2018 Potomac Division Meeting Abstracts

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Diving deeper into recycling water: Exploring the diversity and management of cryptic oomycete pathogens in recaptured irrigation water in nurseries

Justine Beaulieu¹, Johanna M. Del Castillo Munera², Claudia Delgado¹ and Cassandra L. Swett², (1) University of Maryland, College Park, MD, USA, (2) University of California, Davis, CA, USA

Although recycling irrigation water may reduce costs for nurseries, the practice increases risk of recirculating and spreading waterborne pathogens. Using culture-based methods, 17 Oomycete species were found in containment ponds at two Maryland nurseries in 2015, 2016, and 2017. Of these, *Pythium oopapillum*, *Pythium aff. diclinum*, *Phytophthora cryptogea* and *Phytophthora helicoides* were pathogenic in seedling trials (90% damping off). *Pythium oopapillum*, *P. cryptogea* and *Ph. helicoides* increased root rot in chrysanthemum trials (differences not significant $P = 0.06$), and reduced stem growth ($P < 0.05$); the latter two species increased wilt severity ($P = 0.03$). In geranium trials, all species increased root rot severity ($P = 0.0007$) and *Ph. helicoides* significantly affected growth ($P < 0.0001$). Slow sand filtration (SSF) of pond water (site 1) reduced oomycete recovery from 100% of baits in the holding pond to 0%, based on monthly analyses (April–November 2017), although recovery increased to 50% following transport to the greenhouse (species found were primarily non-pathogens). Chlorine treatment (site 2) reduced oomycete recovery from 100% of pond baits to 33% in the spring and summer, and to 0% in the fall (2017). These data indicate that recaptured irrigation water can be a source of diverse pathogens which have not been previously described in Mid-Atlantic nurseries, and that SSF appears to be more effective than chlorine treatment in reducing pathogen load.

Enhancing suppression of dollar spot of creeping bentgrass fairways using organic amendments

Cody Beckley and Joseph A. Roberts, University of Maryland, College Park, MD, USA

Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) is a disease of turfgrass frequently requiring multiple fungicide applications for control. Previous research has shown organic amendments to suppress dollar spot, yet the impact of new organic products when used with fungicides is unknown. This trial was conducted in College Park, MD on creeping bentgrass (*Agrostis stolonifera* L. cv. '007') maintained as a golf course fairway. Main plot treatments included municipal waste compost (MWC) applied at establishment, MWC bi-annual topdressing, biochar applied at establishment, biochar bi-annual topdressing, synthetic fertility, synthetic fertility + vermicompost extract, and none. Main plot nitrogen (N) fertility levels were normalized over the trial period using monthly applications of sulfur coated urea. Sub-plots consisted of fungicide factor and included fluazinam 14 d, fluazinam threshold, fluxapyroxad 14 d, fluxapyroxad threshold, and none. Threshold fungicide treatments were applied once 2 of 4 replicates exhibited symptoms of > 2 infection centers per plot. In the first year of the study, all fertility treatments reduced dollar spot compared to none on 2 of 6 individual rating dates and when comparing area under disease progress curve values. Synthetic fertility + vermicompost provided the longest duration of control for both threshold fungicide treatments.

Aggressiveness and oxalic acid production by *Sclerotinia sclerotiorum* on bean plants

Habtamu Demissie, PhD Student at the University of Maryland, College Park, MD, USA and Kathryn L. Everts, University of Maryland College Park, Lower Eastern Shore Research and Education Center, Salisbury, MD, USA

