coiled-coil (CC)-NB-LRR genes. In this study, we used a homology-based computational method to identify 610 putative NB-containing R genes in the draft genome of switchgrass. As expected, no TIR-NB genes were discovered in the switchgrass genome. Interestingly, 28 genes were identified that contain unique domains other than CC and LRR domains. RNA-sequencing of Dacotah, a rust-susceptible switchgrass cultivar, and Alamo, a rust-resistant switchgrass cultivar, verified the expression of several of these resistance gene candidates. Currently, single nucleotide polymorphisms (SNPs) are being identified in the NB-containing genes that are expressed in both Alamo and Dacotah in order to develop molecular markers for breeding of disease resistance. The results of this study will aid in understanding the genetic mechanisms that control disease resistance in switchgrass and may lead to the development of switchgrass cultivars with improved disease tolerance.

Chemical, biological, and cultural control of pod rot of lima bean caused by *Phytophthora capsici*

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Lima bean is the most important processing vegetable grown in Delaware. Pod rot caused by the oomycete, *Phytophthora capsici*, is one of the most devastating diseases affecting lima beans. With resistance to the widely used fungicide, mefenoxam, and absence of a resistant cultivar, research into additional controls is vital. We conducted a series of experiments to test the efficacy of many chemical, biological, and cultural controls for pod rot of lima bean. Experiments were conducted in Georgetown, Del. in infested fields which were misted to provide an improved climate for *P. capsici*, however, disease levels were low in all trials. Several biological treatments were tested which included nine soil inoculants and ten biofumigant crops. Lowest disease levels were observed in Caliente 199 and Kodiak mustards, with a total of four and six infected pods, respectively. In another trial, lima beans were sown following a spring oat crop in which a range of tillage types were used to achieve various levels of soil cover. Greater soil cover showed promise as an effective cultural control. In a fungicide trial, 13 products were applied at recommended rates and pods were inoculated with *P. capsici* to boost disease; yet, infection remained low. Overall disease was lowest in Dimethomorph (Forum®) and Mandiproamid (Revus®) treatments. Experiments will be repeated in 2014 with alterations to ensure improved disease pressure in an effort to better evaluate treatments.

Development of a lab-on-a-chip real-time PCR system of portable plant disease diagnostics

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The introduction of new pathogens in the US is continuously challenging American agriculture. Consequently, there is a need to develop novel technologies for an earlier and accurate disease diagnosis. Specifically, there is a gap of rapid diagnostics tools to facilitate effective in-field plant pathogen detection. To achieve this, we have developed a real-time microchip PCR system for plant disease diagnosis. The system is composed of a PCR microchip with a reaction chamber and a heater, a fan that accelerates cooling time, a compact fluorescence detector that acquires signal emission of amplified DNA, and a battery powered microcontroller unit that controls PCR reactions and data acquisition. The microchip requires a small sample volume - 8 µl - for a decreased reaction time. The entire system is 25 × 16 × 8 cm³ and weighs 843 g, making the instrument compact and portable. Likewise, we have optimized a DNA extraction protocol feasible for field settings, where no liquid nitrogen, chemicals and other laboratory equipment are necessary. As a proof of concept, we selected *Fusarium* and *Pseudomonas* species as fungal and bacterial pathogens and tested the new extraction method. We obtained an average of 13 µg and 65 µg of DNA per 50 mg of fungal and bacterial biomass, respectively, with a satisfactory quality of genomic DNA for real time PCR. Subsequently, primers were designed to amplify conserved fungal and bacterial regions and tested as suitable targets for diagnosis. PCR analyses showed that primer sets were specific, with no false positives with other species. The developed microchip PCR system and DNA extraction method were tested with six different fungal and bacterial species with 100% success rate. Our results demonstrated that our lab-on-a-chip real-time PCR system has the potential to be a valuable tool for fast and accurate plant disease diagnosis in the future.

