2019 Pacific Division Meeting Abstracts

Abstracts presented at the APS Pacific Division meeting in Fort Collins, Colorado, June 25–27, 2019. The recommended format for citing division meeting abstracts, using the first abstract below as an example, is as follows:

Borland, T., Huerta, A. I., Triplett, L. R., and Leach, J. E. 2019. Generation of *Xanthomonas oryzae* PthXo1 mutants to gain insight into TALE-dependent Xo1-mediated resistance in rice. (Abstr.) *Phytopathology* 109:S3.1. <https://doi.org/10.1094/PHYTO-109-11-S3.1>

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-109-11-S3.1>

© 2019 The American Phytopathological Society

Generation of *Xanthomonas oryzae* PthXo1 mutants to gain insight into TALE-dependent Xo1-mediated resistance in rice

T. BORLAND (1), A. I. Huerta (1), L. R. Triplett (2), J. E. Leach (1), (1) Colorado State University, Fort Collins, CO, USA; (2) Connecticut Agricultural Experiment Station, New Haven, CT, USA

Xanthomonas oryzae pv. *oryzae*, the causal agent of bacterial blight of rice, produces Transcription Activator-Like Effectors (TALEs) that activate target gene expression in the plant by binding to gene promoter regions. To circumvent pathogen infection, plants have evolved TALE-associated resistance, including loss of susceptibility, dominant TALE-dependent, and dominant transcription-independent resistance. The recently described *Xo1* gene from rice is included the latter category. Little is known about this type of resistance. To understand the mechanism of *Xo1*, which is independent of gene activation, we created mutations in the surface-exposed amino acids of PthXo1, a TALE which induces resistance in a rice variety carrying *Xo1*, Carolina Gold. The wild-type alleles triggered resistance when inoculated to Carolina Gold, while the variants lost resistance triggering ability. Our results suggest that the surface exposed amino acids of PthXo1 are critical for triggering *Xo1*-mediated resistance and give a deeper insight into the mechanism governing non-transcriptional resistance in rice.

New occurrence of *Lophodermella* sp. associated with needle cast in limber pine (*Pinus flexilis*) in Colorado, USA

J. ATA (1), K. Burns (2), S. Marchetti (3), J. J. Worrall (3), J. Stewart (1), (1) Colorado State University, Fort Collins, CO, USA; (2) USDA Forest Service, Golden, CO, USA; (3) USDA Forest Service, Gunnison, CO, USA

A potential new species of *Lophodermella* sp. has been recorded to occur in limber pine (*Pinus flexilis*) showing needle cast symptoms in Rocky Mountain National Park, Colorado, USA. Symptoms of infected limber pine needles include discoloration of needles with dark brown coalesced longitudinal ridges. Morphological characterization of the potential pathogen showed significant similarities to species in genus *Lophodermella* causing needle cast in the Rocky Mountain Region. The species had subhypodermal hysterothecia, tanned hypodermal cells, and colorless clavate ascospores. Measurements of microscopic features show significant similarity to *Lophodermella arcuata*. Fungal DNA for genetic analyses was extracted through modified a CTAB method. Molecular characterization and phylogenetic analyses were conducted using three gene loci: internal transcribed spacer, translation elongation factor 1- α , and large ribosomal subunit. Results revealed a distinct species under Rhystimataceae that is genetically different than *Lophodermella arcuata*, *L. montivaga* and *L. concolor*. This is a first report on *Lophodermella* sp. infecting limber pine in Colorado apart from the known *L. arcuata*. This information will help provide additional insights on needle pathogen behavior and ecology causing potential emergence of new diseases.

Apple rootstock genotype influences endophyte and rhizosphere microbial community

C. VAN HORN (1), M. Mazzola (2), (1) USDA-ARS, Wenatchee, WA, USA; (2) USDA Agricultural Research Service, Wenatchee, WA, USA

Apple replant disease (ARD) negatively impacts tree health and productivity in new orchard plantings. Use of disease tolerant rootstocks can diminish growth limiting effects of ARD, however the effect of rootstock genotype on modulating the endophytic and rhizosphere microbiome enabling ARD tolerance is not understood. In this study a series of tolerant and susceptible rootstock cultivars were consecutively planted into orchard replant soil containing a known pathogen complex. Growth measurements, rhizosphere soil and root samples were taken over time. Amplicon sequencing was used to determine simultaneously a broad taxonomic range of organisms and their abundances. T-RFLP data indicated that rootstocks G.41 and G.890 recruit dissimilar rhizosphere bacterial communities from each other and the M.9 and M.26 rootstocks. Endophytic and rhizosphere microbial communities differed for all rootstocks. Several genera known to form ectomycorrhizal associations with other plants were identified as apple root endophytes for the first time in this study. Furthermore, VAM fungal genera represented >20% of OTUs in G.890, G.935 and M.9 endophytic communities. Results from this study will have broad implications for providing guidance to apple rootstock breeding programs in selecting desired traits for soil biotic adaptations. Differences in composition of microbial communities during early stages of rootstock growth may offer insight into ARD tolerance and disease development.

Interactions between potato cropping systems, soil health, and microbial communities

C. E. JAHN (1), J. Stewart (1), S. Essah (2), P. O'Neill (3), Z. Abdo (1), (1) Colorado State University, Fort Collins, CO, USA; (2) Colorado State University, Center, CO, USA; (3) Soil Health Services, Alamosa, CO, USA

A major constraint to potato production is the sustainability of farming systems using short-term rotations that result in a less than optimum crop rotation system for most potato growers. Effective control of soil-borne plant pathogens (plant parasitic nematodes, fungi, phytopathogenic bacteria) and weed pests is a serious challenge to potato producers. Continuous cropping of a susceptible host, or susceptible alternate hosts, can result in the build-up of specific plant pathogens resulting in a decline of crop yield and quality. Studies in potato cropping systems have shown that the rotation frequency and host range of the crops in the rotation influence potato soil-borne disease incidence and severity. The goals of this project are to determine microbial population dynamics in response to two- and three-year rotation schemes by examining changes of both pathogen and total soil microbial populations in response to rotational length and cropping sequence. This research highlights the effect of crop rotations in changing potato yield, and fungal, bacterial, nematode communities. Further, results suggest changes in soil properties that could indicate soil health parameters to increase the sustainability of potato farming systems.

