2013 Potomac Division Meeting Abstracts

Abstracts presented at the APS Potomac Division meeting in Shepherdstown, West Virginia, April 3–5, 2013. 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. 2013. First report of QoI and boscalid resistance of *Botrytis cinerea* in eastern U.S. vineyards. (Abstr.) Phytopathology 103(Suppl. 4):S4.1. <http://dx.doi.org/10.1094/PHYTO-103-10-S4.1>

First report of QoI and boscalid resistance of *Botrytis cinerea* in eastern U.S. vineyards

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Botrytis cinerea causes a serious bunch rot of grapes, as well as diseases of a range of other host plants including fruits, vegetables, and ornamentals. *B. cinerea* in many regions of the world has developed resistance to a variety of fungicides, and recent reports from the southeastern United States document this extensively for populations in strawberries. However, no recent data have been published for grapes in the eastern U.S.A. *B. cinerea* samples were collected in the fall of 2011 and in 2012 from bunch-rot affected grape clusters in 28 vineyards in all regions of Virginia. A total of 178 isolates were tested by the germination/germ tube elongation method of Weber and Hahn (2011, J. Plant Dis. Protect. 118:17), with some modifications in fungicide concentrations in the media. Resistance to trifloxystrobin and to boscalid was present in over 80% of vineyards, with 81% and 64%, respectively, of isolates having a high level of resistance. More than 100 of the isolates were resistant to both compounds, and hence to a popular combination product, Pristine®. Resistance to QoI fungicides and boscalid has also been reported at high frequencies in strawberries in Florida and the Carolinas.

Putting to rest a 75 year old controversy: The true taxonomic placement of the dollar spot pathogens of turfgrass

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Dollar spot is one of the most widespread and economically important diseases of amenity turfgrasses. Since the pathogen was first described as *Sclerotinia homoeocarpa* in 1937, the exact taxonomic placement of this fungus has been the subject of much debate. True *Sclerotinia* species produce apothecia from tuberoid sclerotia, a characteristic lacking in *S. homoeocarpa*. Morphological and rDNA sequence data has suggested that this fungus might be more appropriately placed in the Rutstroemiaceae family; however, additional data is needed to test this hypothesis. In the current study, we used morphological and molecular data to evaluate 85 isolates of the dollar spot fungus, including the original specimens used by Bennett to describe the species, and closely-related fungi in the Rutstroemiaceae (*Ciboria*, *Lambertella*, *Lanzia*, *Poculum*, *Rutstroemia*). ~4300 bp of sequence data were generated from eight markers: actin, β -tubulin, calmodulin, translation elongation factor 1 alpha, the internal transcribed spacer region (ITS), DNA

replication factor *Mcm7*, the large subunit, and the small ribosomal RNA subunit. An additional ~200 bp fragment of the ITS-1 was amplified from 54 fungarium specimens, including the type specimens of *Ciboria*, *Lambertella*, and *Rutstroemia*, to provide anchors for phylogenetic trees. Multi-locus phylogenetic analysis, in combination with morphological assessments, confirmed that the dollar spot pathogen is not a true *Sclerotinia* species. Isolates of the dollar spot fungus formed their own well-supported clade, separate from all other fungal specimens tested. Within this clade, two distinct lineages of the pathogen were present, indicating that at least two species are responsible for dollar spot disease of turfgrass. Although their placement within the Rutstroemiaceae was confirmed, these fungi are not members of any known genus in this family. In this presentation, we will discuss the proposed names for the two causal agents of dollar spot in cool- and warm-season turfgrasses.

Phoma macrostoma var. *macrostoma* from Turkey, a potential biological control agent of field and hedge bindweed

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Bindweeds (*Convolvulus arvensis* L. and *Calystegia sepium* (L.) R. Br.; family Convolvulaceae) are exotic perennial vines that are invasive in the U.S.A. and problematic in Turkey. In July 2011, diseased *C. arvensis* plants were found near Samsun, Turkey. Symptoms were irregular tan-colored necrotic lesions on leaf lamellae. Symptomatic leaves were sent to the quarantine facility of USDA, ARS, FDWSRU in Ft. Detrick, MD where a fungus, 12-041, conforming to the description of *Phoma macrostoma* var. *macrostoma* Mont. was consistently isolated from diseased tissue. Conidial dimensions averaged $10.4 \times 3.7 \mu\text{m}$. DNA sequences for the internal transcribed spacers were deposited in GenBank (KC590613). A phylogram of this and 46 related isolates, based on ITS sequences, was constructed with Mr. Bayes software. The isolate separated into a clade independent from two other *P. macrostoma* isolates reported as weed biological control agents. Conidia from pure cultures were spray-inoculated, in a suspension of 106 conidia ml⁻¹, onto 20 healthy 30-day-old *C. arvensis* and 6 *C. sepium* plants. Plants were placed in a dew chamber at 25°C for 40 h and then placed in a 20–25°C greenhouse. Plants were rated weekly for disease severity on a 0–10 scale where “10” = 100% symptomatic tissue. The disease rating of *C. arvensis* plants ranged averaged 3.6 while *C. sepium* averaged 2.3. *P. macrostoma* var. *macrostoma* was re-isolated from all inoculated plants. Host range and spray formulation tests will determine the potential of this isolate as a biological control agent for these bindweeds.

