2012 Potomac Division Meeting Abstracts

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Sentinel vines to monitor fungicide resistance in grape powdery mildew

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Resistance of grape powdery mildew (PM, *Erysiphe necator*) to demethylation inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides is a serious problem, and the risk of resistance development to several other groups appears moderate to high. Mixing and rotating of different modes of action, recommended to reduce resistance risk, makes it difficult for growers to judge what works and what doesn't. In order to assess the resistance status of PM populations, we tested an approach of placing potted "sentinel" grapevines near vineyards, and treating them weekly with low rates of individual fungicides, in order to find a discriminatory rate that would still control sensitive PM, but not PM with resistance shifts. Sentinel vine results with QoIs agreed with results of laboratory bioassays, and the application rate is not critical since the resistance factor is high. For DMIs, a sensitive population had lower discriminatory rates than PM populations from commercial vineyards, but the correlations between results from sentinel vines, laboratory bioassays, and field trials were only partial. Discriminatory dosages for sensitive PM were estimated for boscalid (5 µg/ml), quinoxifen (7.5–10 µg/ml), and several fungicides that have not yet received commercial registration. This method does require extended time required to obtain results, a consistent time commitment to maintain and treat plants (1–2 hrs/wk), and may not provide satisfactory results in seasons with low PM disease pressure. Advantages are that a grower can implement the procedure with minimal lab support, and that it can detect relatively low frequencies of resistant isolates.

Routine establishment of epidemics of systemic disease of Canada thistle (*Cirsium arvense*) caused by the rust fungus *Puccinia punctiformis*

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Because of observed effectiveness in eliminating Canada thistle (*Cirsium arvense*) patches, the rust fungus *Puccinia punctiformis* was proposed as a biological weed control agent in 1893. Since then, there has been considerable documentation on the effectiveness of the rust in controlling Canada thistle,

but establishment of epidemics of the systemic state of the rust in disease-free thistle patches has not been accomplished. The reason for this failure has been lack of understanding of the disease cycle and the close synchrony between life stages of the host and the rust. We now understand the disease cycle much better and have used this knowledge to establish rust epidemics in fields in Maryland and Greece. In the spring, systemically diseased shoots arise from roots infected with the fungus. The first signs of the fungus on these shoots are orange haploid pycnia (spermagonia) that cross fertilize to form haploid dikaryotic aeciospores. These spores infect leaves of nearby plants, producing local lesions, which give rise to uredinia that produce haploid dikaryotic urediniospores that, in turn, infect other leaves. In the late summer the uredinia transform into telia which undergo nuclear fusion (karyogamy). Through mitotic division the telia give rise to two-celled diploid teliospores. In the late summer and fall, the plants that emerged in the spring senesce and diseased leaves bearing telia dehisce onto newly emerging rosettes. Under conditions of adequate dew the teliospores undergo meiosis and germinate into haploid basidiospores that infect the rosettes. The fungus then develops hyphae that grow into the stem, and ultimately down into the roots of the rosettes where it survives the winter. Systemically diseased shoots emerge from this rootstock the following spring. Inoculating rosettes in the fall with teliospore-bearing leaves is a practical means of producing systemically diseased plants the following spring and initiating a disease epidemic.

***Colletotrichum gloeosporioides*, causing anthracnose of mile-a-minute (*Persicaria perfoliata*) in Turkey, is a potential biological control agent of this weed in the U.S.**

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Mile-a-minute (*Persicaria perfoliata* (L.) H. Gross; family Polygonaceae) is an exotic annual barbed vine that has invaded the northeastern U.S.A., Mississippi, and Oregon. In July of 2010, in a search for potential biological control pathogens, diseased *P. perfoliata* plants were found along the Firtina River near Ardesen, Turkey. Symptoms were irregular dark-colored necrotic lesions along leaf margins and smaller irregular reddish-colored lesions on the lamellae of leaves. Symptomatic leaves were sent to the quarantine facility of USDA, ARS, FDWSRU in Ft. Detrick, MD where a fungus that had morphological characters conforming to the description of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. was consistently isolated from the diseased tissue. Nucleotide sequences for the internal transcribed spacers (ITS 1 and 2) of the fungus were deposited in GenBank (JN887693). A comparison of these sequences with ITS 1 and 2 sequences of the *C. gloeosporioides* epitype using BLAST found 479 of 482 identities with no gaps. Conidia from 14-day-old pure cultures were spray-inoculated, in an aqueous suspension of 1.0×10^6 conidia ml⁻¹ onto healthy stems and leaves of nine 30-day-old *P. perfoliata* plants. Plants were placed in a dew chamber at 25°C for 16 h with no lighting and then placed in a 20–25°C greenhouse. Each plant was rated weekly for disease severity based on a 0–10 rating scale where "10" = 100