The population structure and effector diversity of a United States population of *Puccinia striiformis* f. sp. *tritici*

R. LYON (1), G. Wu (1), D. G. O. Saunders (2), K. Broders (1), (1) Colorado State University, Fort Collins, CO, USA; (2) John Innes Centre, Norwich, UNITED KINGDOM

Since 2010, novel races of *Puccinia striiformis* f. sp. *tritici* (*Pst*), the causal agent of stripe rust on wheat, have been observed overcoming resistances in central U.S. wheat populations. This fungus asexually reproduces in the U.S. and new pathotypes emerge due to events such as mutations within the resident population or introductions of exotic populations. Our goal was to use a field-transcriptomic approach to evaluate the population structure, evolutionary history, and effector diversity of *Pst* within the U.S. In the 2017 growing season, 25 *Pst*-infected wheat leaves from U.S. fields were collected and the transcriptomes sequenced. This data was compared with 300 previously published samples collected from six continents in the last decade. Preliminary results comparing these 2017 U.S. isolates with three older U.S. isolates and 20 *Pst* transcriptomes from northern Europe discriminate seven genetic clusters. The U.S. samples clustered separately from the European samples and an older U.S. genotype. This suggests that recently emergent European races, such as the Warrior race, have not been introduced to the U.S., but rather U.S. diversity may be driven by mutation and adaption within the resident population. The greatest variability in gene expression within the U.S. *Pst* population occurs mainly in candidate effectors, indicating these effectors may be under differential selection pressure and important genes in distinguishing between U.S. sub-populations.

Laboratory virulence assays used to rank potato cultivar tolerance to blackleg provide different results than field observations

D. TAYLOR, Y. Zeng, A. O. Charkowski, Colorado State University, Fort Collins, CO, USA

Seed potato certification data collected in Colorado was used to rank potato cultivar tolerance to blackleg. Five cultivars with different tolerance levels (Chipeta<Algeria<Lamoka<Classic Russet<Yukon Gem) to blackleg were tested to determine if we could validate field data with laboratory assays. A strain isolated from Colorado, *Pectobacterium atrosepticum* CW1-4, and the *P. atrosepticum* type strain, 33260, were used to infect plants through vacuum infiltration and stem inoculation. Disease was assessed with time-lapse video and by measuring lesion length and disease incidence. Through tuber vacuum infiltration, we demonstrated that *P. atrosepticum* 33260 caused soft rot only on Classic Russet, while CW1-4 caused soft rot in all cultivars with different levels of tolerance (Lamoka<Classic Russet@Yukon Gem<Chipeta<Algeria). The incidence of blackleg on the stems of the five cultivars after vacuum infiltration with CW1-4 was higher than with 33260. All cultivars were susceptible after stem inoculation with either strain and showed varying lesion

lengths. Time-lapse video was used to assess Classic Russet and Yukon Gem. CW1-4 resulted in faster occurrence of disease symptoms than 33260 in Classic Russet. Blackleg incidence from either CW1-4 and 33260 in Yukon Gem was insignificant. These results indicate that susceptibility of the five cultivars to *P. atrosepticum* is significantly different than data gathered from field observations.

Distribution and potential impacts of the ring nematode, *Mesocriconema xenoplax*, in vineyards and sweet cherry orchards in British Columbia

T. A. FORGE (1), P. Munro (2), A. Midwood (3), L. Phillips (4), K. Hannam (2), M. Jones (3), (1) Agriculture and Agri-Food Canada, Summerland, BC, CANADA; (2) Agriculture and Agri-Food Canada, Summerland, CANADA; (3) University of British Columbia - Okanagan, Kelowna, CANADA; (4) Agriculture and Agri-Food Canada, Harrow, CANADA

Production of wine-grape and sweet cherry have increased rapidly over the past 20 years in the Okanagan Valley, British Columbia. Although soil-related vigour decline problems have been noted, the distribution of plant-parasitic nematodes in these crops in the region had not been determined. The ring nematode, *Mesocriconema xenoplax*, is of particular interest as it is known to be damaging to grapevine and other *Prunus* species fruit trees. In 2018, populations of plant-parasitic nematodes were analyzed from 57 vineyards and 25 cherry orchards in the region. Complementary analyses of soil microbial and physico-chemical properties were obtained from 25 and 21 of the vineyard and orchard samples, respectively. In 2012, similar analyses were performed on samples from 35 cherry orchards. Ring nematodes were found in 82% of vineyards sampled in 2018, and in 80% and 77% of cherry orchards sampled in 2018 and 2012, respectively. Ring nematode population densities were correlated with soil percentage sand but no other soil properties. Preliminary results from field microplot studies and analyses of relationships between ring nematode population densities and indices of tree vigour in 2012 suggest that ring nematodes have negative impacts on cherry tree growth. Considering the widespread distribution of ring nematodes and their potential impacts on grapevine and cherry, we suggest that they should be a primary focus of future nematological research in the region.

Wheat curl mite-transmitted viruses in Colorado

B. Poon (1), P. Nachappa (2), T. ALBRECHT (2), (1) Colorado State University, CO, USA; (2) Colorado State University, Fort Collins, CO, USA

Wheat curl mites (WCMs), *Aceria tosichella*, transmit three different viruses of economic impact on wheat production in Colorado: *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV) and *High plains wheat mosaic virus*. Yield losses from WSMV average between 5–10% annually in the Central Great Plains, with some areas experiencing 100% loss affected by severe infections. Two genetically distinct genotypes of WCM have been characterized based on ribosomal *ITS1* and mitochondrial *Cytochrome oxidase I/II* partial sequences: type 1, initially identified from South Dakota, Kansas, Montana, Nebraska and Texas, and type 2, from Nebraska. The genetic differences in WCM populations correspond to altered response to wheat resistance genes and variable transmission efficiencies of different wheat viruses. To understand WCM populations in Colorado, which have not been characterized, wheat samples were collected across eastern Colorado. Samples were tested for presence of all three WCM-transmitted viruses using RT-PCR and examined for the presence of the microscopic WCM vector. These results characterize WCM types present in Colorado, the virulence of each type and the corresponding viruses they vector. This information is valuable to wheat producers to assist in varietal selection decisions and to support research involving screening of wheat varieties for resistance or tolerance phenotypes.

Discovery of novel viruses in apple by RNA-seq

A. WRIGHT, S. J. Harper, Washington State University, Prosser, WA, USA

Apple decline has been observed in Washington state, particularly on Honeycrisp trees grown on G935 rootstock. In this disease the trees exhibit dieback with necrosis at the graft union and in the rootstock. The cause of this disease is unknown. To identify viruses that may be involved in the disease, RNA-seq was performed on six trees: four trees exhibiting decline and two healthy trees. Across the six trees, eight known viruses and viroids were detected. None of the known viruses or viroids has been consistently associated with this disease. To explore the possibility that a novel virus (or viruses) may be associated with apple decline, a BLASTx analysis was performed on the RNA-seq data. Seventeen putative novel viruses were detected, most of which likely infect either apples or associated fungal species. These included an ilarvirus, two tombus-like viruses, a barna-like virus, a picorna-like virus, three ourmia-like viruses, three partiti-like viruses, and two narna-like viruses. Four additional novel viruses were detected that could not be assigned to a family. None of these novel viruses appeared to be associated with apple decline in that they were not consistently observed in declining trees and absent in healthy trees. That seventeen putative novel viruses representing multiple viral families were discovered in sequencing only six apple trees underscores both the diversity of viruses infecting or associated with apples and how few viruses are known.