Emerging tomato virus diseases in Hawaii

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Tomato spotted wilt, caused by *Tomato spotted wilt virus* (TSWV; family *Bunyaviridae*, genus *Tospovirus*), has long been a major hindrance to local tomato production in Hawaii. Recently, additional virus diseases have emerged in Hawaii's tomato fields. Tomato yellow leaf curl disease, caused by *Tomato yellow leaf curl virus* (TYLCV; family *Geminiviridae*, genus *Begomovirus*), was first identified in 2009 on the island of Oahu. TYLCV has since been discovered on the islands of Maui and Hawaii. Genetic data

suggests the TYLCV isolates present in Hawaii originated in Asia and/or North America. We have screened 28 common agricultural weed and crop species to determine if they were alternative hosts for TYLCV. Of these species, 10 were putatively identified as hosts of the virus. In 2010, a tomato plant with unusual virus-like symptoms from Hawaii was found to harbor Southern tomato virus, a dsRNA virus with similarities to totiviruses and partitiviruses. In 2011, tomato fruits displayed mottling symptoms similar to that caused by TSWV were found during a tomato variety trial on Oahu. Foliage from affected plants, however, appeared symptomless. Foliar samples from symptomatic plants were negative for TSWV. Molecular and serological assays, however, indicated the present of *Pepper mottle virus* (PepMoV; family *Potyviridae*, genus *Potyvirus*). The incidences of PepMoV ranged from 4.8% (1/21) to 47.6% (10/21) for the different tomato varieties, with an overall incidence of 19.9% (58/292). In 2013, *Capsicum chlorosis virus* (CaCV; tentative member of the family *Bunyaviridae*, genus *Tospovirus*) was discovered in an ornamental hoyo plant on Oahu. CaCV is an important pathogen of tomato in Asia and Australia, although it has not yet been detected in Hawaii's tomato fields.

Characterizing the infection conditions of grape ripe rot (*Colletotrichum acutatum* and *Colletotrichum gloeosporioides*) on wine grape clusters

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The lack of basic epidemiological knowledge on ripe rot of grape, caused by *C. acutatum* and *C. gloeosporioides*, has led to uncertainty in management recommendations. To determine temperature and wetness duration requirements, point inoculation experiments were conducted on table grapes. Temperatures from 15 to 35°C, combined with 6 to 30 hours of wetness were tested. Disease incidence varied from 0% to 17% and the mean disease incidence of *C. gloeosporioides* was low (<3%) with short (<12 h) wetness duration, and increased with the length of wetness duration. The optimal temperature was around 25°C; however, there was some infection (6–8%) at 30°C and 35°C with longer (>24 h) wetness. To investigate varietal sensitivities and cluster maturity, inoculations of clusters were performed in the field and on potted vines during the 2012–2013 growing seasons. We have identified that infection could take place on all varieties tested from bloom to the near harvest, and there were significant differences in disease incidence among varieties. In the field, mean disease severity of Merlot was low at 10–15% while Chardonnay and Cabernet Franc were 32–33% and Cabernet Sauvignon was the most sensitive with 42% disease severity. The potted vine study found that disease severity was lower on Cabernet Sauvignon (6%), Chardonnay (18%) and Merlot (1%). In both studies, clusters were more susceptible at fruit set and near harvest; however in the field study, disease development was very high when inoculation was held at bloom. Results from this study help us to determine the critical period for ripe rot management and conditions that required for the infection.

Biological control of invasive *Ailanthus altissima* using formulated and natural *Verticillium nonalfalfae* inoculum

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In Pennsylvania, *Verticillium nonalfalfae* Inderbitzin et al., causal agent of Verticillium wilt, has proven a safe and highly effective biocontrol agent for invasive tree-of-heaven, *Ailanthus altissima* (Mill.) Swingle. To obtain control, basal or stem wounds of *Ailanthus* are currently treated with a *V. nonalfalfae* conidial suspension, which must be prepared in the laboratory and has a short shelf life. To avoid these shortcomings, we evaluated the biocontrol efficacy of a soil-water formulation of inoculum, which can maintain viable *V. nonalfalfae* propagules for >2 years. We also tested several natural forms of inoculum. In May 2013, three soil-water formulation treatments were field-tested on ten *Ailanthus* stems per treatment. A sterile soil-water formulation was used as a control, and our standard conidial suspension was used for comparison. Concurrently, infested soil, as well as infected *Ailanthus* stems, branches, wood chips, and leaves, were applied to wounds on healthy *Ailanthus* stems. In these treatments, controls consisted of uninfested soil and non-infected *Ailanthus* wood chips, leaves, and tree parts. By September 2013, Verticillium wilt resulted from all three soil-water formulation treatments. In addition, wilt and vascular discoloration occurred on 4 of 10 wood chip-inoculated stems and on 1 of 5 stems that had been treated by placing infected tree parts around the wounded base. *Verticillium nonalfalfae* was successfully isolated from all symptomatic stems. Symptoms were not observed from other natural inoculum treatments. Additional observations will be made during summer 2014 to evaluate further symptom