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percent symptomatic tissue. The average disease rating of the plants ranged from 4.3 to 4.8. *C. gloeosporioides* was re-isolated from all inoculated plants. Results of host range tests with this isolate will determine its potential as a biological control agent for *P. perfoliata*.

Evidence for differential susceptibility of non-native *Rubus* species from Oregon to rust disease by *Phragmidium violaceum*

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Non-native invasive blackberry in the Western United States (U.S.) is likely several species of *Rubus*. In 2005, *P. violaceum* was discovered in Oregon, raising the possibility for biological control of this weed. Variability has been noted in the amount of disease on blackberry within the region, and variation within host or pathogen may be contributing factors. For this reason, collection of blackberry accessions and rust isolates was made west of the Cascade Mountains of Oregon in 2010 as part of a comparative disease study. Replicated inoculations have been made by an isolate from the Elk River region increased on an accession of blackberry from Lincoln City. All inoculations involve cut canes growing in water, and an accession known to be susceptible is included in each inoculation as a standard. One-third of the accessions inoculated to date, i.e., 30% of the collection, were not diseased. Greenhouse-grown plants with different disease responses also differ in terms of leaf pubescence. Plans are to gather additional plant material from sites of these test accessions for morphological identification and DNA sequence analysis. From this it seems clear that distinct variation within *Rubus* in Oregon is evident, and that such is likely to affect results of biological control with the rust fungus currently on blackberry in the U.S.

Grapevine yellows from New York to Virginia: Multilocus molecular genetic characterization of phytoplasmas affecting commercial vineyards

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Grapevine yellows (GY) is a term that is used to refer to any of several diseases that are currently attributed to infection of grapevine (*Vitis vinifera* L.) plants by pathogens known as phytoplasmas (formerly termed mycoplasma-like organisms, MLOs). Although the symptoms are very similar among the different grapevine yellows diseases, available data indicate that several different phytoplasma species cause GY. To date, GY diseases have been reported in Europe, the Middle East, Africa, Australia, New Zealand, and South America, and in eastern North America - where the term North American grapevine yellows (NAGY) has been applied. We initiated work to investigate NAGY phytoplasmas in eastern U.S., based on the need to characterize the pathogens and based on the hypothesis that NAGY is more widely distributed than is currently recognized. DNA-based technologies were used for detection and multilocus genetic characterization of the phytoplasmas. The results confirmed association of phytoplasmas with NAGY in cultivated grapevines in Virginia and New York State and revealed the presence of phytoplasmas in diseased grapevines exhibiting symptoms characteristic of NAGY in Maryland and Pennsylvania, where NAGY had not been previously reported. Nucleotide sequence and phylogenetic analyses of 16S rRNA, *secY*, and ribosomal protein genes distinguished two 'Candidatus Phytoplasma' species among the NAGY phytoplasmas, and revealed within one of the species distinct genotypes marked by SNPs. The availability of these new molecular markers will facilitate future efforts to identify insect vectors and alternative plant hosts of the NAGY phytoplasmas, aiding the design of management practices for combating GY.

Occurrence of *Xylella fastidiosa* subsp. *multiplex* on Washington D.C. street trees

