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 of fungicides, and recent reports from the southeastern United States documented this extensively for populations in strawberries. However, no recent data are available for grapevine isolates. In the current study, we used 1,886 isolates from eastern U.S. 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 tuberous sclerotia, a characteristic lacking in *S. homoeocarpa*. Morphological and dDNA 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 weeds 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 × 3.7 μm. 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 *P. macrostoma* var. *macrostoma* 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 “0” = 100% symptomatic tissue. The disease rating of 3.6 in the first assessment 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
diseases, including flavescence dorée, bois noir, and other GD 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 GD 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 GD 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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Phytopathology 103(Suppl. 4):S4.2

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 hemibiotrophic- 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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Phytopathology 103(Suppl. 4):S4.2

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, black gum, American holly), 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 until November 2012. Seven random necrotic sections were cut from each leaf bait and plated onto clarified V8-juice based PARPN selective media that have been found to discriminate between Phytophthora and Pythium spp. The tissue status (dead vs. live) was not a significant factor for Phytophthora isolation and 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.

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lead us gain deep insight about the virulence function of AvrRxv1, 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 Mat1-2, that 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 a different pattern of 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 a 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. The fungal population Inspire XT (dimethomorph + 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 need for fungicide registration. 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 < 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 was that associated with DMI resistance was the previously described Y136F mutation, but three cyp51-1 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 fenamidol. 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-1 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 been fully characterized. Inspire XT (dimethomorph, easC), and easC, were PCR amplified from A. fumigatus and transformed into the model organism Aspergillus nidulans, which does not contain ergot alkaloid synthetases. When investigated by HPLC and LC/MS, transformed strains containing the four ergot alkaloid synthesis genes accumulated chanoclavine-1 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.

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