Examination of chemical effects on limiting the incidence of powdery scab and *Potato mop-top virus* in potato

Y. ZENG, A. C. Fulladolsa, A. Cordova, A. O. Charkowski, Colorado State University, Fort Collins, CO, USA

Spongospora subterranea (Ss) and its transmitted *Potato mop-top virus* (PMTV) are soil-borne pathogens that limit production of healthy tubers in North American potato production and negatively impact seed and fresh market potato exports. The biphasic life cycle

of Ss and the durability and viability of its resting spores makes disease management particularly difficult. We investigated the effects of two chemicals, Omega®500F and FOLI-R-PLUS RIDEZ™, on PMTV and powdery scab caused by Ss and on the reduction of Ss inoculum in naturally infested fields and potting mix using different potato cultivars. There was no significant difference related to chemical treatments on powdery scab and PMTV incidence. However, RIDEZ significantly increased the amount of Ss inoculum in the soil or potting mix although it varied across farms and among potato cultivars. Russet cultivars were more tolerant to powdery scab than the yellow- or red-skinned cultivars. PMTV incidences on tubers collected from Classic Russet and Russet Norkotah ranged from 0 to 80%, even though the tubers lacked powdery scab lesions. In addition, 93% of plants were infected by PMTV in the greenhouse potting mix experiment, indicating PMTV transmission was high under optimal conditions for powdery scab disease development. These results expand our current understanding of powdery scab and PMTV and support that RIDEZ and Omega should not be part of integrated management practices for these important diseases.

Root lesion nematode resistance (*Pratylenchus neglectus*) has been advanced in winter wheat lines

E. GUNNINK TROTH, D. May, A. T. Dyer, Montana State University, Bozeman, MT, USA

The root lesion nematode *Pratylenchus neglectus* (RLN) presents a substantial threat to wheat production worldwide. Because management options are limited, the development of RLN-resistant wheat cultivars is highly desirable. To this end, a series of crosses were made in combination with recurrent disease selection using Iranian landrace ‘Persia 20’ as the resistant parent. Initially, Persia 20 was backcrossed into ‘Yellowstone’ winter wheat. Progeny were then selected based on agronomic performance and recurrent disease testing. Ultimately, this resulted in the selection of two lines that yielded 84.9 and 81.3 bu/acre (Yellowstone = 70.1 bu/acre, LSD_(0.05) = 11.3). These lines also displayed low nematode reproductive factors (RF) < 2.0 (Yellowstone RF = 14.9). Unfortunately, the lines were > 7 cm taller than their recurrent Yellowstone parent. To decrease plant height, the two lines were crossed with the winter wheat cultivar ‘Warhorse’. The F₁ progeny from this cross were used to produce double haploid (DH) lines. Repeated nematode screening of these DH lines yielded 10 resistant lines with a mean RF of 1.06 (SE = 0.32), and 10 susceptible DH lines with a mean RF of 4.73 (SE = 1.52). For comparison, the susceptible control Machete had a mean RF of 5.80. The 20 lines are currently being amplified for field testing starting in fall 2019.

Pathogenicity of *Phytophthora* on grape

P. FUJIYOSHI (1), A. L. Fabritius PhD (2), K. Baumgartner (1), (1) USDA ARS, Davis, CA, USA; (2) AL&L Crop Solutions Inc, Vacaville, CA, USA

Phytophthora is a recently described genus intermediate between *Phytophthora* and *Pythium* that includes species newly recognized as plant pathogens. *P. vexans* has been shown pathogenic on grapevine, but is isolate- and environment-dependent in virulence. We conducted greenhouse virulence trials of two *P. vexans* isolates from the California Central Valley on three grapevine rootstocks. There was no significant effect of *P. vexans* inoculation on shoot dry weight, possibly due to the length of the trial. *P. vexans* was re-isolated from rootstocks S04 and 1103P non-inoculated controls at a rate of 0–20% and from 3309C 45–60%, compared to 58–100% for inoculated plants. Cross-contamination by fungus gnats was possible, and rootstock may have played a role in the establishment of *P. vexans* in this manner. There was a clear decrease in shoot weight due to flooding across all inoculation treatments in 1103P and 3309C, potentially due to opportunistic oomycete pathogens. Other oomycetes isolated from roots included *P. helicoides*, *P. chamaeophyon*, and *Pythium irregulare*.

Genomic analysis of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koa*) for the development of tools for early detection and monitoring

J. DOBBS (1), M. S. Kim (2), N. Dudley (3), T. C. Jones (3), A. Yeh (3), R. K. Dumroese (4), P. G. Cannon (5), R. Hauff (6), N. B. Klopfenstein (7), J. Stewart (1), (1) Colorado State University, Fort Collins, CO, USA; (2) USDA Forest Service, Corvallis, OR, USA; (3) Hawaiian Agricultural Research Center, HI, USA; (4) USDA Forest Service, Rocky Mountain Research Station, ID, USA; (5) US Forest Service, Vallejo, CA, USA; (6) Hawaii State Dept of Land & Natural Resources, Honolulu, HI, USA; (7) Rocky Mountain Research Station, USDA Forest Service, Moscow, ID, USA

Koa (*Acacia koa*), Hawai‘i’s second most common endemic tree that is ecologically, economically and culturally important to Hawai‘i, is under threat from koa wilt disease caused by *Fusarium oxysporum* f. sp. *koa* (Fo koae), particularly in the low- to mid-elevation forests. *Fusarium oxysporum* is a cosmopolitan, vascular-wilt pathogen of diverse plant hosts. Morphological identification methods have little utility for pathogenic and non-pathogenic *F. oxysporum* strains because differences are unreliable. After screening for pathogenicity, genetic analyses of 24 isolates showed a clear and well-supported clade of pathogenic Fo koae isolates. These results were confirmed by sequencing six loci and identifying SNPs that distinguish pathogenic isolates from non-pathogenic isolates. Because highly virulent strains of Fo koae are genetically but not morphologically distinct from non-pathogenic isolates, our objectives were to develop a DNA-based probe to detect pathogenic Fo koae and examine putative protein-encoding genes associated with virulence. Using whole-genome sequencing of one highly virulent isolate and one non-pathogenic isolate of Fo koae, a SNP-based DNA probe will be developed to identify pathogenic strains of Fo koae. This probe will be verified on characterized Fo koae isolates and closely related *Fusarium* spp. The results will provide guidance to koa restoration efforts, including resistance-breeding and screening programs for koa.