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Street trees in Washington D.C. were surveyed for the incidence of *Xylella fastidiosa*, the causal agent of bacterial leaf scorch. In total, 175 scorched trees, representing 19 different species, were sampled. Leaf samples were tested with *X. fastidiosa*-specific ELISA and infected trees were rated for scorch severity. In total 56.6% (99 out of 175) tested positive. Greatest incidence of *X. fastidiosa* was found on *Quercus palustris* (95.2%), *Quercus rubra* (75.5%), *Plantanus occidentalis* (66.7%), and *Ulmus americana* (54.3%). Highest average scorch was found on the following trees: *Q. palustris* (78%) and *Q. rubra* (59%), *U. americana* (58%), and *P. occidentalis* (41%). Additionally, *Liriodendron tulipifera* and *Acer platanoides*, two previously unreported hosts of *X. fastidiosa* were identified. A strain-specific, multiprimer polymerase chain reaction was used to differentiate among *X. fastidiosa* subspecies. All samples tested were identified as *X. fastidiosa* subsp. *multiplex*. Our study shows that *X. fastidiosa* subsp. *multiplex* is widespread in Washington D.C. and is associated with common street trees. Future planting should consider increasing the diversity of street tree species to include fewer known hosts of *X. fastidiosa*.

Searching for the sensor of reactive oxygen species in the rice blast fungus: The story of *Tmpl*

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Magnaporthe oryzae, commonly called "Rice Blast," is a fungus that infects rice, wheat, rye, and barley. It is estimated that Rice Blast destroys enough rice to feed 60 million people yearly. In order to combat this plant pathogen it is crucial to understand what makes it virulent and how it reacts to stress from fungicides, weather, and plant defenses. This project is meant to investigate a small piece of this puzzle. *Tmpl* is a protein found to regulate reactive oxygen species through interactions with HYR1 and YAP1 proteins in similar species of plant-pathogenic fungi. Our hypothesis is that it senses reactive oxygen species and transmits a signal to these other proteins. By studying the effects of its homolog in *Magnaporthe* we can discern if it has the same function. In the most recently annotated version of the *Magnaporthe* genome, *Tmpl* has been split into two separate genes, one of which we believe to have knocked out. We are currently conducting experiments to determine whether the absence of *Tmpl* affects virulence and response to oxidative stress. Furthermore, we will test whether expression of *HYR1* and *YAP1* genes are impacted in the *tmpl* mutant and discuss results from these experiments, along with how the *Tmpl* gene fits into our model of reactive oxygen species in the rice blast fungus.

Extract of the brown seaweed *Asophyllum nodosum* reduces Bacterial Spot of four varieties of tomatoes

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Crop losses due to Bacterial Spot are important to Eastern tomato producers and many have an increased interest in natural methods to manage this disease such as through improved genetic resistance, as well as softer control measures in the field. In a 2011, a field study was conducted in Waynesville, NC, to study the effect of *Asophyllum nodosum* seaweed extract on Bacterial Spot (*Xanthomonas axonopodis* pv. *vesicatoria*) in four tomato varieties (i.e. NC-144, NC-244, Cherokee Purple, RG-220). *A. nodosum* seaweed extract treatments applied in weekly alternations with Nordox (copper), significantly reduced severity of Bacterial Spot in all four tomato varieties compared to the unsprayed control. In addition, bacterial spot severity in the *A. nodosum*/copper treatment was not significantly different from the organic standard (Serenade combined with Regalia and applied with weekly alternations with Nordox). Area under the disease progress curves were significantly decreased and marketable yields were significantly increased in both the *A. nodosum* treatment and the Regalia/Serenade/copper standard across all tomato varieties. No treatment vs. variety interactions were observed in this factorial experiment. The results are in conformity with published work on the protective effect of *A. nodosum* seaweed extract against bacterial disease. Further, earlier research suggests that the *A. nodosum* seaweed extract elicits jasmonic acid dependent systemic resistance. It is likely that the Bacterial Spot suppression observed in the *A. nodosum* treatment in this study was also an elicited response; however other modes of action may also be involved.

The use of a non-invasive inoculation method of tomato fruit with *Geotrichum candidum* to evaluate post-harvest disease management strategies

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Geotrichum candidum is the causal agent of sour rot in tomato and other fresh produce. This disease is a limiting factor of tomato production on the Eastern Shore of Virginia and can cause major losses in the field and especially during post-harvest handling. Infections by *G. candidum* are most prevalent during periods of wet harvest conditions or abrupt temperature changes and if improper post-harvest handling procedures are employed. There are currently no in-field treatments targeted to prevent sour rot infections so post-harvest treatments are used to prevent further losses. Currently, the methodology utilized for screening materials on harvested tomato fruits is invasive and involves severe wounding to inoculate with *G. candidum*. This practice does not accurately reflect natural infections that are usually internal and latent, so response to post-harvest treatments may differ under these artificial methods. This compelled us to develop a method of infection without wounding. A vacuum method of internalizing *G. candidum* spores was developed to insert spores into tomato fruit to imitate infected fruit for further management studies. Validation studies were done using the vacuum method to compare fungicide efficacy and the ability to infect different stages of fruit, as well as screen for differences in susceptibility among cultivars.

