2021 Potomac Division Meeting Abstracts

Abstracts presented at the APS Potomac Division and Northeastern Division joint meeting held virtually, March 10–12, 2021. The recommended format for citing division meeting abstracts, using the first abstract below as an example, is as follows:

Collum, T., Grando, M., Stone, A. L., Sherman, D., Culver, J. N., Dardick, C. D., Stover, E., and Rogers, E. E. 2021. Characterization of phloem specific host responses to *Candidatus Liberibacter asiaticus* in tolerant and susceptible citrus cultivars. (Abstr.) *Phytopathology* 111:S1.16. <https://doi.org/10.1094/PHYTO-111-9-S1.16>

The abstracts are published as a supplement to *Phytopathology* for citation purposes. They were not reviewed by the *Phytopathology* Editorial Board and were not edited by the APS editorial staff.

<https://doi.org/10.1094/PHYTO-111-9-S1.16>

© 2021 The American Phytopathological Society

Characterization of phloem specific host responses to *Candidatus Liberibacter asiaticus* in tolerant and susceptible citrus cultivars

T. COLLUM (1), M. Grando (2), A. L. Stone (1), D. Sherman (1), J. N. Culver (3), C. D. Dardick (4), E. Stover (2), E. E. Rogers (1), (1) USDA, Agricultural Research Service, Foreign Disease-Weed Science Research Unit, Frederick, MD, USA; (2) USDA, Agricultural Research Service, U.S. Horticultural Research Laboratory, Fort Pierce, FL, USA; (3) University of Maryland, College Park, MD, USA; (4) USDA, Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, WV, USA

Huanglongbing (HLB) is a devastating disease of citrus worldwide. HLB has been associated with the bacterium *Candidatus Liberibacter asiaticus* (CLas). CLas is a phloem-limited pathogen that is spread by Asian citrus psyllids. Within the phloem, CLas manipulates host gene regulation to facilitate its life cycle. However, accurately characterizing host phloem responses has been challenging due to the technical difficulty of isolating this tissue. To address this issue, we have adapted translating ribosome affinity purification (TRAP) methodologies for use in citrus trees. TRAP uses tissue-specific promoters to express FLAG-tagged ribosomal proteins for immuno-capture of cell-specific mRNAs. In this study, HLB-susceptible sweet orange, HLB-resistant Poncirus, and HLB-tolerant Carrizo (sweet orange × Poncirus) were transformed to express the FLAG-tagged ribosome protein L18 (RPL18) under the control of two phloem-specific promoters (pSULTR2;2 and p396ss), as well as the constitutive CaMV 35S promoter. Tagged ribosome complexes were purified from citrus leaves using anti-FLAG conjugated magnetic beads. High-quality translatome RNA was recovered from citrus expressing FLAG-RPL18 but not from non-transformed control plants. Efforts are underway to identify phloem genes that are altered during infection. Identified genes will represent unique phloem specific targets for disrupting the CLas life cycle.

Management of the root-associated oomycetes, *Pythium myriotylum* and *Phytophthora nicotianae*, in burley tobacco production

R. Pearce (1), W. Barlow (1), E. E. PFEUFER (2), (1) University of Kentucky, Lexington, KY, USA; (2) USDA ARS FDWSRU, Frederick, MD, USA

Several *Pythium* spp. cause Pythium root rot (PRR), the most common disease in transplant production of tobacco (*Nicotiana tabacum*). To understand the impact of transplant PRR on tobacco establishment and survival, six experiments were undertaken over five seasons. Transplants were started in segregated float trays, then a single *Pythium myriotylum* isolate was inoculated into water of select trays. Two labeled treatments, Terramaster and Oxidate 2.0, and one unlabeled fungicide, Ridomil Gold SL, were utilized singly in float water to manage PRR. An ordinal 0–5 scale was used to rate root ball quality (RBQ) prior to transplant. In the greenhouse-to-field trials, four fields had no soilborne disease history, while two were black shank “nurseries,” with established *Phytophthora nicotianae* pressure. Nonparametric tests indicated Terramaster and Ridomil improved median RBQ in inoculated plants. Using mixed models with pooled data, average transplant RBQ positively associated with plant establishment and survival in *P. nicotianae*-free fields. Ridomil use in at-transplant water was positively associated with establishment and survival in fields with *P. nicotianae*; RBQ had little effect. These results associate PRR’s negative influence on field tobacco populations and deemphasize at-transplant fungicide treatments in fields

with *P. nicotianae*, which causes black shank. These proactive approaches can manage PRR and black shank, influential tobacco diseases caused by oomycetes.