Detection of Grapevine virus A in wild grape in Kazakhstan

D. GRITSENKO, K. Aubakirova, N. Deryabina, S. Baizhumanova, N. Galiakparov, Institute of Plant Biology and Biotechnology, Almaty, KAZAKHSTAN

Huge economic losses within vine sector are caused by viral infections, which might infect 80% of the field. Grapevine virus A (GVA) might lead to financial losses, which constitute 22% of the crop harvested from the field. Timely detection of the virus will prevent the spread of infection in the vineyard as well as to identify the natural reservoirs of the virus. In this work, a test system based on reverse transcription combined with PCR for GVA detection was developed. Specific primers for GVA were designed by analyzing nucleotide sequences of capsid proteins of 12 GVA isolates from the NCBI database. 54 plants of wild grape growing in Tulkubas mountainous area were examined for the presence of GVA. Total DNA and RNA were isolated from leaves. DNA was used to perform genotyping of wild grape with 6 SSR markers. Genetic analysis of the genotyping results showed that wild grape formed a cluster with European and Asian cultivars. GVA was detected in 2 plants of 54 samples. The nucleotide sequences of the capsid proteins of these two isolates were determined. Phylogenetic analysis showed that GVA isolates from wild grape form a cluster with isolates from Europe (NCBI). The highest genetic homology of 92.43% with 100% coverage was noted with isolate SK13 (Slovakia). Genetic analysis of GVA isolates from wild grape will allow a better understanding of the molecular mechanisms of virus evolution and host – pathogen interaction.

Genomic, transcriptomic and proteomic analyses of *C. leucostoma* isolated from peach during infection

J. IBARRA CABALLERO, J. Stewart, Colorado State University, Fort Collins, CO, USA

Cytospora leucostoma is the causal agent of leucostoma canker, the main disease limiting peach production in Colorado. Recent surveys have found the pathogen infecting trees in 100% of orchards in western Colorado, where peaches are grown. Little is known about the genetic background that allows this species to be a successful opportunistic pathogen on peach trees. In this study, we sequenced and annotated the genome of *C. leucostoma* isolated from infected peach trees. In addition, we performed *in vitro* experiments to examine expressed genes and proteins secreted during colonization of peach branches or during saprophytic growth in culture medium. Within the genome, a large arsenal of enzymes are encoded that could help *C. leucostoma* during infection and colonization of its host. We also found significant differences in the sets of expressed transcripts, and many different secreted proteins when comparing the pathogenic and saprophytic conditions. Our methodology could be useful to get a deeper understanding of the infection strategy of *C. leucostoma* and of the response of the host to the pathogen.

From the file drawer: Null results embellish the nuanced biology of *Verticillium dahliae*

D. WHEELER (1), D. A. Johnson (2,3), (1) Montana State University, Bozeman, MT, USA; (2) Washington State University, WA, USA; (3) Washington State University, SPOKANE, WA, USA

Evidence that the fungus *Verticillium dahliae* adapts towards some hosts and away from others during serial inoculations has been published by independent researchers since the 1960's. These observations have pragmatic implications but need to be reproduced in local contexts before recommendations are warranted. The objective of this research was to generate attenuated phenotypes of *V. dahliae*. Three hosts, including potato, barley, and mustard were repeatedly inoculated, for four generations, with three different isolates of *V. dahliae* that varied in aggressiveness. For each generation, each isolate was recovered from each host and used to inoculate new individuals of the same host. Potato plants were subsequently inoculated with all of the isolates recovered from all hosts and generations. Tuber yields, disease expression, and stem infection were quantified but, contrary to expectations, were not affected by the history of the isolates. These results remind us that categorical recommendations should be tempered and conditioned on local contexts. Although this is a null result, it underscores the nuanced biology of *V. dahliae*. Moreover, failure to disseminate such null results, would only further contribute to the 'file drawer problem' and introduce additional bias into science. Finally, withholding such null results from publications might enable the continuation of disease management strategies that are not supported by empirical evidence.

Monitoring potato pathogens in the Plant Diagnostic Clinic at Colorado State University

A. C. FULLADOLSA, A. O. Charkowski, Colorado State University, Fort Collins, CO, USA

In recent years, *Dickeya dianthicola* (potato soft rot and blackleg) and *Spongospora subterranea* (powdery scab) have become pathogens of major concern for early generation seed potato production and trade. Since 2018, the Plant Diagnostic Clinic at Colorado State University has tested over 800 plant, water, potting mix, and field soil samples for the presence of *D. dianthicola* and/or *S. subterranea*. Samples originated from different production systems (tissue culture, hydroponics, greenhouse pot production, field) in ten U.S. states. Detection of *D. dianthicola* in plant and water samples was performed using a conventional PCR to detect the *Dickeya* genus and a species-specific real-time PCR assay. *D. dianthicola* was not detected in any of the samples tested. These data indicate that greenhouse irrigation water is not a major risk factor for blackleg and that *D. dianthicola* has not been the main pathogen causing blackleg disease on plant samples. *S. subterranea* detection in water, field soil, and potting mix samples was performed by quantitative PCR. Samples with low pathogen levels were further tested with a tomato bioassay. *S. subterranea* was detected in 0/35 water, 12/97 potting mix, and 108/208 soil samples, demonstrating that potting mix spreads *S. subterranea* and the pathogen is common in field soil. *S. subterranea* detection data are being used by growers to determine which potting mixes to use and which cultivars to plant in infested fields.

Understanding the disease ecology of the corn bacterial leaf streak pathogen *Xanthomonas vasicola* pv. *vasculorum*

M. C. ORTIZ-CASTRO (1), G. Lavden (1), T. Albrecht (1), C. Wee (1), T. A. Jackson-Ziems (2), A. E. Robertson (3), J. E. Leach (1), K. Broders (1,4), (1) Colorado State University, Fort Collins, CO, USA; (2) University of Nebraska, Lincoln, NE, USA; (3) Iowa State University, Ames, IA, USA; (4) Smithsonian Tropical Research Institute, PANAMA

Bacterial leaf streak, caused by *Xanthomonas vasicola* pv. *vasculorum* (Xvv), is an emerging disease of corn in North and South America. Based on the combined \$52.4 billion value of the corn industry, early reports of Xvv disease severity, and lack of management methods, this emerging pathogen represents an economic threat to corn production in the United States. The primary goal of this research is to provide a basic understanding of the infection ecology and survival of the corn bacterial leaf streak pathogen. Through infiltration of the pathogen on corn plants, we found that there is a significant interaction between Xvv isolates and two corn varieties. By evaluating the bacterial fitness across representative isolates of Xvv, we showed that 22°C is the optimal temperature for bacterial growth in culture. We also evaluated the interaction of Xvv with the endophyte *Pantoea ananatis* and found that the presence of the endophyte significantly decreases Xvv's disease response. Finally, through litter studies at multiple locations, we demonstrated that infected residue left on the surface of the soil harbored significantly greater quantities of Xvv than infected residue buried 10 cm below the surface. These findings will be useful to understand the bacterial leaf streak disease cycle and aid in the development of management strategies that may limit the distribution of Xvv within corn fields and prevent its spread to other corn producing regions.