Progression of thousand cankers disease (TCD) on black walnut in Tennessee in 2010 and 2011 and possible association with drought stress

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Eastern black walnut is an important landscape and forest tree in the eastern United States. In July, 2010, thousand cankers disease (TCD) of black walnut, previously known to occur in the West, was discovered in Knoxville, TN. Following this discovery, I initiated on September 14 a study in Knoxville on the progression of TCD on black walnut with an emphasis on trees with a high per cent live crown. This study is part of a project with co-investigators, Scott Schlarbaum and Mark Coggeshall, on identifying TCD resistance in black walnut. In 2011, from mid-May until mid-September, I observed little or no new TCD development (foliage wilt or branch dieback) on black walnuts in Knoxville. On some trees early in the growing season, recovery, or increase in per cent live crown, was observed, suggesting that environmental factors may have affected per cent live crown ratings on some trees in September, 2010. Rainfall in Knoxville during the growing season (April to September) was 53% greater in 2011 than in 2010 when severe TCD symptoms were observed. These findings suggest that environmental factors, such as drought stress, may be involved in per cent live crown ratings and TCD development on this mesic tree species.

'Ca. Liberibacter asiaticus' genes orthologous with pSymA-borne genes of *Sinorhizobium meliloti*: Suggested roles in eukaryotic host interactions

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'*Ca. Liberibacter asiaticus*,' is a psyllid-vectored, obligate phytopathogen associated with citrus huanglongbing disease with a greatly reduced genome. *Sinorhizobium meliloti* strain 1021, a nitrogen-fixing, root-nodulating bacterial microsymbiont of alfalfa, has a 3.5 Mbp circular chromosome and two megaplasmids including 1.3 Mbp pSymA carrying nonessential 'accessory' genes for nitrogen fixation (*nif*), nodulation and host specificity (*nod*). In this work we compare the gene content of the '*Ca. Liberibacter asiaticus*' chromosome with that of the pSymA plasmid of *S. meliloti*. Most genes from '*Ca. Liberibacter asiaticus*' with orthologs on pSymA also have orthologs on the *S. meliloti* chromosome. In general, proteins encoded by pSymA genes are more similar in sequence alignment to those encoded by *S. meliloti* chromosomal orthologs than to orthologous proteins encoded by genes carried on the '*Ca. Liberibacter asiaticus*' genome. Only two '*Ca. Liberibacter asiaticus*' genes were identified as having orthologous genes carried on pSymA without orthologs on the chromosome of *S. meliloti*. These two orthologous gene pairs encode a Na⁺/K⁺ antiporter and an efflux protein for Co⁺⁺, Zn⁺⁺ and Cd⁺⁺ cations. Also essential in maintaining homeostasis, a redox-regulated K⁺ efflux pump regulates cytoplasmic pH. The pSymA and '*Ca. Liberibacter asiaticus*' orthologs of the latter protein are much more similar in amino acid alignment than are pSymA and *S. meliloti* chromosomal homologs. About 182 pSymA encoded proteins have sequence similarity (<E-10) with '*Ca. Liberibacter asiaticus*' proteins, often present as multiple orthologs of single '*Ca. Liberibacter asiaticus*' protein. These proteins are involved with amino acid uptake, cell surface modifications, chaperonins, electron transport, export of bioactive molecules, homeostasis, regulation of gene expression, signal transduction and synthesis of amino acids and metabolic cofactors. The presence of multiple orthologs on pSymA suggests that these proteins are of particular importance in host/microbe interaction and facilitates the ongoing evolution of these genes.