development in all treatments. These findings confirm that the soil-water formulation of inoculum is an effective biocontrol agent against *Ailanthus*, and has a long shelf life while maintaining pathogenicity. We also confirmed that natural inoculum sources can be used to establish new *Verticillium* infections in healthy *Ailanthus* stands, eliminating the dependence on the laboratory-prepared *V. nonalfalfae* conidial suspension.

Management of lawn broadleaf weeds with indigenous fungal isolates of *Sclerotinia minor*

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Ground ivy and clover are common invaders of lawns in most parts of West Virginia and is regarded as the most difficult-to-control weed problems in home lawns. We used isolates of *Sclerotinia minor* recovered from local lawns to evaluate their efficacy as mycoherbicide for controlling broadleaf lawn weeds. Colonized oat grain inocula were applied at two different levels with a non-treated control in a randomized complete block design (RCBD) in four replications. Within 60 days of inoculation ground ivy was significantly ($P < 0.001$) suppressed at higher and lower levels of inocula by 70% and 55% compared to the non-treated control. In order to further characterize the isolates and assess their myco-herbicide potential, host specificity, optimum growth temperature, over-wintering capacity, and DNA analyses were performed. The initial host specificity trial indicated that the fungal organism was basically a pathogen of ground ivy killing >90% weed population both in the lawn and growth chamber. However, this isolate was weakly pathogenic on clover and did not show any pathogenicity to dandelion, broad leaf plantain, lamb's quarter, yellow rocket, or prostrate knotweed indicating it has a narrow host range, whereas similar isolates previously characterized by other investigators had a broad host range with major focus on dandelion. The optimum growth temperature range was 27–29°C for this isolate; a range that is significantly higher than other documented *S. minor* strains used as myco-herbicides.

Droplet digital PCR detection of aneuploidy in *Plasmopara obducens* causing impatiens downy mildew disease

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Impatiens downy mildew (IDM), caused by the fungus-like oomycete *Plasmopara obducens*, is an aggressive, newly emergent disease affecting cultivated impatiens (*Impatiens walleriana*) in the U.S. The first U.S. IDM outbreak was reported in 2004. By 2012, IDM was reported from 33 states, resulting in millions of dollars in losses. The factors driving these destructive outbreaks are unknown. It is unclear whether IDM is caused by the same organism recorded on wild impatiens throughout the 20th century, or if disease outbreaks are due to a new pathotype or species. In this work, we used droplet digital PCR (ddPCR™) technology and whole genome sequencing to compare pre-epidemic and modern populations of IDM. We identified five single nucleotide polymorphisms (SNP) by performing Sanger sequencing of the nuclear ribosomal DNA of 134 *P. obducens* samples. Taqman® hydrolysis probes were designed for four SNPs and used to genotype 830 *P. obducens* samples, including 87 herbarium specimens collected between 1881-2005. The ddPCR™ analyses revealed allelic ratios departing from 1:1 for multiple heterozygote samples, with 100 of the 449 samples analyzed to date exhibiting a 3:1 allelic skew. For two of the SNPs analyzed, this 3:1 allelic ratio was observed in 30 to 38% of all the modern samples and only in 4 to 6% of herbarium specimens. This observed 3:1 allelic ratio departs from the expected 1:1 ratio in diploid organisms. Single sporangium genotyping through ddPCR™ of a modern isolate confirmed the presence of aneuploidy in a modern specimen. An increase in ploidy levels in modern samples could have an impact on the genetic diversity of the pathogen and thus contributed to the recent outbreaks of IDM. Further analyses are underway to confirm this potential increase in ploidy in modern samples of IDM.