Reduction of Phytophthora blight severity and its relation to temporal changes in total phenolic content in Jalapeño pepper inoculated with *P. capsici*

S. DURA (1), P. A. Lujan (2), I. Guzman (1), S. Sanogo (2), R. Steiner (1), (1) New Mexico State University, Las Cruces, NM, USA; (2) New Mexico State University, Las Cruces, NM, USA

Phytophthora capsici is a destructive pathogen, which causes Phytophthora blight disease in many vegetable crops including chile pepper. Our research aims at identifying the factors associated with reduction of Phytophthora blight in Jalapeño cultivars and one associative factor could be an increase in the production of phenolic compounds. The Folin-Ciocalteu reagent method was used to extract the total phenolics from six Jalapeño and two non-Jalapeño genotypes at 12 and 24 hours post-inoculation. Control plants were not inoculated. A significant difference was observed between inoculation levels and among genotypes, but not between time periods. Total phenolic content was higher in inoculated plants than non-inoculated plants. Except for NuMex Pumpkin Spice and NuMex Jalmundo, total phenolic content was higher at 12 hours post-inoculation. Likewise, a sharp decline in total phenolic content was observed for Early Jalapeño and NM 6-4 at 24 hours post-inoculation. Lower disease severity in NuMex Pumpkin Spice may be due to continuous increase in production of total phenolic content. For TAM Jalapeño and NuMex Vaquero, lower disease severity could be due to the expression of resistance genes along with increase in production of phenolic compounds since they had a similar phenolic content trend as CM-334. Additional experiments will focus on determining the concentration of total phenolic content at early post-inoculation periods of 3 and 6 hours.

Insect-Bacteria-Plant Interactions: Microbiomes of Russian wheat aphid contain bacteria that increase virulence to wheat

E. K. LUNA (1), J. Shipp (1), C. Chang (2), J. Ziegler (2), C. Heiner (2), D. Caldwell (3), A. Iyer-Pascuzzi (3), J. E. Leach (1), (1) Colorado State University, Fort Collins, CO, USA; (2) Pacific Biosciences, CA, USA; (3) Purdue University, West Lafayette, IN, USA

Phenotypic responses to biotic stresses are often studied as interactions between two species; however, in the phytobiome, these responses frequently result from complex interactions involving several organisms. We previously demonstrated that variation in chlorosis on wheat caused by feeding of Russian wheat aphid is determined, in part, by aphid-associated bacteria. The saliva of the aphid contains proteins mainly from bacteria in the families *Enterobacteriaceae* and *Erwiniaceae*. Using Pacbio SMRT sequencing, seven bacterial isolates associated with the aphids were sequenced. Genome annotation of relatively abundant types of bacteria show they contain a repertoire of virulence factors such as: type III and type VI secretion systems and quorum sensing genes. When each of these seven bacterial species were inoculated to wheat and barley alone or in a mixture containing all of the bacteria, no symptoms (chlorosis, water-soaking or necrosis) were visible even after 72 hours. Overall, these results show that the bacteria themselves are not virulent to wheat, and are consistent with the hypothesis that these bacteria may contribute to aphid virulence by suppressing the plants' insect defense mechanisms.

Manipulation of transcriptional *cis*-regulatory modules (CRMs) to improve disease resistance in rice

F. MARTIN, Colorado State University, Fort Collins, CO, USA

Defense response (DR) genes are often members of gene families and are associated with genomic regions that are part of QTLs. Identifying and increasing the number of QTLs has the potential to improve plant defense responses against diverse pathogens and stabilizing disease resistance in the field. However, trait complexity and lack of reliable genetic markers often makes the identification and transferring of favorable genes within QTLs a daunting process. Interestingly, not all DR genes within a QTL are equally active during the defense response mainly due to their transcriptional regulation; which is mostly influenced by the repertoire of *cis*-regulatory elements found in their promoter regions. We hypothesize that presence of unique groups of *cis*-regulatory elements, called *cis*-regulatory modules (CRMs), can determine the efficacy and strength of DR genes in their response to pathogen attacks. Through the

integration of data from multiple gene expression studies, we identified a series of well conserved CRMs associated with DR genes genome-wide, indicating a broad transcriptional response against diseases. Initial analyses indicate that the presence/absence of certain CRMs can influence temporal expression of DR genes. Using genome editing technologies, we are testing the possibility of improving CRM content in susceptible cultivars to enhance resistance and potentially use these signature modules as reliable genetic markers to improve breeding practices.

It's not easy being green: Improvements to algae control in rice hydroponic growing systems

T. CRUE (1), J. Shipp (1), S. P. Cohen (2), J. E. Leach (1), (1) Colorado State University, Fort Collins, CO, USA; (2) The Ohio State University, Columbus, OH, USA

We need a comprehensive understanding of how plants respond to environmental stresses to protect crops with the onset of climate change. Bacterial blight of rice, a disease caused by *Xanthomonas oryzae* pv. *oryzae*, is more severe during high temperature stress. Previous experiments suggested that the plant hormone abscisic acid (ABA) may have a role in the interaction. In order to rapidly assess methods for combined heat stress and ABA experimentation, we developed a hydroponic system. This system is soil-free and plants are grown in water supplemented with fertilizer. However, a new problem arose with this system: algae contaminated the water, potentially affecting the outcome of the stress/hormone experiments. In this study, several potential solutions are explored to control and prevent algal contamination in the hydroponic system. This study will provide future directions for a more controlled rice hydroponic system to be developed.