Survey for maize viruses in the United States

L. R. STEWART (1), M. Willman (2), K. Willie (2), (1) USDA-ARS, Foreign Disease-Weed Science Research Unit, Frederick, MD, USA; (2) USDA-ARS Corn, Soybean and Wheat Quality Research Unit, Wooster, OH, USA

Viruses are among pathogens that can impact production of maize (*Zea mays* L.) in the United States and globally. In 2018, maize samples were selected and collected from various states in the U.S. to assess viruses by RNA sequencing. Maize samples were selected from sweet corn due to its greater virus susceptibility than observed for field corn. Samples from sweet corn plants, weedy monocots within or adjacent to maize fields, or both were collected from Ohio, Indiana, Iowa, Illinois, Kentucky, Tennessee, Pennsylvania, Nebraska, New Hampshire, Louisiana, and Washington states, and RNA was Illumina sequenced and analyzed using an in-house bioinformatics pipeline for virus sequence identification. Sequences with top hits to Maize dwarf mosaic virus (MDMV), Foxtail mosaic virus (FoMV), and High Plains wheat mosaic virus (HPWMoV) were each identified in multiple sweet corn samples. Sequences matching Brome mosaic virus (BMV) and Maize chlorotic dwarf virus (MCDV) were identified in weedy monocots but not in the sampled maize. Distribution and sequence comparison of the identified virus sequences provides information on variability and distribution of maize viruses in the United States, although sampling was not extensive enough to indicate the absence of any virus by lack of detection.

Sorting through the boxwood blights

N. Shishkoff (1), M. L. Daughtrey (2), E. SMALLWOOD (3), (1) USDA ARS FDWSRU, Frederick, MD, USA; (2) Cornell University, Long Island, NY, USA; (3) USDA ARS FDWSRU, Fort Detrick, MD, USA

For years U.S. growers of boxwood have been familiar with Volutella blight (VB), a poorly understood disease. The interest in a newer disease, boxwood blight, caused by two *Calonectria* species, has renewed interest in VB. Volutella blight has been known since 1815, when it was ascribed to *Tubercularia buxi*. VB is caused by two pathogens, neither of which is now in the genus *Volutella*; instead, *Pseudonectria buxi* and *P. foliicola* have been reported as the causal agents. Both are endophytes that can cause die-back of boxwood branches and death of leaves, which turn straw colored and typically do not fall off the plant. Both fungi are considered to infect wounded or stressed plants. Boxwood leaves and twigs affected by VB, if incubated under moist conditions, will develop salmon-colored sporodochia that dot the tissue as pink viscous cushions of spores (setose for *P. buxi* and non-setose for *P. foliicola*). If incubated longer, setose perithecia may develop for *P. buxi* infections; the perithecia are greenish when young and brownish when mature. Perithecia for *P. foliicola*, which was named only recently, in 2015, have not yet been observed. Occasionally perithecia of *P. coronata* are also seen on leaves colonized by *P. foliicola* or *P. buxi*. Learning to distinguish among these comingled fungal structures and better understanding of the ecological role and interactions of the fungi on boxwood will help us to improve the management of boxwood blights.