Survival of microsclerotia of *Calonectria pseudonaviculata* (the boxwood blight pathogen) treated with a variety of sterilants

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Boxwood blight is caused by *Calonectria pseudonaviculata*, a newly introduced pathogen causing concern in the nursery industry. Infected boxwood leaves and stem tissue contain microsclerotia that appear to be

persistent in soil and debris. A number of commercially available sanitizing agents were tested for their ability to kill discrete microsclerotia (produced after 2 mo on glucose-yeast extract-tyrosine agar covered with a sheet of cellophane). The first set of tests exposed microsclerotia to sanitizers at label rates for 0, 10, 30 or 60 min; the most effective were selected for tests at 0, 5, 10 and 15 min. Of the products tested, 70% ethanol was the most effective, killing all microsclerotia after 5 min; Zerotel 2.0 (Hydrogen Dioxide 27.1%) was immediately effective at the maximum rate (1:50 dilution), and fairly effective (killing ca. 80% of microsclerotia) after 15 min at the rate for cleaning pots or drenching soil (1:100). Oxidate, Sanidate, X3, Greenshield, Lysol Concentrate (all of these at label-recommended rates) and 10% household bleach were less effective.

Sampling techniques and detection methods for developing risk assessments for root-knot nematode (*Meloidogyne incognita*) on lima bean (*Phaseolus lunatus*) in the Mid-Atlantic region

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Lima bean, *Phaseolus lunatus*, is a cornerstone crop in the Mid-Atlantic region and *Meloidogyne incognita*, the southern root knot nematode (RKN) causes significant yield loss. The RKN has become more pervasive as toxic nematicides have been removed from the market and risk evaluation research is needed to offset crop losses. In 2013 eight lima bean production fields, 7 in southern Delaware and 1 from Maryland's eastern shore, were divided into geo-referenced 1 hectare grids and sampled three times a year. Samples were taken: before planting of crop, after lima beans had pinned and podded, and directly after harvest. Edaphic features: pH, EC, temperature, moisture, elevation and texture were measured during the first sampling period and additional soil was collected to conduct a bioassay for use in measuring dormant RKN. Subsequent samples of soil and plant material were taken for laboratory analysis of root galls and second-stage juveniles (J2) in soil. Preliminary findings indicate that a spring, pre-planting bioassay is better correlated with population numbers of post harvest nematodes than are spring samples of J2 from soil. Correlations between edaphic features and nematode population numbers were inconclusive. Pigweed, *Amaranthus hybridus*, a suitable host for RKN, was found to correlate with higher levels of galling on lima bean and higher population numbers of J2 in soil. In addition to sampling during the growing season, weather and moisture data is currently being collected to measure overwinter survival rates and RKN levels will be measured in March 2014. Data collected on RKN population dynamics will be used to create a risk assessment model.

Incidence of *Colletotrichum coccodes* in ripe tomato fruit after feeding by Brown Marmorated Stink Bug (*Halyomorpha halys*)

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Colletotrichum coccodes (Ascomycota) causes anthracnose of tomato fruits. *Halyomorpha halys* (Brown Marmorated Stink Bug) is a newly invasive Asian insect that injures tomato fruits with a piercing/sucking mouthpart. To determine if *H. halys* feeding increases incidence of anthracnose of tomato fruit, detached cherry tomato fruits in plastic containers were incubated for 21 days at 22°C. Fruits in Treatment 1 and 2 were sprayed with a suspension of 10^6 spores of *C. coccodes*/mL. *H. halys* nymphs were placed inside containers in Treatment 2. In treatment 3, *H. halys* was infested by incubating for 24 hours in a petri dish of *C. coccodes* on PDA. In Treatment 4 fruits were incubated with *H. halys* (with no inoculum) for 7 days, after which insects were removed and fruit sprayed with the *C. coccodes* spore suspension. Treatment 5 was untreated. Fruits in Treatments 2 and 4 showed greater anthracnose incidence than those in Treatments 1 and 5 ($p < 0.05$). A 12-day greenhouse trial was conducted using intact plants and the same treatments, except that Treatment 4 was changed to a positive control of *H. halys* feeding with no inoculum. Fruits with *H. halys* and sprayed with *C. coccodes* (treatment 2) had higher levels of anthracnose than other treatments ($p < 0.05$). Disease incidence in Treatments 1 and 4 did not differ significantly but was greater than in untreated controls or fruits with *H. halys* only. In a 2013 field trial, insect netting sleeves were fitted around tomato fruit clusters, and 0, 1, 3, and 9 juvenile stinkbugs were placed inside for periods of 5 and 10 days. Dead insects were replaced daily. At both 5 and 10 days, anthracnose incidence increased with increasing *H. halys* numbers. Results from these studies indicate that feeding by *H. halys* increases incidence of *C. coccodes*.