Foliar leachate of *Vicia villosa* increases growth rate of *Fusarium oxysporum* f. sp. *niveum* and *Trichoderma harzianum* in vitro

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Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f. sp. *niveum* (FON), can significantly reduce fruit yield. Triploid watermelon cultivars lack resistance to *Fusarium* wilt, which has led to the search for sustainable management practices, such as the use of cover crops for disease suppression. A legume cover crop, *Vicia villosa*, significantly suppressed *Fusarium* wilt in trials in the mid-Atlantic region of the U.S. However, the mechanism of this suppression is unknown. Elevations of the growth rates of *Trichoderma* spp. and nonpathogenic *Fusarium* spp. have been associated with disease suppression. Foliar leachates that are released during the decomposition of the cover crop may directly affect FON growth. To test this hypothesis, foliar leachates from three cover crops (*V. villosa*, *Trifolium incarnatum* and *Secale cereale*) were collected from fresh biomass by a simulated rainfall and used in Petri dish assays to explore the effect of leachate on the growth of FON and *Trichoderma harzianum*. The leachates were amended to Potato Dextrose Agar along with the control treatment (no amendment) and pH adjusted to 3.5 and 6. *Trichoderma harzianum* grew significantly faster than FON across all leachate treatments; both fungi grew faster at pH 6. At a pH of 3.5 the linear growth rate of FON was significantly higher on *V. villosa* leachate treatments than on the nonamended controls. The growth rate of *T. harzianum* followed a similar pattern where in all trials the highest mean growth rate was on *V. villosa* treatments, though this was not consistently significant. The growth rates of FON and *T. harzianum* were generally significantly lower on *T. incarnatum* and *S. cereale* leachate amended plates compared *V. villosa* treatments. These results indicate that, in-vitro, *V. villosa* leachate stimulates the linear rate of FON growth at pH 3.5 while the growth of *T. harzianum* is enhanced at both pH's.

Documentation of grapevine leafroll-associated virus-2, -3, and grapevine fleck virus in wine grape varieties and native grape species in Virginia

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Grapevine leafroll-associated virus-2 (GLRaV-2), GLRaV-3, and grapevine fleck virus (GfKV) are three of the most problematic grapevine viruses found worldwide. These viruses can cause significant crop loss and affect wine quality by reducing sugar accumulation and compromising skin color. Moreover, a mixed infection of GLRaV-3 and GfKV was shown to aggravate disease symptoms. Presence and prevalence of viral diseases of grapevines in the state of Virginia were unknown; therefore, a statewide survey was conducted in 2009–11 seasons. Petiole samples were collected from random vines from various commercial vineyards. Using a one-tube, one-step RT-PCR and PCR method, we tested over 1300 vine samples (39 different wine grape varieties) from 136 locations in and around Virginia for GLRaV-2, GLRaV-3, and GfKV. Also, 65 wild grapevines samples were examined. Testing results showed 7.3%, 24.6%, and 0.5% of sampled vines were positive for GLRaV-2, GLRaV-3, and GfKV respectively. Of all vineyards surveyed, 61% were found to be positive with at least one of the tested viruses. A majority of these infected vines were planted prior to the 1990s; however, some new plantings were also found to be positive, indicating movement of the viruses among vineyards and also potential contamination prior to the planting. All wild grapevines tested have been found to be free of these three viruses. This study is the first to examine multiple grape viruses in VA, and thus, will serve as a baseline for the level of viral grape disease infections in the state. The high level of virus-infected vines emphasized the importance of clean plant materials, as well as management of vector insects (mealybugs and scale insects).

Update on the limited effects of foliar insecticidal treatments for the control of mealybugs on grape in Virginia

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Two field experiments were conducted at the experimental farm (AHS AREC at Winchester, VA) and a commercial vineyard (Orange, VA) to investigate the effectiveness of foliar insecticide sprays on controlling grape mealybug populations. The AHS AREC vineyard block has 21-year old 'Cabernet Sauvignon' grapevines infected with grapevine leafroll disease (specifically GLRaV-2 and -3). Within each row, all but one of the infected vines were removed and re-planted with certified 'Cabernet Franc' cuttings at approximately 5 and 10 feet away from each infected vine. After planting, foliar insecticide treatments were applied in a randomized block design with six