The pathogen *S. sclerotiorum* causes significant losses in bean yield in the mid-Atlantic region. In order to study the diversity of local *S. sclerotiorum* populations, we correlated aggressiveness to lesion size and measured virulence by quantifying oxalic acid (OA) production. *Phaseolus lunatus* (lima bean, LB), *P. vulgaris* (common bean, CB), and *Glycine max* (soybean, SB) were examined. Aggressiveness was evaluated by inoculating the top of cut stems of one-month-old bean plants with one-week-old cultures of *S. sclerotiorum*. Lesion size was measured after 8 days in the greenhouse. To test virulence, OA from infected tissues was quantified using a diagnostic kit (BioVision). Data were analyzed using proc mixed in SAS. Aggressiveness varied significantly among cultivars ($P = 0.0001$), but not among crops ($P = 0.24$). The mean lesion sizes were 4.6, 7.2, 4.7, 4.8, 5.9, and 6.5 cm for 6122 (CB), Beryl (CB), Celite (LB), Dixie (LB), NKS 1990 (SB), and Williams82 (SB) cultivars, respectively. Oxalic acid production varied significantly among isolates ($P = 0.038$), but not among crops ($P = 0.87$) or cultivars ($P = 0.35$). Spearman's correlation indicated a weak ($r = 0.15$) but significant ($P = 0.001$) correlation between lesion size and OA. This may indicate that low and high levels of OA are required to produce large lesions on susceptible and on resistant crops, respectively. Alternatively, it may indicate that the role of OA in aggressiveness is more complex than previously thought.

Synthetic biology for plant viral diagnostics: Application to maize lethal necrosis disease

Susan Gachara, M.S. student, University of Delaware, Newark, DE, USA and Randall J. Wisser, University of Delaware, Newark, DE, USA

Plant viruses are a risk to many economically important crops, including maize which serves as a staple food source for smallholder farmers throughout the world. Indeed, a current epidemic of maize lethal necrosis (MLN) is affecting food security across eastern and central Africa. The disease is caused by a synergistic interaction of Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV; or other potyviruses). Timely disease detection is imperative to reduce spread and yield losses. To facilitate timely and rapid detection of hyper-mutable viruses, emerging disease diagnostic technologies from the human health sector can be modified for use in agriculture. The Toehold switch technology is a prime example, which has already been demonstrated for the detection of Zika virus. This technology offers a promising, but untested solution for plant viral detection as it can be rapidly designed to sense any RNA sequence. Using publicly available whole-genome sequences for 15 and 73 global isolates of MCMV and SCMV, respectively, 637 and 208 conserved 36-mers were identified in each genome as candidate sensor sequences for isolate non-specific viral detection. After further filtering to match the canonical structure of a Toehold switch, 457 MCMV and 159 SCMV sequences form the basis for toehold switch construction. In parallel, as a proof-of-concept, we have independently validated the technology *In vitro*, based on previously published Toehold switches. Looking ahead, Toehold switches can be freeze-dried on cell-free paper, providing an inexpensive, field-deployable platform for viral detection. This project seeks to demonstrate the potential of toehold switches in plant disease diagnostics using MLN as an example.

Analysis of resistance to mefenoxam in isolates of *Phytophthora phaseoli* from the mid-Atlantic region

Adam Ginn, Terence Mhora, Nancy F. Gregory, Thomas A. Evans and Nicole M. Donofrio, University of Delaware, Newark, DE, USA

Downy mildew caused by the oomycete pathogen *Phytophthora phaseoli* has destroyed lima bean crops throughout Delaware and the mid-Atlantic region of the United States for over a century. When left untreated, *P. phaseoli* epidemics can cause extensive yield losses. The fungicide mefenoxam (Ridomil Gold), the primary source of chemical defense against the most recent physiological race of *P. phaseoli* (Race F), inhibits oomycetes by disrupting RNA synthesis via RNA polymerase interference. To determine the threat of fungicide resistance in local isolates infected lima bean samples were collected primarily from southern Delaware but also from Salem County, NJ and Cecil County, MD. Samples were isolated using rye isolating agar and maintained on potato dextrose agar. Single sporangial isolates were obtained from each field isolate with the purpose of acquiring homogeneous representatives of composite isolates. Composite field isolates and their corresponding single sporangial isolates were grown for 28 days on lima bean agar containing 0ppm, 5ppm, 50ppm and 100ppm of mefenoxam. At weekly intervals, the plates were measured, and 28 day measurements were used to assign a label of 'sensitive', 'intermediate' or 'insensitive'. Out of 119 field isolates, three had a rating of intermediate mefenoxam sensitivity, and one insensitive, respectively. Single sporangial isolates are currently being tested, and of those completed, all are sensitive. This data will provide crop consultants and growers with better information on effective chemical control utilizing mefenoxam for *Phytophthora* on lima beans.