Progress towards understanding the window for cranberry fruit rot control

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In the control of cranberry fruit rot the critical time for application of protectant fungicides is during and shortly following the bloom period, with applications between 30%–60% open bloom having the greatest effect. Since the highest level of disease control for many cranberry fruit rotting pathogens is accomplished via fungicide applications during the bloom, it is reasonable to hypothesize that plant signals produced during bloom play a critical role in the sporulation and infection processes. The objectives of this study were to investigate the effects of cranberry cv. Stevens water-soluble floral-extracts on the cranberry fruit rot fungi; *Coleophoma empetri*, *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. An *in vitro* glass coverslip assay was used to evaluate the effects of flower extracts on conidial germination and growth. Morphological data were collected after 24-hours of exposure for each pathogen and a time course assay was conducted over a 24-hour period for *C. empetri*. Next, the effects of flower extract on the infection of ripe non-wounded Stevens fruit were investigated. We followed the microscopic stages of infection by conidia on the surface of intact Stevens fruit at 0, 12 and 24 hours post inoculation with or without flower extract. These experiments suggest that cranberry floral-extracts have the capacity to increase virulence by reducing the minimum required wetness period. Specifically, the floral-extracts increased the rate of spore germination and appressorium formation as well as altered hyphal thickness and caused formation of melanized hyphae. Spore numbers also increased as a result of micro-cyclic sporulation. *In vivo* experiments demonstrated that addition of floral-extracts to spore suspensions increased fruit rot development on ripe fruit. Thus, floral extracts provide a critical stimulus to the development of cranberry fruit rot and may provide insight to the timing of infection for this important disease.

A transcription factor AIN2 manipulates plant immunity by regulating the plant stomatal aperture size

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Rice bacterial leaf streak disease caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) is one of most important rice bacterial diseases in rice fields. A type III effector gene *avrRxol* was isolated from the genome of *Xoc*. Ectopic expression of *avrRxol* in *Arabidopsis* could suppress plant basal defense responses, which suggesting *AvrRxol* has virulence function to inhibit plant immunity. In aim to identify the virulent targets of *AvrRxol*, we screened two *Arabidopsis* yeast two-hybrid cDNA libraries by using *AvrRxol* as the bait. As a result, *AIN2* was identified as a putative transcription factor that has two homologues in the *Arabidopsis* genome. Double homozygous T-DNA knock-out mutant of *Ain2* was more susceptible to virulent pathogen *Pseudomonas syringae* pv. *tomato* DC3000. While *AvrRxol*'s virulence function was compromised on the mutant of *ain2*. To identify *Arabidopsis* genes regulated by *AvrRxol* and *AIN2*, we performed RNA-seq analysis on the *AvrRxol* transgenic and *ain2* mutant *Arabidopsis* plant, which allows us to identify a collection of *AvrRxol* induced genes (AIG). We further demonstrated that *AvrRxol* stabilized *Ain2*, in order to induce its direct target *AIG-1* gene's expression level. Overexpression of *Aig-1* in *Arabidopsis* inhibited stomata closing, and suppressed plant immunity. Our result illustrated *AvrRxol* manipulated *Ain2* to compromise *Arabidopsis* defense system, which suggested *Ain2* plays an important role in plant immunity. Further characterization of *Ain2* in *Arabidopsis* may help us gain new insight of plant immunity and develop new strategy to achieve durable disease resistance in crop plants.