replications. The treatments were (i) an acetamiprid (Assail, 2.5 oz/A) at delayed dormant stage, (ii) an acetamiprid (Assail, 2.5 oz/A) at delayed dormant stage plus a pyrethroid (Baythroid XL, 3 oz/A) at bloom and (iii) a control (no spray). At the commercial vineyard, a single row of variety 'Chardonnay' was used. Treatments were assigned in a completely randomized design with four replications. Two foliar neonicotinoid treatments, dinotefuran (Movovento, 6 oz/A) and spirotetramat (Scorpion, 4 oz/A), were tested. Following treatments, mealybug numbers were visually assessed spending 5 minutes per vine. The experiment at the AHS AREC resulted in the increase of mealybug populations on all vines regardless of treatment, where the vines treated with two-time application of insecticide resulted in significantly higher mealybug count ($P < 0.05$). On the other hand, treatments at the commercial vineyard resulted in a significant time and treatment interaction ($P < 0.05$) where two insecticide treatments showed significantly faster decline of mealybugs count over time. The results indicated the importance of insecticide selection for grape mealybug management.

Small fluctuations in the recovery of fusaria across consecutive sampling intervals with unmanned aircraft 100 m above ground level

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Many *Fusarium* spp. are well-suited for atmospheric dispersal, yet the aerobiology of these fungi are poorly understood. Recent work has highlighted the role of atmospheric transport barriers (ATBs) in the movement of fusaria in the lower atmosphere. Here, we extend this work by examining the relationship between the length of atmospheric sampling intervals with autonomous unmanned aerial vehicles (UAVs) and the recovery of fusaria. UAVs were equipped with an array of eight microbe-sampling devices with four 'inner' sampling arms and four 'outer' sampling arms. Each set of arms was used to collect consecutive aerobiological samples for periods of 10 minutes at 100 m above ground level at Kentland Farm in Blacksburg, Virginia. A total of 102 consecutive sampling intervals (51 flights) were conducted in 2011. A correlation analysis showed that counts of fusaria did not vary between the inner and outer sampling arms from consecutive sampling periods of 10 min ($r = 0.93$, $P < 0.001$). This work supports the idea that atmospheric populations of fusaria are well-mixed, and large changes in the recovery of fusaria in the lower atmosphere may be attributed to large-scale phenomena (e.g., ATBs) operating across varying temporal and spatial scales. This work may contribute to effective control measures for diseases caused by fusaria in the future.

***Fusarium* fungi dominate isolations from blocky cankers not associated with beech bark disease on American beech in the central Appalachians**

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Species of *Fusarium* cause cankers on numerous hardwoods, notably maples (*Acer* spp.) and oaks (*Quercus* spp.). They have been isolated from bark of American beech (*Fagus grandifolia*) but not associated with cankers. Blocky, cracked cankers regularly have been observed in the Appalachian region on beech stems affected or unaffected by beech bark disease (BBD). This study attempted to identify fungi associated with these cankers. During the study 1,565 bark plugs were collected at sites in Pennsylvania, West Virginia, and Tennessee from 403 blocky cankers on 140 beech stems that lacked signs of BBD. *Fusarium* isolates were recovered from 85% of cankers on 95% of stems. *Phomopsis* spp. were the next most abundant but only were isolated from 20% of cankers on 37% of stems. *Neonectria* fungi that contribute to BBD also were isolated. When paired in culture with *Fusarium* fungi, *Neonectria* spp. were easily outcompeted and overgrown. These results indicate *Fusarium* spp. are predominant in the unusual blocky cankers and their ability to compete may influence *Neonectria* infection. Pathogenicity tests have been initiated by inoculating healthy beech with a single spore *Fusarium* isolate obtained from a blocky canker.

***Moniliophthora roreri*: The link between cacao disease and physiological conditions**

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Moniliophthora roreri causes frosty pod a devastating disease of *Theobroma cacao* L. *M. roreri* infection is limited to cacao pods. Initial symptoms of *M. roreri* infection are yellowing and deformation of pod tissue in the form of localized swellings. As disease progresses, malformations undergo tissue death and the pod tissue rots. This unique agaric basidiomycete lacks the ability to produce basidiocarps. Instead, infected mature pods become covered with millions of meiospores. Young cacao pods (~70 days old) can be lost to

cherelle wilt, a natural physiological thinning. Cherelle wilt occurs when the natural formation of vessel occlusions stops pod development. Pods become yellow, then turn brown and mummify, remaining on the tree. It is thought that *M. roreri* may play a role in cherelle wilt, but there is no scientific support. Through metabolite and QPCR analysis, we found that infection of young pods with *M. roreri* does not drastically change the physiology of pods, but has a pronounced effect on older, more mature pods. The physiological changes brought about during cherelle wilt are those of senescence. Additionally, *M. roreri* in pods undergoing cherelle wilt as well as mature infected pods, the fungus is in a highly stressed environment. Overall, this study describes the physiology of cherelle wilt and *M. roreri* infection of cacao pods.