North American grapevine yellows phytoplasma NAGYIII and ‘Candidatus Phytoplasma pruni’: Intraspecific evolutionary divergence or distinct species?

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In cultivated grapevines (*Vitis vinifera* L.), phytoplasmas induce diseases collectively known as grapevine yellows (GY) diseases. The different GY

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diseases, including flavescentia dorée, bois noir, and other GY diseases, are caused by different ‘*Candidatus Phytoplasma*’ species, distinguished from one another by sharing <97.5% nucleotide sequence identity of 16S rRNA genes. Our findings indicate that the NAGYIII phytoplasma causing North American GY disease in eastern U.S. and the peach X- disease causal agent, ‘*Ca. Phytoplasma pruni*’, share >97.5% 16S rRNA gene sequence identity, a level above the threshold widely accepted for separating ‘*Candidatus Phytoplasma*’ species. The close relatedness of this GY phytoplasma to ‘*Ca. Phytoplasma pruni*’ provokes the question of how NAGYIII phytoplasma should be properly termed. According to traditional guidelines and common practice, the high (>97.5%) nucleotide sequence identity of 16S rRNA genes dictates that the NAGYIII phytoplasma should be classified as a ‘*Ca. Phytoplasma pruni*’-related strain or as a strain of that species. Conversely, results from multi-locus genotyping revealed evolutionary divergence of NAGYIII phytoplasma and ‘*Ca. Phytoplasma pruni*’. Distinctness of NAGYIII phytoplasma based on analyses of non-rRNA genes, and the apparently non-reciprocal sharing of peach (*Prunus persica* L.) and grapevine as hosts, challenge previous guidelines for recognizing distinct ‘*Candidatus Phytoplasma*’ species. Resolution of this apparent conflict may lie in the recognition of a lineage, such as that of NAGYIII phytoplasma, as an incipient species, if not an evolved, distinct species.

The TmpL homolog in the rice blast fungus: A gene predicted to sense reactive oxygen species

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Magnaporthe oryzae, commonly called “Rice Blast,” is an ascomycete fungus that infects rice, wheat, rye, and barley. It is unique in that it is a hemibiotroph- having both a biotrophic and a necrotrophic phase. In order to colonize its host, *M. oryzae* must be able to ameliorate the effects of a plant-generated oxidative burst through suppression or scavenging of ROS (Reactive Oxygen Species). We identified a homolog of the TmpL protein, originally isolated in two other fungi by the Lawrence lab, and was found to regulate ROS through interactions with the transcription factor YAP1 in other species of ascomycete fungi. By characterizing its homolog in *M. oryzae* we can discern if it has the same function. To test its function, we first had to re-sequence this gene which was originally mis-annotated, and subsequently delete it. Here, we report results on virulence and abnormal growth.

Isolation of Phytophthora and Pythium spp. from stream ecosystems: The impact of host material, tissue status and sampling period

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The objective of this study was to understand the host preferences of *Phytophthora* and *Pythium* spp. at stream ecosystems. Host plants used for bait material included conifers (hemlock and white pine), deciduous (red oak, red maple and American elm) and evergreen plants (rhododendron and holly). Tissue status (dead vs. live), baiting period and the effects of few abiotic factors on the isolation rates was also investigated. Each month, two mesh bags each containing four leaves of dead or live plants were deployed and collected after five days. This was repeated twice for each month from August to November 2012. Seven random necrotic sections were cut from each leaf bait and plated onto clarified V8-juice based PARPN selective media that included hymexazol or not. There was no significant differences when hymexazol was in the isolation media when *Phytophthora* spp. were targeted, although, addition of hymexazol significantly influenced the isolation rate for *Pythium* spp. The tissue status (dead vs. live) was not a significant factor for *Phytophthora* isolation. In contrary, when we attempted to isolate *Pythium* spp., dead tissue baits resulted with significantly greater isolations than the live tissue. Conifers consistently gave lower isolation rates for *Phytophthora* and *Pythium* compared to evergreen and deciduous hosts. The isolation rates using the latter two plant groups usually gave similar isolation rates. Overall, when a particular plant species is considered, the isolation rates of *Phytophthora* and *Pythium* spp. were not different from each other throughout the eight-week of baiting period. In general, isolations were greater with increasing temperatures; particularly with *Pythium* species. Increased temperatures had only an effect on *Phytophthora* isolation when live conifer baits or dead evergreen plants were used. Dissolved oxygen and turbidity were negatively correlated whereas pH was positively correlated when significant differences occurred.