Effect of mulching on splash dispersal of *Calonectria pseudonaviculata* and boxwood blight in a private garden

T. Michael Likins¹, Ping Kong² and **Chuanxue Hong**², (1) Chesterfield County Extension, Chesterfield, VA, USA, (2) Hampton Roads Agricultural Research and Extension Center, Virginia Tech, Virginia Beach, VA, USA

As the boxwood blight pathogen, *Calonectria pseudonaviculata* (*Cps*), continues to spread via contaminated stock plants, greeneries, landscaping equipment and tools, there is an urgent need for effective tools to salvage established boxwood plantings from devastation of this disease. The objective of this study was to evaluate the efficacy of mulching as a physical barrier to prevent soil inoculum from

splashing onto healthy boxwood foliage. A field trial consisting of two treatments: mulched and nonmulched with five replicates each, was conducted for 2 years in a well-established boxwood garden where diseased boxwood were removed to leave a 18-cm stump. Mulching efficacy was assessed by monitoring and comparing boxwood blight development on detector plants - containerized Justin Brouwers rotating through mulched and control plots at 2-week intervals. A total of 14 sets of monitoring was performed in 2016 starting on April 20 and 16 sets were done in 2017 beginning on April 21. Boxwood blight was observed on detector plants in eleven monitoring periods of 2016 while only three in 2017. Boxwood blight was significantly reduced by mulching in both years. These results indicate that mulching is an effective tool for salvaging boxwood plantings from devastation of boxwood blight following an accidental pathogen introduction.

A new pathotype of *Magnaporthe oryzae* causing devastating wheat blast disease in multiple continents

Md Tofazzal Islam, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, BANGLADESH

The blast fungus *Magnaporthe oryzae* is comprised of lineages that exhibit varying degrees of specificity on about 50 grass hosts. A new pathotype, *Triticum* was first evolved and emerged as a devastating pathogen of wheat in Paraná state of Brazil in 1985 through host jump from a local grass. It rapidly spread to several neighbouring countries of South America. In 2016, wheat blast suddenly emerged in Bangladesh and devastated more than 15,000 hectares of wheat. Application of field pathogenomics and open data sharing by 31 researchers from 4 continents revealed that the emergence of wheat blast in Bangladesh was caused by a South American lineage of *M. oryzae Triticum* (*MoT*). This finding suggests that genomic surveillance can be rapidly applied to monitor plant disease outbreaks and provide valuable information regarding the identity and origin of the infectious agent. The wheat blast fungus already spread to new areas in Bangladesh and neighboring West Bengal of India. It is now a threat to the food security of both Asia and South America. In Asia, wheat blast strains are now co-occurring with rice blast pathogen raising the possibility of genetic recombination between these destructive pathogens. We found that recently developed *MoT*₃ primer is not specific for the *Triticum* pathotype. Multiplex amplicon sequencing seems a reliable method to distinguish the Bangladeshi lineage of *MoT* from others. To develop blast resistant wheat varieties, the genome editing of the *S*-genes in wheat by CRISPR/Cas9 technique is now underway. The whole genome sequences of a large number of *MoT* strains from the fields of Bangladesh were uploaded in the open wheat blast website. A concerted global effort is urgently needed to combat this fearsome pathogen.

The effects of reducing chlorothalonil use in fungicide spray programs on melons

Jake G. Jones and Kathryn L. Everts, University of Maryland College Park, Lower Eastern Shore Research and Education Center, Salisbury, MD, USA