Comparison of metabolite levels between healthy *Prunus persicae* trees and trees inoculated with *Plum pox virus* or exposed to *Myzus persicae* aphids

D. SHERMAN (1), C. M. Wallis (2), W. Schneider (3), L. R. Stewart (4), E. E. Rogers (1), (1) USDA, Agricultural Research Service, Foreign Disease-Weed Science Research Unit, Frederick, MD, USA; (2) USDA ARS, Parlier, CA, USA; (3) FIK9, Yalaha, FL, USA; (4) USDA-ARS, Foreign Disease-Weed Science Research Unit, Frederick, MD, 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, phenolics, sugars, and amino acids may impact feeding duration and quality. In this study, we compared the levels of soluble phenolics between healthy (no insect feeding), mock-inoculated (healthy *Myzus persicae* feeding), and *M. persicae*-transmitted *Plum pox virus* (PPV)-infected *Prunus persicae* (peach) trees at 30, 60, 90, and 120 days post-inoculation or treatment. Additionally, aphid feeding behavior and preference for healthy versus infected leaves were monitored using *M. persicae*, a known PPV vector. Results from this initial study showed some significant phenolic differences between treatments and time points. However, aphid behavior and preference assessments were inconclusive and further investigation is needed.

The effect of *Trichoderma koningiopsis* and *Calonectria* spp. on survival of *Pseudonectria buxi* in boxwood leaf debris incubated at 0, 10, or 20°C

N. SHISHKOFF (1), E. Smallwood (2), M. Camp (3), (1) USDA ARS FDWSRU, Frederick, MD, USA; (2) USDA ARS FDWSRU, Fort Detrick, MD, USA; (3) USDA, Beltsville, MD, USA

To explore a biological control-based management option, an experiment was conducted to determine the effect of a *Trichoderma koningiopsis* drench over boxwood blight-infected leaves. Treated and untreated infected and uninfected leaves were placed as a litter layer over sand and incubated at 0, 10, or 20°C and sampled monthly over 6 months. Because *Pseudonectria buxi* (a poorly understood endophyte of boxwood) was always present to some degree in boxwood foliage, the experiment also accidentally determined the effect

of co-infection with *Calonectria* spp., of incubation temperature, and of the presence of *Trichoderma* on survival of *P. buxi*. Analysis of variance indicated that temperature strongly influenced sporulation of *P. buxi* ($P = 0.0009$), with sporulation increasing over time with incubation at 0°C. *Trichoderma* had little effect on sporulation of *Pseudonectria*, but the presence of *Calonectria* significantly reduced the number of leaves with visible sporulation of *Pseudonectria*. These results confirm the suspicion that *Pseudonectria* is a cool-weather pathogen and suggests that it cannot out-compete *Calonectria* in leaf litter. These implications may affect end-user prioritization of management factors associated with the two pathogen genera based on prevailing local climate conditions.

Lima bean leaf shape and plant architecture effects on the severity of downy mildew caused by *Phytophthora phaseoli*

A. GINN (1), E. Ernest (2), N. F. Gregory (1), A. Koehler (2), N. M. Donofrio (1), T. A. Evans (1), (1) University of Delaware, Newark, DE, USA; (2) University of Delaware, Georgetown, DE, USA

Lima bean has been a valuable part of Delaware's economy for nearly a century. Unfortunately, the oomycete pathogen, *Phytophthora phaseoli*, thrives in the same climactic conditions present during lima bean maturation and harvest. Most commercial lima bean cultivars have sprawling growth patterns with ovate leaf shapes. These phenotypes make lima bean highly susceptible to *P. phaseoli* via splash inoculations and elevated canopy humidity. Six pairs of lima bean near isogenic lines with lanceolate and ovate leaf shapes, along with five inbred experimental varieties with upright architectures were planted in the early July of 2019 and 2020 to assess how these phenotypes affected disease severity. Two rounds of *P. phaseoli* inoculum were applied in mid-September, with visual ratings and pod acquisition for qPCR analysis happening 3 weeks post-inoculation. Plants were harvested in early October at the succulent seed stage. Canopy climate data were simultaneously collected in a separate control field. Lanceolate leaf shapes showed significantly lower disease severity over both years when compared to their ovate peers, while the architecture cultivars showed no significant difference between years. No significant differences were observed in yield data between phenotypes. Canopy humidity was significantly lower in lanceolate lines. Pod samples are currently being tested via qPCR to determine pathogen amount per plant. The data will be used to inform future breeding.