Insights into population structure of *Botrytis cinerea* infecting small fruit in the Pacific Northwest

O. KOZHAR (1), N. Grunwald (2), T. L. Peever (1), (1) Washington State University, Pullman, WA, USA; (2) USDA ARS, Corvallis, OR, USA

Botrytis cinerea, causal agent of gray mold, is a major pathogen of small fruit (berry) crops worldwide. Despite its ability to infect different hosts, some studies have demonstrated host specificity while others have not, and it remains to be determined if populations of this fungus are specialized to berry hosts. The objective of this study was to test the hypothesis that *B. cinerea* is a broad host range pathogen with no specialization to berry hosts. Twenty single-conidial isolates were sampled from each of 34 small fruit fields in WA and OR, USA and genotyped using 7 microsatellite markers and one fungicide resistance marker. No association of genotype with host was observed however AMOVA indicated significant population differentiation by location. Significant differentiation between *B. cinerea* populations in adjacent berry fields indicated that they are highly localized and the presence of private alleles in adjacent fields provided further evidence of limited movement in space. Fungicide resistance alleles were negatively correlated with genotypic diversity of neutral markers suggesting selection through fungicide use. Our findings provide a better understanding of *B. cinerea* population biology and will be used to improve gray mold management strategies by developing customized programs based on fungicide resistance profiles in Pacific Northwest berry fields and elsewhere.

Out of the box and through the rows: Intelligent Spray Systems research on grape and hazelnut at Oregon State University

B. WARNEKE (1), J. W. Pscheidt (1), R. L. Rosetta (2), L. Nackley (2), (1) Oregon State University, Corvallis, OR, USA; (2) Oregon State University, Aurora, OR, USA

The Intelligent Spray System (ISS) includes Lidar and ground speed sensors to scan the plant canopy and adjust the application volume of an air-blast sprayer automatically. A Rear's 50 gallon axial fan air-blast sprayer was retrofitted with ISS components in 2018 and, in consultation with vineyard managers, four sulfur treatments were evaluated in a block of 'Pinot Noir' grapes for management of Grape Powdery Mildew (GPM, *Erysiphe necator*). After a single season, the ISS mode did not control GPM on leaves or clusters as well as the standard sprayer mode on the same sprayer. Factors that contributed to this result included severe GPM pressure, use of the non-systemic fungicide sulfur and use of initial ISS settings. Further testing is planned for 2019. Responsivity of the ISS at 2, 3, and 5mph was evaluated on hazelnut shoot tips using kaolin clay (Surround WP) as a spray tracer. At each speed, coverage was compared to the standard air-blast setting. Within ISS and standard air-blast settings there were no significant differences in coverage among the three speeds tested, however when the ISS was compared with standard air-blast at each speed, the ISS treatment had significantly lower coverage in all cases ($p < 0.01$, Z test). With some adjustment and consideration of its limitations, the ISS should prove to be an effective tool for use in specialty crop systems.

Genomic acquisitions in emerging populations of *Xanthomonas vasicola* pv. *vasculorum* infecting corn in the U.S. and Argentina

A. PEREZ-QUINTERO (1), M. C. Ortiz-Castro (1), G. Wu (1), J. M. Lang (1), S. Liu (2), T. Chapman (3), C. Chang (4), J. Ziegler (4), Z. Peng (5), F. F. White (5), M. C. Plazas (6), J. E. Leach (1), K. Broders (7), (1) Colorado State University, Fort Collins, CO, USA; (2) Kansas State University, Manhattan, KS, USA; (3) Biosecurity and Food Safety, NSW Department Primary Industries, Narellan, NSW, AUSTRALIA; (4) Pacific Biosciences, CA, USA; (5) University of Florida, Gainesville, FL, USA; (6) Universidad Catolica de Cordoba, Cordoba, ARGENTINA; (7) Smithsonian Tropical Research Institute, PANAMA

Xanthomonas vasicola pv. *vasculorum* (Xvv) is an emerging bacterial plant pathogen that causes bacterial leaf streak on corn. First described in South Africa in 1949, reports of this bacteria have greatly increased in the past years in South America and in the U.S.,

where it is now present in all the corn producing states. Phenotypic characterization showed that the emerging U.S. and South American *Xvv* populations may have increased virulence in corn compared to earlier strains. To understand the genetic mechanisms behind the increased virulence in this group, we used a comparative genomics approach to identify gene acquisitions in *Xvv* genomes from the U.S. and Argentina. We sequenced 41 genomes of *Xvv* and the related sorghum-infecting *X. vasicola* pv. *holcicola* (*Xvh*). A comparison of all available *X. vasicola* genomes showed the phylogenetic relationships in the group and identified clusters of genes associated with the emerging *Xvv* populations. The newly acquired gene clusters showed evidence of horizontal transfer to *Xvv* and included candidate virulence factors. One cluster, in particular, corresponded to a prophage possibly transferred from *Xvh* to all *Xvv* from Argentina and the U.S. The prophage contained putative secreted proteins, which represent candidates for virulence determinants in these populations and await further molecular characterization.

Evaluation of difenoconazole as postharvest fungicide to control ten major pathogens of pome fruit

A. AMIRI, L. Pandit, Washington State University, Wenatchee, WA, USA

Difenoconazole (DFC) is demethylation inhibitor (DMI) fungicide recently registered to control postharvest diseases of pome fruit. Herein, we report on its efficacy against ten major postharvest pathogens which are *P. expansum*, *Mucor piriformis*, *Lambertella corni-mar*, *B. cinerea*, *Neofabraea perennans*, *Phacidiopycnis washingtonensis*, *Phacidiopycnis pyri*, *Phacidium lacerum*, *Sphaeropsis pyriputrescens* and *Alternaria alternata*. To mimic natural infection pathways, DFC was applied preventively for the first three pathogens on harvested and wounded fruit. For the remaining seven orchard pathogens, non-wounded fruit were inoculated in the orchard with spore suspensions of each pathogen at 10^5 spores/ml two weeks before harvest and DFC was applied curatively postharvest. Trials were conducted on non-treated Fuji apples from a research orchard in Wenatchee, WA. Treated fruit were stored at 1°C in a regular atmosphere and disease incidence was evaluated after 6 months. Incidence of *S. pyriputrescens*, *N. perennans*, *B. cinerea*, *A. alternata*, *L. corni-mar*, *P. washingtonensis*, *P. pyri*, *M. piriformis*, *P. lacerum*, and *P. expansum* in the non-treated fruit was 18, 22, 30, 33, 45, 59, 72, 83, 98, and 98%, respectively, after 6 months. The respective incidences in DFC-treated fruit were 4, 8, 5, 7, 18, 6, 9, 60, 43 and 30%. Our results indicate that DFC reduced the incidence of nine pathogens significantly and should be an effective additional tool.