Fungicides are costly, but essential for the production of the 1,500 hectares of melons grown in Maryland annually. Chlorothalonil (Bravo), one of the most common broad-spectrum fungicides, was recently linked to increased honeybee susceptibility to Nosema. We designed an alternative fungicide spray program to examine the effects of reducing the use of chlorothalonil on three melon crops ('Athena' muskmelon, 'Snow Leopard' honeydew melon, and 'Crunchy Red' watermelon) in 2016 and 2017. Chlorothalonil was replaced in the tank-mix of weekly sprays of targeted fungicides, with either a polyoxin D zinc salt (Oso) or an extract of *Reynoutria sachalinensis* (Regalia). Powdery mildew (PM- *Podosphaera xanthii*) and gummy stem blight (GSB- *Stagonosporopsis* spp.) were the most prevalent diseases and among those rated for severity throughout the trials. Each disease was differentially affected by treatments, however where chlorothalonil was replaced by Oso and Regalia within a program, PM severity was not significantly different than where chlorothalonil was applied in the same program (both <1% compared with 56% severity in the untreated control in muskmelon, $P < 0.001$). Also, on honeydew melon, the inclusion or replacement of chlorothalonil resulted in statistically similar GSB severities 7% and 5%, respectively, compared to the untreated control at 79% ($P < 0.001$). Therefore, the proper replacement of chlorothalonil, may not reduce the efficacy of fungicide spray programs.

Agronomic and economic evaluation of fungicide seed treatments for soybean production in the mid-Atlantic United States

Andrew Kness, University of Maryland Extension, Forest Hill, MD, USA and Nathan M. Kleczewski, University of Illinois, Urbana, IL, USA

Soybean (*Glycine max*) is a major agricultural commodity in the Mid-Atlantic region. Although seedling pathogens such as *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* occur sporadically in the region, some growers routinely utilize fungicide seed treatments. Reasons for this include perceived plant health benefits and insuring disease management. To address whether commonly used seed treatments provide benefits in the absence of disease, field trials were established in 2017 at four locations (Georgetown, DE; Queenstown, MD; Beltsville, MD; and Keedysville, MD) lacking history of seedling diseases. Treatments included metalaxyl + fluxapyroxad + pyraclostrobin; fluopyram; trifloxystrobin; and an untreated control applied at commercial rates to the variety SS4514NR2 by a local seed dealer. Treatments were replicated five times per site and arranged in a spatially balanced, random complete block design. Emergence 14 days post planting and yield were recorded, and data analyzed using a mixed model. Treatment effects were separated using Fisher's LSD. Seed treatments marginally increased emergence ($P = 0.06$) but did not significantly increase yield and resulted in a 3–14% yield decrease when compared to the controls in Georgetown ($P = 0.04$) and Queenstown ($P = 0.02$). Economic analysis indicated that seed treatments reduced net income by \$337 hectare⁻¹. This study will be repeated in the future to better understand the impacts of seed treatments in disease-free situations.

Impact of two *Fusarium* spp. causing melon rot on *Salmonella enterica* Newport survival and growth on melon rinds

Robert Cheruiyot Korir¹, Kathryn L. Everts¹ and Shirley A. Micallef², (1) University of Maryland College Park, Lower Eastern Shore Research and Education Center, Salisbury, MD, USA, (2) University of Maryland College Park, College Park, MD, USA

Fusarium fruit rot is a common disease in melon production, causing quality losses. Fruit rot may also impact the ability of *S. Newport* to survive and grow on melon fruit. The objective of this study was to evaluate the impact of *Fusarium* spp. on *S. Newport* survival and growth on melon. Eight *Fusarium* isolates were collected in Maryland and molecularly identified as *F. armeniacum*, *F. fujikuroi*, or *F. oxysporum*. To evaluate their impact on *S. Newport* growth, *F. fujikuroi* and two *F. oxysporum* isolates were inoculated separately on melon rind (100 µl of 6 log spore ml⁻¹), or water. The treatments were 1) no *Fusarium* (control), and *Fusarium* with 2) *F. oxysporum*-A, 3) *F. oxysporum*-B, and 4) *F. fujikuroi*. Four days after *Fusarium* inoculation, *S. Newport* adapted for rifampicin resistance was inoculated at 100 µl (~10⁴ CFU ml⁻¹). The treatments were replicated six times ($n = 6$ rinds). *Salmonella* Newport counts in Log CFU ml⁻¹ were analyzed using a mixed model (JMP) and the means were separated using Tukey Kramer HSD. No *S. Newport* was recovered from water or *Fusarium* only treatments. On rinds where *S. Newport* was inoculated, counts were significantly lower for *F. fujikuroi* compared to *F. oxysporum* ($P < 0.03$). This result contradicts our previous trial, where fruit were riper, and *S. Newport* counts were significantly higher following *F. fujikuroi* inoculation, but only on the melon cultivar 'Jaune'. *Fusarium* spp. presence influenced the survival and growth of *S. Newport*.