Coinfection of satellite and defective RNA of *Bamboo mosaic potexvirus* in nature

T. Yeh, G. P. CONTRERAS, Agricultural Biotechnology Laboratory, Auxergen Inc., Baltimore, MD, USA

Bamboo mosaic potexvirus (BaMV-Yoshi) was isolated from Beechey bamboo (*Bambusa beecheyana*) showing mosaic symptoms in San Marcos, CA, USA. Genomic nucleotide sequences of BaMV-Yoshi share 79.0–80.3% identity with other known BaMV isolates. Phylogenetic analysis revealed that BaMV-Yoshi diverged from a common ancestor earlier than all Asian isolates and became a distinct group by itself. BaMV satellite (satBaMV-Y) RNAs show 93.2–95.1% identity to other isolates and contain noncanonical polyadenylation signal AAUAAU or AAGAAU instead of conserved AAUAAA. Northern blotting using BaMV 5' untranslated region-specific riboprobe also detected 1.2- and 0.9-kb RNA species in virions, indicating that they are encapsulated defective RNAs (D RNAs). Two BaMV D RNAs (Y1 and Y2) consist of 1,162 and 845 nucleotides in length with a single internal deletion of the helper virus genome. However, their RNA recombination sites (Y1, 525/5,730; Y2, 185/5,709) are different from D RNAs generated by serial passages of BaMV-S in tobacco. To our knowledge, this is the first report of full-length BaMV genomic sequences outside Asia, and new recombination hotspots of BaMV D RNAs occurring in the bamboo host. This is also the first coinfection of two BaMV subviral RNAs identified in nature. Contribution of mixed infection of satBaMV and D RNAs to replication competence and evolution of BaMV warrants further investigation.

Can we expand the spray window to control FHB in wheat using a new fungicide Miravis-Ace?

L. SINGH, J. White, L. Thorne, J. Crank, N. Rawat, University of Maryland, College Park, MD, USA

Fusarium head blight (FHB) is a devastating disease of wheat and barley that not only lowers the crop yield, but also impairs grain quality with associated mycotoxins such as deoxynivalenol (DON). Chemical control options for FHB and DON include the application of triazole fungicides at anthesis stage. This presents practical challenges for growers in managing FHB, as the appropriate timing window typically lasts only 3–4 day. This study was conducted over two years (2019 and 2020) to test the efficacy of a new fungicide from Syngenta labeled as Miravis-Ace (pydiflumetofen + propiconazole) at 50% head emergence (Feeke's 10.3), anthesis (Feeke's 10.5.1) and end of flowering (Feeke's 10.5.3) stages in controlling FHB and DON content. Prosaro 421 SC, a standard FHB control fungicide, was used for comparison. Miravis-Ace application at 50% head emergence provided significant control over the non-treated check for FHB incidence (2020), FHB severity (2019), and DON content (2019) with control efficacies as high as 51%, 69% and 52%, respectively. However, with the highest mean control percentages relative to check, anthesis was the most effective application stage to control FHB and DON.

Monitoring season long impacts of early-season *Pythium* infection in Delaware corn

J. T. BICKEL, A. Koehler, University of Delaware, Georgetown, DE, USA

Corn (*Zea mays* L.) is the most important grain crop produced in Delaware. Widespread use of no-till agriculture and wet spring planting conditions have favored *Pythium* infection in recent years. In addition to losses from early season death, infected plants that survive through the season will produce smaller ears with fewer kernels. With no previous work studying interactions of *Pythium*

species in the Mid-Atlantic, 60 1/1,000 acre segments across three corn fields with a history of *Pythium* were marked and tracked throughout the growing season. Plants displaying symptoms of *Pythium* infection were flagged at V2-V3 and paired with an asymptomatic neighbor. At R1, stalk biometrics were collected on nine paired sets where *Pythium*-infected plants had lower force/deflection slopes than the asymptomatic controls in all pairs ($P < 0.00001$). These same sets were evaluated at R5 for biometrics and destructively sampled to measure first node diameter, root mass, and kernel set. Stalk circumference measurements were significantly larger ($P < 0.0001$) in the control (77.6 mm) than *Pythium*-infected plants (53.6 mm). Root mass ($P = 0.0013$) and average kernel estimates ($P = 0.0002$) were larger in the control (236.6 g, 587) than infected plants (89.8 g, 231). A total of seven different species of *Pythium* were isolated from corn tissue in the fields tested: *P. attrantheridium*, *P. catenulatum*, *P. inflatum*, *P. graminicola*, *P. perillium*, *P. tardicrescens*, and *P. torulosum*.