Bacterial Leaf Scorch of amenity trees caused by Xylella fastidiosa in Washington, DC: Distribution, host range and presence of the pathogen within street trees

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In 2011 and 2012, 95 sites in Washington DC were surveyed for Bacterial Leaf Scorch (BLS) caused by *Xylella fastidiosa*. Street trees with characteristic BLS symptoms and neighboring asymptomatic trees were sampled. In addition, we sampled asymptomatic foliage from symptomatic trees. Samples were first screened using *X. fastidiosa*-specific ELISA tests. Subsets of ELISA-negative samples were then further tested with PCR to confirm the absence of bacterial DNA. Out of 18 genera, *Quercus palustris*, *Q. rubra*, *Q. phellos*, *Platanus occidentalis*, *Ulmus americana*, *Liriodendron tulipifera*, and *Morus rubra* were found to be ELISA-positive for *X. fastidiosa*. *Q. palustris* and *Q. rubra* had the highest ELISA readings. Higher ELISA readings positively correlated with an increased level of crown dieback with *Q. palustris*, *Q. rubra*, *P. occidentalis* and *U. americana*. The bacterium was also found within the asymptomatic foliage of infected *M. rubra*, *Q. palustris*, *Q. rubra* and *U. americana* trees. Interestingly, asymptomatic trees adjacent to infected trees were generally free of *X. fastidiosa*. The only exceptions occurred with *Q. rubra* and *Q. palustris*. When a subset of ELISA-negative samples of *Q. rubra*, *Q. palustris*, *P. occidentalis* and *U. americana* were tested using PCR, results were mostly in agreement with ELISA findings. Oaks were the exception; 10% of ELISA-negative asymptomatic tree samples were PCR-positive. Based on our data, *X. fastidiosa* does not appear to be confined to the symptomatic portion of *Q. palustris*, *Q. rubra*, *M. rubra*, and *U. americana* street trees, and removing infected branches based on BLS symptoms may not eliminate the pathogen.

Watermelon root arbuscular mycorrhizal colonization is enhanced following Vicia villosa and Trifolium incarnatum cover crops

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Triploid watermelon cultivars, grown on more than 1800 ha in Maryland and Delaware, have little resistance to *Fusarium* wilt of watermelon (*Fusarium oxysporum* f. sp. *niveum*). A *Vicia villosa* cover crop suppresses *Fusarium* wilt of watermelon but the mechanism of suppression is unknown. *Vicia villosa* has been shown to increase mycorrhizal colonization of subsequent crops, which may play a role in suppression. This study examined the effect of *V. villosa* and the biocontrol Actinovate AG (*Streptomyces hydicus* WYEC 108) on arbuscular mycorrhizal colonization in a subsequent watermelon crop. Field experiments at Beltsville and Salisbury, MD in 2010 examined the impact of fall-planted, spring tilled cover crops (*V. villosa*, *Trifolium incarnatum*, *Secale cereale*, *Brassica juncea*), or bare ground, and Actinovate on the mycorrhizal root colonization of the following watermelon crop. Watermelon roots were stained with trypan blue and fine roots were examined for the presence of arbuscules. The percentage of watermelon roots colonized by arbuscular mycorrhizae was significantly higher following *V. villosa* and *T. incarnatum* green manures compared to that of all other treatments, as much as 71% and 44%, respectively. Actinovate had no significant effect on arbuscular mycorrhizal root colonization of watermelon. Enhancement of watermelon root mycorrhizal colonization following a *V. villosa* and *T. incarnatum* green manure may be a factor in *Fusarium* wilt disease suppression.