Plant-parasitic nematodes present in Virginia vineyards

Caroline Lucernoni, Noah Adamo, Jonathan D. Eisenback and Anton Baudoin, Virginia Tech, Blacksburg, VA, USA

Soil samples from six Virginia vineyards representing the largest four of Virginia's five physiogeographic regions were collected at grape budbreak and veraison in 2017 and samples from ten vineyards across three physiogeographic regions were collected during the growing season in 2015. Samples were processed using semi-automatic elutriation and centrifugation, and plant-parasitic nematodes were morphologically identified using the *Identification Guides for the Most Common Genera of Plant-Parasitic Nematodes* (J.D. Eisenback). *Xiphinema* sp., *Hoplolaimus* sp., *Pratylenchus* sp., *Mesocriconema* sp., *Meloidogyne* sp., and *Paratrichodorus* sp. were found in populations large enough to damage grapevine growth. In addition to restricting plant growth, large populations of these nematodes increase the severity of existing vine diseases by contributing to vine stress. *Xiphinema* sp. were found in 35% of sites sampled. *Xiphinema* sp. are especially damaging to vines even in small numbers due to their ability to function as vectors for major grapevine viruses: grapevine fanleaf virus and tomato ringspot virus. High populations of nematodes parasitic to grapes were found in conjunction with large numbers that were probably feeding on groundcover vegetation. Management options are most effective pre-plant but strategies exist for established vineyard nematode control. Established vineyard control focuses on reducing vine stress to increase the vines' tolerance to nematode feeding and minimizing nematode spread in the vineyard.

Getting the upper hand on bitter rot of apples: Methods for understanding bitter rot epidemiology

Phillip Martin¹, Teresa Krawczyk², Brian L. Lehman² and Kari A. Peter^{1,2}, (1) Penn State University, University Park, PA, USA, (2) Penn State Fruit Research and Extension Center, Biglerville, PA, USA

Bitter rot of apples caused by fungi in *Colletotrichum* spp. complexes was primarily a southern disease, but has become an increasing problem in the mid-Atlantic and northeastern US. Several causes for this increase have been hypothesized, including increased tolerance of widely used fungicides and fungicide use restrictions, planting of susceptible apple cultivars, climate change, and changes in orchard management. In this research project, *Colletotrichum* spp. from across PA were identified to species based on partial sequences of the beta-tubulin, GAPDH, and calmodulin genes. Isolates were tested for tolerance to commonly used fungicides using mycelial growth assays. Culturing based methods were used to search for *Colletotrichum* in the orchard environment to elucidate overwintering sites and spore dispersal periods. Freezing was used to detect quiescent infections on immature apples. *Colletotrichum fioriniae* and *C. fructicola* were the dominant species identified and increased tolerance to trifloxystrobin fungicide was observed. *Colletotrichum* does not appear to overwinter in apple buds and culturing proved ineffective in detecting *Colletotrichum* in the environment. Freezing detected *Colletotrichum* in immature apples by early July, even though no natural rot was seen until mid-August. Culturing and PCR based methods of detecting *Colletotrichum* spp. in the orchard environment and in quiescent infections are compared and discussed in terms of bitter rot epidemiology and future research objectives.

Disruption of the genome of Rice Blast to identify genes involved in production of Reactive Oxygen Species

Danielle Mikolajewski, Jessica Pancake and Nicole M. Donofrio, University of Delaware, Newark, DE, USA

Rice Blast caused by the fungus *Magnaporthe oryzae*, causes severe losses to crops worldwide. It's a virulent pathogen, which can cause complete devastation when conditions are favorable. To better understand how to take advantage of basal immunity, a mechanism by which plants can protect themselves even against virulent pathogens, we are performing forward genetic screens with rice and *M. oryzae*. Previous work in our lab used a live sensor gene called Hyper-sensor (HS) to measure reactive oxygen species (ROS) in a well-characterized strain of *M. oryzae* 4091. The HS gene gives 4091 the ability to fluoresce when in the presence of ROS, thus providing a quantifiable measurement of ROS. We are in the process of randomly disrupting the genome of 4091-HS by transformation with a DNA insert constructed with a gene for resistance to the drug Hygromycin (Hyg). This construct has two degenerate ends so that it will generate random insertions in the genome, disrupting as many genes as possible including genes that play a role in ROS production. Our

screen could potentially identify these genes directly linked to basal immunity. Through screening the randomly mutagenized library of 4091-HS, we will be able to identify changes in ROS production using confocal microscopy. Currently we have 700+ transformed candidates, 400 being characterized. Results will be presented on our screening strategy and some preliminary data from a similar screen on a previously mutagenized population.