Investigating late-season rots: A challenge for wine grape production in the Mid-Atlantic

S. D. COSSEBOOM, University of Maryland, College Park, MD, USA

Mid-Atlantic vineyards are threatened by late-season fruit rots annually, and many species of fungi have been presumably associated. Samples of rotten grapes were collected from the Mid-Atlantic, and 265 isolates were collected and classified into 15 genera. Three understudied fungi in these genera, *Alternaria alternata*, *Aspergillus uvarum*, and *Neopestalotiopsis rosae* were evaluated for pathogenicity over two seasons in a vineyard. The effect of wounding, inoculation timing, and cultivar on pathogenicity of these fungi were assessed. *Aspergillus uvarum* was found to infect wounded and non-wounded clusters, causing significantly higher disease severity than the control. *Alternaria alternata* and *N. rosae* were able to consistently cause significant disease severity on wounded clusters. The inoculation timing of veraison and pre-harvest with *Aspergillus uvarum* resulted in higher *Aspergillus* fruit rot severity than bloom timing. The sensitivity of five *Aspergillus uvarum* and five *N. rosae* isolates to three fungicides was assessed. *Aspergillus uvarum* isolates were found to vary in sensitivity to azoxystrobin, while being sensitive to boscalid and difenoconazole. *N. rosae* isolates were resistant to azoxystrobin and boscalid, while sensitive to difenoconazole. In conclusion, *Aspergillus uvarum* was found to be a prevalent and understudied fungus associated with late-season rots in the Mid-Atlantic and was demonstrated to be a primary pathogen that may have developed resistance to the commonly applied fungicide azoxystrobin.

Performance of several bio-rational compounds in managing Verticillium wilt on eggplant

M. RAHMAN, West Virginia University, Morgantown, WV, USA

Vascular wilt disease caused by soilborne fungal pathogen *Verticillium dahliae* is one of the most devastating diseases of eggplant that can drastically reduce yield. Small growers who do not have enough acreage to rotate or leave the infested area fallow for a few years, in most cases attempt to grow crop like eggplants despite potential risks posed by the soilborne pathogen. In addition, backyard and organic growers prefer organically acceptable products, especially those that can be applied by themselves compared to synthetic fumigants that would need custom applicators. This study was conducted to evaluate the efficacy of several bio-rational compounds for management of Verticillium wilt of eggplant with six different treatments such as i) non-treated check; ii) anaerobic soil disinfestation with 50:50 mix of Biofence (*Brassica juncea*) and *Sinapis alba* mustard meal pellets; iii) seedlings grown on Terragrow treated pasteurized planting mix + Terragrow applied in planting holes; iv) seedlings grown on RootShield treated planting mix; v) seedlings grown on Terragrow-treated regular organic planting mix + Terragrow applied in planting holes; and vi) mustard cover crop (MCC). Sixty days after planting, plants in all treatments except MCC showed enhanced vigor and height compared to non-treated check. Consistent with plant vigor and height, significantly higher ($P < 0.02$) total yield/plant was obtained from all treatments except MCC compared with non-treated control.

Identification and molecular characterization of a new environmental Iflavirus member using high-throughput sequencing of apple leaves

L. CARVALHO COSTA (1), K. Stevens (2), X. Hu (1), B. Atha III (1), R. Slocum (1), M. O'Connell (1), M. Al Rwahnih (3), A. Diaz-Lara (4), J. A. Foster (1), O. P. Hurtado-Gonzales (1), (1) USDA-APHIS Plant Germplasm Quarantine Program, Beltsville, MD, USA; (2) Department of Evolution and Ecology, University of California-Davis, Davis, CA, USA; (3) University of California-Davis, Davis, CA, USA; (4) Tecnológico de Monterrey, School of Engineering and Sciences, Queretaro, CA, MEXICO