Genetic and biochemical characterization of the ACC oxidase in plant immunity

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The gaseous hormone ethylene is known to have role in plant disease development and program cell death in both tomato and Arabidopsis. However, it is still unclear how bacterial pathogen regulates plant ethylene production during pathogenesis. We identified a bacterial type III effector, AvrRxol1, from *Xanthomonas oryzae* pv. *oryzicola* that can directly interact with Arabidopsis ACC oxidase 2 (ACO2) in yeast two-hybrid assays. This interaction was also confirmed by Co-immunoprecipitation assay in vivo. ACO2 encodes the enzyme that catalyzes the final rate-limiting step in ethylene biosynthesis. It belongs to a gene family with three confirmed members in Arabidopsis. The biological roles of each member of the ACO gene family in Arabidopsis immunity haven't been studied. To this end, we identified homozygous T-DNA knockout lines of *aco1*, *aco2* and *aco4*, and developed double and triple T-DNA knockout mutants. Disease assay demonstrated that *aco2* single mutant plants have increased resistance to *Pseudomonas syringae* pv. *tomato* DC3000(D28E), but more susceptible to the same bacterial strain carrying the *avrRxol1* gene. We speculate the AvrRxol1 also interact with other ACO enzyme to manipulate the ethylene production. Further characterization of other *aco* single, double and triple mutant plants in response to *Pseudomonas syringae* pv. *tomato* DC3000(D28E) carrying the *avrRxol1* gene will

lead us gain deep insight about the virulence function of AvrXo1, and understand the role of ethylene biosynthesis enzymes in plant immunity.

Mating type genes expression during sexual reproduction of *Magnaporthe oryzae*

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The sexual reproduction of *Magnaporthe oryzae* is regulated by mating type genes, which are organized in idiomorphs Mat1-1, that encodes Mat1-1-1, Mat1-1-2 and Mat1-1-3, and in Mat1-2, which encodes Mat1-2-1 and Mat1-2-2. While gene expression during the asexual infective stage of this plant pathogen is well-studied, less is known about gene expression during sexual development. Our goal was to verify if the mating type transcripts have the same pattern of expression during a sexual reproduction time-course, and how this pattern relates to morphological changes. Sexually compatible lines were crossed by placing: plugs of mycelia approximately 4 cm apart on OA. These were kept at 25°C under continuous fluorescent white light until hypha meet, for 7 days. They were then transferred to 18°C under continuous black light (near UV). The samples were harvested on 7th, 14th, 21st and 28th days, for real time qRT-PCR analysis and microscopic observations. We observed that the relative level of gene expression had the same pattern for all mating type genes studied, with a high increase between 7th and 14th days, followed by a continuous decrease until the 28th day. The 14th day revealed differential expression between genes, with Mat1-1-1 being highly expressed over 1-1-2 and 1-1-3, and Mat1-2-2 being highly expressed over 1-2-1. Microscopically, we observed some sexual elements, including croziers hooks and protoperithecia, on the 14th day, and on the 21st day, observed some perithecia with exudation of ascospores. We discuss these results with regard to how timing of gene expression aids in induction of differentiation of sexual tissues.

Correlative imaging of fungal pathogenesis in maize: Linking disease symptoms to cellular-level infection events

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Genetic resistance is paramount among the approaches available to sustainably combat diseases of crop species. Phenotypic variation in quantitative disease resistance is associated with numerous genes of diverse functions, but the mechanisms of action of these genes are largely unknown. As part of a multi-way effort aimed at linking information about the genetics of host plant resistance to mechanisms of gene action, we are investigating variation in tissue and cellular-level pathogenesis events. One component of this effort is to relate macroscopic disease symptoms visible to the naked eye with microscopic events of pathogenesis. Corn leaf punches 15 mm in diameter containing phenotypically visible symptoms of Northern leaf blight (*Setosphaeria turcica*) were sampled, fixed, and processed for imaging. Images were recorded: 1) using a digital camera and 2) by scanning laser confocal microscopy (SLCM). A procedure was developed to align both the camera image and SLCM image (multi-layer image data) using specialized plugins originally developed for neurobiology research in the open source software ImageJ. The resulting correlated images are expected to provide new insights regarding the causes of disease symptoms and pathogen infection. Here, we demonstrate the potential of this approach and steps being taken to improve the precision of image alignment. This approach has the potential of being used to correlate other types of images too, such as different types of light microscopy, electron microscopy and their variants; this would be beneficial for a wide range of applications designed to better understand mechanisms of resistance and pathogenesis.