Comparison of metabolite levels between healthy *Prunus persicae* trees and Plum Pox infected trees after vernalization

Diana Sherman¹, William Schneider¹, Christopher M. Wallis² and Elizabeth E. Rogers¹, (1) USDA ARS FDWSRU, Fort Detrick, MD, USA, (2) USDA ARS, Parlier, CA, USA

Previous research suggests potyvirus infection alters host metabolic levels. These adjustments may in turn influence vector feeding behaviors as changes in host quality occur with changes in plant compound levels. For example, plant-feeding insects use volatile compounds (terpenoids) as feeding cues. Likewise, phenolics, sugars, and amino acids may impact feeding duration and quality. In this study, we compare the levels of terpenoids, soluble phenolics, sugars and amino acids between healthy and Plum Pox virus (PPV) infected *Prunus persicae* (peach) trees 30, 60, 90 and 120 days post vernalization. Additionally, aphid feeding behavior and preference for healthy versus infected leaves were monitored using *Myzus persicae*, a known PPV vector. Results from this initial study show some significant metabolite differences. However, aphid behavior and preference assessment were inconclusive, further investigation is needed.

Infection of germinating seeds of *Impatiens walleriana* by oospores of *Plasmopara obducens*, the *Impatiens* downy mildew

Nina Shishkoff, USDA ARS FDWSRU, Frederick, MD, USA, **Emily Smallwood**, USDA ARS FDWSRU, Fort Detrick, MD, USA and Farivar Eskandari, FDWSRU/ARS/USDA, Frederick, MD, USA

Impatiens downy mildew is a pathogen that kills bedding *impatiens* (*Impatiens walleriana*) and is known to infect plants by means of its sporangia; the role of the oospore stage is less clear. In these studies, seeds (*I. walleriana* 'Baby White') were pre-germinated for 4 days then planted in Turface[®] soilless media and covered with sterile water and stem tissue containing oospores (cold-conditioned at 0 C for 1 month). Controls were treated with sterile water alone. Inoculated and control plants were placed in separate trays and covered with clear plastic humidity domes for 3 days, then incubated under greenhouse conditions (22 C) for one month. At this time, plants had 4–6 true leaves and showed no symptoms of downy mildew except stunting and chlorosis. To examine plants for presence of the pathogen, they were placed in petri dishes containing moist filter paper in a growth chamber (20 C) for 24 hrs, and then examined for sporulation. Any non-sporulating seedling were left in a moist chamber for an additional 16 days, after which final results were recorded. There were three trials of 36–48 inoculated seedlings and 8 controls in each trial. Over all, sporulation was recorded for 53 out of 126 inoculated seedlings (43%) and for none of the controls. We observed necrosis and browning inside the roots, stems, and petioles. Typical sporangiophores and sporangia were observed on some parts of the inoculated symptomatic plants. The results showed that zoospores released from these oospores are able to infect germinating seeds and later under the right environmental conditions produce sporangiospores on foliage.