In the past decade, high-throughput sequencing (HTS) has had a major role in virus diagnosis as well as in the characterization of the virome of different species. In the present study, a novel viral species possibly associated with an eriophyid mite species was determined in HTS analysis from total RNA extractions of apple leaves. The genome sequence was fully completed using rapid amplification of cDNA ends followed by sanger sequencing, resulting in a genome of 8885 nucleotides in length. Phylogenetic analysis suggested that the novel virus belongs to the genus *Iflavirus*, and it was most closely related with the unclassified *Iflavirus* "Tomato matilda associated virus" (TMAV). Nonetheless, the new virus shows enough differences to be considered as a new species, sharing a maximum sequence identity of 58% in replicase with TMAV, and it was tentatively named "Eriophyid mites iflavirus" (EMIV). Although this novel virus was identified from total RNA extractions of apple leaves, the detection of this virus was not consistent in different extractions of the same apple accessions in different seasons or years, which indicated that it might not be a plant virus. The high number of reads from the mite *Tetra pinnatifidae* in all EMIV-positive samples suggest this insect as the most likely host of the novel described virus. Indeed, iflaviruses are known to infect insects. This study highlights the importance of a careful biological study before assigning a new virus to a host.

Insights from the past: Trends in microbial-based weed biological control research

M. FULCHER (1), M. Tancos (2), (1) USDA-ARS, Frederick, MD, USA; (2) USDA ARS Foreign Disease Weed Science Research Unit, Fort Detrick, MD, USA

Research on the use of plant pathogens as weed biocontrol agents has grown exponentially over the last 20 years, making comprehensive literature reviews challenging. Summarizing and synthesizing this body of research is further complicated by the diverse range of pathosystems under study, including hosts from 23 plant orders and over 180 pathogen species. Text analysis can augment traditional literature reviews by condensing information from large document collections, but these methods have not been applied widely in the fields of plant pathology or weed management. With 748 abstracts on the use of plant pathogens for weed biocontrol, we compared the performance of automatic and manual abstract categorization, described long-term trends in the field, and identified opportunities for future research. Automatic and manual annotations matched in 86% of cases. Only 18% of noxious weeds in the US appeared in the literature, and just three host species were the subject of more than 10 published studies. The weed biocontrol potential of bacterial and viral plant pathogens is virtually unexplored, and three of the largest orders of invasive plants (Poales, Fabales, Lamiales) remain understudied. Automated text analysis was an effective way to summarize decades of pathogen-based biocontrol research and could be applied to the review of other sub-disciplines.

Serological and recombinase polymerase amplification-based assays to diagnose boxwood blight

X. YANG, M. B. McMahon, M. Carter, D. G. Luster, Foreign Disease-Weed Science Research Unit, USDA, ARS, Fort Detrick, MD, USA

Boxwood blight is a global disease of *Buxus* spp. that has no known cure. Early detection of its causal pathogens, *Calonectria henricotiae* (*Che*) and *C. pseudonaviculata* (*Cps*), is critical to conduct prompt disease containment measures. Traditional diagnosis of boxwood blight relies on disease symptoms and pathogen identification based on morphology and PCR-based technologies. However, symptoms can be obscured during non-growing season, while molecular identification can be time-dependent. There is an urgent need for on-site diagnostic assays. In this study, two lines of on-site assays for detecting *Che* and *Cps* are under development. First, polyclonal antibodies were raised against two partial amino acid sequences derived from a high-abundance protein unique to *Che* and *Cps*. They were found to react with proteins extracted from *Buxus* leaves infected with *Che* and *Cps*, but not to those infected with *Pseudonectria buxi*, another boxwood fungal pathogen, or healthy leaves. Second, five sets of primers and probes were designed for recombinase polymerase amplification (RPA) targeting a P.38 putative gene unique to *Che* and *Cps*. In a preliminary test, four out of five sets amplified their respective DNA targets in a 30-min RPA assay without producing any false positive detections against non-targets. Additional evaluations are underway to determine the specificity, sensitivity, and field performance of these two assays.