Improved management of blackberry cane blight caused by *Leptosphaeria coniothyrium*

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Cane blight is a serious fungal disease of blackberry (*Rubus fruticosus*) especially on winter frost or mechanically injured canes. The fungus overwinters on infected canes (stumps) that may serve as a ready source of inocula

to following year's primocanes. Once inocula are on the floricanes, it is necessary to spray fungicide to prevent infections of primocanes. In the first year of this study, fungicides from existing recommendations did not provide satisfactory level of disease control even though applied in combination with early fruiting cane removal. In vitro screening of new fungicides showed higher efficacy with significantly lower EC₅₀ value compared to that of currently recommended ones. Fungicide Inspire XT (difenoconazole + propiconazole) had the lowest EC₅₀ value with 0.008 ppm followed by Quilt Xcel (azoxystrobin+propiconazole) and Quadris Top (azoxystrobin+difenoconazole) with 0.04 and 0.05 ppm, respectively. Two other fungicides, Pristine and Cabrio from the current recommendations did not attain EC₅₀ value with the highest concentration (10 ppm) used in the in vitro study indicating a potential resistance in the fungal population. The three best performing fungicides were then tested in the field together with a non-treated control in the main plot in three replicates and removal of blighted canes without leaving any stump at two levels in the sub plots during the summer 2012. At the end of the season, percentages of primocanes with blight symptoms were counted from each treatment combination. In vitro efficacy of fungicides showed a significant positive correlation with field performance. There was a significant strong interaction ($P \leq 0.001$) of fungicide and cane removal indicating that both removal of blighted second year canes early in the season and spraying highly efficacious fungicide may be necessary to prevent primocane infection.

Target site mutation and *cyp51* over-expression as mechanisms of DMI resistance in *Erysiphe necator*

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Long-term, intensive use of demethylation inhibitors (DMIs) in managing the grapevine powdery mildew (*Erysiphe necator*) has resulted in some loss of efficacy due to resistance in pathogen populations. We investigated the target gene (*cyp51*) sequence of 24 eastern U.S. isolates, and conducted gene expression assays to understand DMI resistance mechanisms. The only mutation detected that was associated with DMI resistance was the previously described Y136F mutation, but three *cyp51* genotypes for the 136th codon were found: TAT or wildtype, and two mutant genotypes, TTT and TWT. The TWT genotype indicated the presence of both wildtype and mutant alleles in the same individual. The mutant isolates had a range of resistance factors, and the mutation only partially explained resistance to tebuconazole, myclobutanil and fenarimol. *Cyp51* over-expression was associated with the presence of Y136F, with relative copies of the target gene ranging from 1.4- to 19-fold more in eight mutant than in three wildtype isolates. Our findings support at least two mechanisms of DMI resistance in *E. necator* - the Y136F mutation which may be working in conjunction with *cyp51* overexpression, possibly due to copy number variation, for enhanced resistance.

Analysis of the early steps of the ergot alkaloid pathway by heterologous gene expression in *Aspergillus nidulans*

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Ergot alkaloids are tryptophan-derived mycotoxins that are pharmaceutically and agriculturally important. Several species of fungi produce these secondary metabolites including *Claviceps* spp. which infect rye and other grasses, *Neotyphodium* spp. which are mutualistic symbionts of grasses, and *Aspergillus fumigatus*, a common saprotroph and opportunistic human pathogen. Although ergot alkaloid profiles differ among these fungi, the synthesis of the early intermediate chanoclavine-I appears to be evolutionarily conserved among all ergot alkaloid-producers. Based on gene knockout studies, four genes, *dmaW*, *easF*, *easE*, and *easC*, are required for steps prior to chanoclavine-I; however, it is presently unknown whether these genes are sufficient for synthesis of chanoclavine-I. Roles for DmaW and EasF in the first two steps of the pathway are firmly established; the functions of EasC and EasE have not yet been fully characterized. Four genes, *dmaW*, *easF*, *easE*, and *easC*, were PCR amplified from *A. fumigatus* and transformed into the model organism *Aspergillus nidulans*, which does not contain ergot alkaloid synthesis genes. When investigated by HPLC and LC/MS, transformed strains containing the four ergot alkaloid synthesis genes accumulated chanoclavine-I and earlier pathway intermediates. Transformation of *A. nidulans* with subsets of three genes (*dmaW* and *easF* along with either *easC* or *easE*) did not result in chanoclavine-I production, but the strain containing *dmaW*, *easF*, and *easC* produced a previously uncharacterized alkaloid. Mass spectrometry and carbon 13 labeling studies provided information on this alkaloid and indicated that EasC can act as a decarboxylase. We conclude that *dmaW*, *easF*, *easE*, and *easC* are sufficient to make *A. nidulans* synthesize chanoclavine-I and that transformation of ergot alkaloid synthesis genes into *A. nidulans* provides an opportunity to further characterize the early steps of the ergot alkaloid pathway.