A tale of two transporters: Cellobiose transporters as the key to trigger virulence in the plant pathogenic bacterium *Streptomyces scabies*

S. GUPTA (1), R. Villagrana (1), J. Salazar (1), R. Loria (2), I. Francis (1), (1) California State University, Bakersfield, Bakersfield, CA, USA; (2) University of Florida, Gainesville, FL, USA

Pathogenicity by the plant pathogenic *Streptomyces scabies*, the causative agent of common scab disease, on various important root crops is triggered by cellobiose, a subunit of plant cell wall polymer cellulose. Cellobiose induces production of thaxtomin A, the main virulence factor of this species. This phytotoxin affects cellulose synthase leading to stunted growth. The ABC transporter CebEFG-MsiK has been shown to be the primary importer of cellobiose, hence, a deletion of this transporter showed a significant decrease in thaxtomin production and a consequent attenuated virulence on plants. However, bacteria missing this transporter were still viable on minimal medium with cellobiose as the only carbon source (TDMc). Homology searches revealed there to be two other transporter candidates. The transporter with the highest homology seems to be absent in other pathogens, while a third transporter is located within the pathogenicity island of the pathogenic species. Mutants in the genes coding for these transporters showed, under the conditions tested, at least one other transporter is important during the pathogenic interaction with the plant host. Under more complex conditions, as would be in plant-microbe interactions, the loss of this transporter could not be compensated for by the first transporter as shown by the results of plant bioassays and the inability of this mutant to produce toxin on plant-based media known to induce thaxtomin production.

Growing plants inoculated with nitrogen-fixing bacteria in Martian regolith as a method to increase plant biomass and accessible nitrogen

F. HARRIS (1), J. Dobbs (2), J. Stewart (2), (1) Colorado State University-Bioagricultural Science and Pest Management, Fort Collins, CO, USA; (2) Colorado State University, Fort Collins, CO, USA

Due to increasing population growth and declining arable land here on Earth, astroagriculture is required to terraform inorganic material on the surface of Mars, known as Martian regolith for potential settlement. Nitrogen-fixing plants and their symbionts have been shown to increase plant viability. These plants have displayed the ability to increase soil fertility. In 2012, Wamelink et al. successfully grew N-fixing plants in simulated Martian regolith. In this study, we tested plant-bacterial symbiosis for nitrogen fixation in simulated Martian regolith to influence plant growth by inoculating clover and vetch with their respective N-fixing symbionts and measuring plant biomass and root nodules. Vetch (*Vicia sp.*) and clover (*Melilotus sp.*) were chosen for this study for their hardiness as these species have a symbiotic relationship with nitrogen-fixing bacteria, *Rhizobium leguminosarum* and *Sinorhizobium meliloti*, respectively. Initial results have indicated that there was successful symbiosis between clover and its symbiont in simulated Martian regolith. We observed greater biomass for clover with nodules than clover without nodules in simulated Martian regolith. No nodules formed on Vetch in simulated Martian regolith, while nodules formed on the Vetch in soilless potting mix. These results warrant further testing in astroagricultural settings to continue the development of soil amendment techniques for Martian regolith, a necessity for Martian colonization.

Dagger nematode diversity in fruit production systems in the Pacific Northwest

I. A. ZASADA (1), A. Peetz (2), C. N. Hesse (3), M. Kitner (4), (1) USDA ARS, Corvallis, OR, USA; (2) USDA ARS HCRU, Corvallis, OR, USA; (3) USDA-ARS, Corvallis, OR, USA; (4) USDA-ARS, OR, USA

Dagger nematodes (*Xiphinema americanum*-species complex) are commonly found in perennial fruit crops in the Pacific Northwest (PNW) of the United States and Canada. The risk of these nematodes to fruit production in the PNW is that they are vectors of several NEPO viruses. Viruses can be introduced into production fields via contaminated planting material. Spread within a location is mediated by dagger nematodes, leading to the question of whether all dagger nematodes are capable of vectoring viruses. This research was undertaken to begin to characterize the diversity of dagger nematodes present in the PNW, with the final goal of understanding the virus-vectoring potential of this group of nematodes. Populations of *X. americanum*-species complex were collected from 10 fruit production fields in the region. The populations were then morphologically and molecularly characterized. The character-based phylogeny indicated substantial overlap of morphometric characters among populations. For molecular characterization, genus-specific primers targeting a 2,800 base pair (bp) mtDNA region were designed from accessions deposited in GenBank which included portions of the cytochrome oxidase 1 (CO1) gene, partial sequences from the 3' end of the ribosomal small subunit (mtSSU), and the cytochrome b (CYTB) gene. Preliminary results indicate that there are at least five species from this complex present in the PNW. The virus vectoring capacity of these species remains unknown.

Biological control of canker diseases in the fruit and nut crops

L. A. HOLLAND (1), R. Travadon (1), D. P. Lawrence (1), M. Nouri (2), F. P. Trouillas (3), (1) University of California, Davis, CA, USA; (2) Department of Plant Pathology, UC Kearney Ag. Research and Extension Center, Parlier, CA, USA; (3) University of California, Kearney, Parlier, CA, USA

Canker diseases caused by pathogenic fungi constitute an important problem for fruit and nut crop production in California. Pruning wounds serve as the primary infection court for canker-causing pathogens. Protection of these wounds using chemical fungicides can be ineffective due to the short persistence of the active chemical products on pruning wounds. Challenging also is the expansive diversity of fungal pathogens that cause canker diseases. Thus, control products need to be efficacious against a broad spectrum of taxonomically divergent canker-causing pathogens, including *Botryosphaeria dothidea*, *Ceratocystis destructans*, *Cytospora sorbicola*, *Diaporthe ampelina*, *Eutypa lata* and *Neofusicoccum parvum*. The goal of this study was to assess the efficacy of *Trichoderma* biological control agents as pruning wound protectants in comparison to the effectiveness of chemical fungicides, sealants and paints. Field trials were conducted over three consecutive years in commercial almond orchards and two consecutive years in cherry orchards as well as in a vineyard in California. Results show that *Trichoderma*-based products reduced disease incidence by 60% to 100% and was equivalent to that of thiophanate-methyl fungicide. This work suggests that *Trichoderma*-based products can be as or more effective for the management of canker-causing fungi in the fruit and nut crops while providing environmentally safe solutions for use in conventional as well as organic production systems.

LAMP detection of *Neofabraea perennans*, the causal agent of Bull's eye rot of pome fruit

D. ENICKS, A. Amiri, Washington State University, Wenatchee, WA, USA

Neofabraea sp. are quarantine, fungal pathogens which cause bull's eye rot (BER), a major economic postharvest disease of apple and pear. In the growing regions of the Pacific Northwest (PNW), BER is mainly caused by the species *N. perennans*. The fungus infects fruit preharvest and because of the dry growing season in the PNW infections remain latent to express symptoms only after 3–4 months of storage when incidences as high as 20% can be seen in rainy seasons, especially on susceptible cultivars. To detect the infections more efficiently and quickly before symptoms become visible at point-of-care locations, a portable diagnostic tool termed LAMP, Loop mediated isothermal amplification, has been developed for early detection of *N. perennans*. The LAMP technique was optimized and tested for specificity and sensitivity using extracted DNA from pure culture and was used to analyze apple fruits inoculated with *N. perennans* at different concentrations and timings pre and postharvest. Our preliminary results indicate that LAMP was able to detect DNA of *N. perennans* at concentrations as low as 0.0001 ng/μl after only 10 min. Further validation of the LAMP assay using a portable thermocycler (Genie II®), indicates that, beside some variability in its sensitivity between samples, LAMP can detect the fungus on apples inoculated at 10³ spores/ml prior to commercial maturity and could be adapted for early, quick, and real-time detection in commercial settings.