Efficacy of *R. vitis* ARK-1 as a biological control agent for *Rhizobium vitis* isolates causing crown gall in Virginia vineyards

Alexander Thomas Wong, Virginia Tech, Winchester, VA, USA and Mizuho Nita, Virginia Polytechnic Institute and State University, Winchester, VA, USA

Crown gall of grapevine, *Vitis* spp., is one of the most economically important diseases affecting wine grape production around the world. The bacterial pathogen *Rhizobium vitis* (= *Agrobacterium vitis*) causes gall formation which can result in vine death due to girdling of the vascular system. Replanting diseased vines is the standard management recommendation for crown gall, but is unsustainable due to high cost. Also, the market preference for wine grapes leads to planting *V. vinifera* cultivars, which are more susceptible to crown gall. Recently, the use of a novel non-tumorigenic *R. vitis* strain (ARK-1) reduced gall formation by 90% in grapevine in Japan. To test the efficacy of ARK-1 as a biological control against US *R. vitis* isolates, we conducted studies in which ARK-1 and a Virginia *R. vitis* isolate were co-inoculated (1:1 cell ratio suspension) into artificial wounds in stems of three year old *V. vinifera*, Cabernet Sauvignon. Four *R. vitis* isolates of different geographic origins were tested. The effect of isolate, treatment (ARK-1 vs. water), and their interaction on gall incidence and size was analyzed using the generalized linear mixed model (SAS ver. 9.4, PROC GLIMMIX). A significant isolate by treatment interaction ($P \leq 0.05$) was observed and co-inoculation with ARK-1 resulted in reductions in both gall incidence (average of 90%) and size (1 cm average). Also, there were significant differences in gall incidence and size among *R. vitis* isolates ($P \leq 0.05$). To our knowledge, this is the first study to demonstrate the efficacy of ARK-1 against US (VA) native *R. vitis* isolates when inoculated into the woody tissue of a wine grape cultivar.

Signature sequences of *Phytophthora* species

Xiao Yang¹ and Chuanxue Hong², (1) Virginia Tech, Virginia Beach, VA, USA, (2) Hampton Roads Agricultural Research and Extension Center, Virginia Tech, Virginia Beach, VA, USA

DNA sequencing has become a standard procedure for identifying *Phytophthora* cultures in both research labs and plant disease clinics. The culture identity determined using this procedure is as good as the quality of reference sequences. To this goal, we sequenced 328

isolates representing 142 described species and 43 provisionally named taxa of *Phytophthora* including 114 ex-types and 164 authentic isolates on seven genetic markers. These included 60S Ribosomal protein L10, beta-tubulin, elongation factor 1 alpha, enolase, heat shock protein 90, 28S ribosomal DNA, and *tigA* gene fusion protein. Here we present how these signature sequences were deposited in the GenBank to help first responders, diagnosticians and researchers to find and use this valuable resource, and identify *Phytophthora* cultures with confidence. We also discuss how the resolution of different genetic markers may vary with *Phytophthora* clade to help select the best one in lieu with the internal transcribed spacers to identify *Phytophthora* cultures of interest to species level in the most timely and economic fashion possible. With your support, we together can reduce the portion of erroneous reference sequences currently in public repositories and make this standard procedure a reliable tool for identifying *Phytophthora* species.

Effects of growth media and temperature on conidial production by the boxwood blight pathogen, *Calonectria pseudonaviculata*

Shu Yang and Chuanxue Hong, Hampton Roads Agricultural Research and Extension Center, Virginia Tech, Virginia Beach, VA, USA

Boxwood blight caused by *Calonectria pseudonaviculata* (*Cps*) is an emerging disease of great concern to the ornamental horticulture industries and the public. The objective of this study was to evaluate culture media and conidial induction temperatures in order to further improve a lab protocol based on potato dextrose broth (PDB) for conidial production, which is an important step in investigations into *Cps* biology, disease epidemiology and management. Specifically, this study assessed the effects of four different types of fresh potato with and without carrot addition, and three different induction temperatures. Two *Cps* isolates 11D05 and 12A02 recovered from boxwood and sweet box respectively were used in this study. Overall, *Cps* produced the most conidia (3.1×10^7 per dish) on media made of the Green Giant White potato in the absence of carrot. *Cps* produced significantly more conidia on media prepared with the Yukon Gold potato with carrot than without carrot (3.1×10^7 per dish vs. 1.1×10^7 per dish). Carrot addition also improved mycelial strength and attachment, adding ease to broth culture washing and conidia harvesting steps. Among three temperatures of 18, 20 and 23°C evaluated, most conidia were produced at 20°C. These results indicate that the performance of the PDB protocol can be further improved by using media made of the Yukon Gold potato with carrot, and by placing culture dishes at 20°C during the conidial induction stage.