Effect of early floricanes removal and fungicides on primocane blight of blackberry caused by *Leptosphaeria coniothyrium*

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Cane blight is a serious fungal disease of blackberry especially on mechanical or winter frost injured canes. The fungus overwinters on infected canes that serve as a ready source of inocula to following year's primocanes. Once inocula is on the floricanes, it is necessary to spray fungicide to prevent infections of primocanes. However, established infections on floricanes can't be removed or cured by fungicide spray. This study was conducted in a factorial split plot design in a four year old blackberry plantation in Monongalia County, WV that had severe cane blight. The study included three fungicides and 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 to determine the efficacy of fungicides combined with blighted cane removal in preventing primocane infection. Blighted canes were removed at soil level followed by two applications of fungicides during the summer 2011. At the end of the season, percentages of primocanes with blight symptoms were counted from each treatment combination. Pristine (pyraclostrobin + boscalid) treated plots combined with early removal of blighted second year cane had significantly ($P \leq 0.005$) lower blighted primocanes compared to the non-treated control. There was a significant strong interaction ($P \leq 0.0001$) of fungicide and cane removal indicating that both removal of blighted second year canes early in the season and spraying highly efficacious fungicide seems to be necessary to protect primocanes from being infected.

The role of Hsp104 in pathogenicity and gene regulation in the rice blast fungus

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Magnaporthe oryzae, better known as rice blast fungus, is an extremely successful pathogen that is not only able to gain resistance to methods used to combat it, but it is also able to overcome environmental stress as well. These characteristics have allowed this cereal fungus to continually devastate vital crops for centuries, and its ability to survive comes from its genetic structure. One very important protein that is synthesized in *M. oryzae* and all other living organisms is HSP104, or the heat-shock protein. HSP104 has been found to increase survival in organisms when exposed to multifarious environmental stressors by allowing the refolding of damaged proteins, or protein complexes. The *HSP104* gene was deleted in *M. oryzae*, and the mutants exposed to a heat shock to determine the effect of this gene. The test samples were allowed to recover for various periods of time, and then tested for gene expression using reverse transcription PCR. We observed changes in the expression of other chaperone-type genes in the mutant versus the wild type, indicating that *HSP104* impacts gene expression during heat stress and recovery. Further efforts in expanding our knowledge on the role of HSP104 in *M. oryzae* could help in identifying more successful ways to control this pathogen.

Natural products for suppression of damping-off pathogens in organic cucumber production

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Disease control measures need to be developed for organically produced cucumbers as growers have few disease management options. Ethanol extracts of antibiotic-producing beneficial bacteria were tested for suppression of the soilborne damping-off pathogens *Pythium ultimum* and *Rhizoctonia solani*.

Extracts from *Serratia marcescens* isolates N4-5 and N2-4 inhibited *P. ultimum* sporangial germination in Nutrient broth while extracts from *Burkholderia cepacia* BC-F and BC-2 inhibited growth of *R. solani* on Potato Dextrose Agar. Extracts from *B. cepacia* BC-1 and *Pseudomonas fluorescens* PF-5 were only slightly inhibitory to these pathogens. Ethanol extracts from isolate N4-5 also suppressed damping-off of organic cucumber seedlings in growth chamber studies when applied as seed treatments. Growth chamber experiments are currently being conducted to determine if ethanol extracts

from these beneficial bacteria suppress damping-off of cucumber caused by *R. solani*. PCR followed by DNA sequence analysis and TLC were used to confirm that pyrrolnitrin was produced by isolates N4-5, BC-F, BC-2, and PF-5. Prodigiosin was previously shown to be produced by N4-5. Pyrrolnitrin and prodigiosin are ethanol soluble and inhibitory to *P. ultimum* and/or *R. solani*. These studies demonstrate that the use of OMRI-listed solvents for the isolation and seed application of natural products is promising for use in organic cucumber production systems for the control of soilborne plant pathogens.