The effect of increasing soil salinization on resistance to Fusarium wilt of tomato

E. HELLMAN, University of California, Davis, Davis, CA, USA

California produces over 95% of the processing tomatoes grown in the United States. Fusarium wilt (FW) of tomato, caused by the soilborne fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* race 3 (Fol R3), drastically decreases marketable yield of processing tomato; in some cases, causing greater than 50% yield loss one year after detection. Many of the highest producing counties in CA, including Fresno County, are also facing issues with soil salinization. In infested fields, Fol R3 is managed by planting tolerant (F2) or resistant (F3) cultivars (cvs); however, FW R3 has been positively diagnosed in symptomatic F3 cvs from areas where abiotic stress may be playing a role. Soil salinity may be increasing FW severity and compromising host resistance. In 2018, field trials were conducted to look at the effects of soil salinity on FW development under saline and non-saline field conditions. At 64 days post-transplanting, FW

symptoms were observed in 3.70 – 6.67% of F3 cvs under saline treatment (1.09 ± 0.16 mS/cm) compared with 0% incidence in non-saline (0.18 ± 0.02 mS/cm). There was no significant difference in fruit rot proportion between saline and non-saline treatments. Additional research is focused on elucidating the effects of salinity on disease risk thresholds in the soil, and on Fol R3 survival. This research aims to inform the development of risk-assessment tools for the co-management of Fusarium wilt and soil salinity.

Emerging diseases of cannabis and their management

Z. K. PUNJA, Simon Fraser Univ, Burnaby, BC, CANADA

Plant pathogens on marijuana plants (*Cannabis sativa* L.) that infect the roots, crown and foliage of the crop in Canada were studied over multiple years of sampling. A PCR-based assay using primers for the internal transcribed spacer region (ITS) of ribosomal DNA confirmed identity of the cultures. Root-infecting pathogens included *Fusarium oxysporum*, *F. solani*, *F. proliferatum*, *F. brachygybbsum*, *Pythium dissotocum*, *P. myriotylum*, *P. ultimum* and *P. aphanidermatum*. Symptoms included root browning, discoloration of crown and pith tissues, stunting and yellowing, and in some instances, plant death. On foliage, powdery mildew, caused by *Golovinomyces cichoracearum*, was the major pathogen observed. On inflorescences, penicillium bud rot (caused by *P. olsonii*, *P. copicola* and other species), botrytis bud rot (*B. cinerea*) and fusarium bud rot (*F. solani*, *F. oxysporum*) were present to varying extents, depending on the cannabis strain. Approaches to management of powdery mildew and fusarium root and crown rot were investigated through application of biological control agents and nonfungicidal treatments. *Gliocladium catenulatum* (Prestop) and *Bacillus subtilis* (Rhapsody) significantly reduced development of fusarium root rot and powdery mildew, respectively, when applied prior to disease onset. Multiple weekly application of Regalia Maxx, Milstop and Zeritol, and daily exposure of leaves to UV-C light, also significantly reduced incidence and severity of powdery mildew.

Improved detection of the potato pathogen *Clavibacter michiganensis* subsp. *sepedonicus* using droplet digital PCR

S. KARIM (1), F. Zink (2), A. O. Charkowski (1), L. Tembrock (1), (1) Colorado State University, Fort Collins, CO, USA; (2) Colorado State University, Fort Collin, CO, USA

The Gram-positive bacterium *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) causes bacterial ring rot in potato. There is zero tolerance for Cms in seed potato production, and the presence of a single infected plant on a farm may disqualify it from seed production for that year. Effective management of Cms is aided by accurate and sensitive molecular detection methods. Ambiguous test results have led to costly delays in the past. In addition, false negatives have resulted in large losses in subsequent growing seasons. In this study, we developed a droplet digital PCR (ddPCR) assay for accurate and sensitive detection of Cms. The assay was compared empirically to existing methods for detection including conventional PCR, real-time PCR (RT-PCR), and ELISA. The ddPCR assay improved detection capacity by at least 10-folds using genomic DNA dilution in sterile water compared to RT-PCR. The ddPCR assay is sensitive enough to consistently detect one infected potato tuber core among 800 uninfected tuber cores whereas RT-PCR is limited to one infected core in 400. As such large potato seed lots can be more comprehensively sampled using ddPCR. Because of the improved sensitivity of ddPCR, Cms was found on potatoes, in the course of assay development, sold for consumption in grocery stores. This discovery highlights the improved detection capabilities of ddPCR and the need for expanded Cms monitoring.

Development of microsatellite markers for the biotrophic fungus *Podosphaera leucotricha*

L. GANAN (1), A. Amiri (1), T. L. Peever (2), (1) Washington State University, Wenatchee, WA, USA; (2) Washington State University, Pullman, WA, USA

Podosphaera leucotricha causes powdery mildew, one of the most important diseases of domesticated apples (*Malus × domestica*) in the Pacific Northwest. Despite its importance, not much is known about the genotypic diversity of *P. leucotricha* populations mostly due to the lack of suitable molecular markers. Microsatellites, or simple sequence repeat (SSRs), are one of the most important markers commonly used to solve ecological and evolutionary questions, and their development for *P. leucotricha* studies will provide novel insights about the population biology and epidemiology of this obligate biotroph. In this study, high throughput sequencing was used to obtain a de novo genome assembly of *P. leucotricha*. The assembly was then used to identify and isolate potentially amplifiable SSR loci (PALs) using a post-sequencing bioinformatics pipeline. Primers were developed and the amplification of 18 selected PALs was validated through independent PCR using genomic DNA from representative *P. leucotricha* isolates collected from Washington State apple orchards. Results were confirmed through Sanger sequencing of the amplified SSR loci. These markers will be labeled with fluorescent dyes and tested for their reproducibility and polymorphism using fragment analysis. We conclude that both the *P. leucotricha* draft genome and the isolated SSR markers will allow researchers to address a range of ecological and evolutionary questions associated with this important pathosystem.

Associational susceptibility: Having the wrong neighbors increases plants' vulnerability to insect, pests and diseases

B. POON (1), T. Albrecht (2), A. C. Fulladolsa (2), W. S. Cranshaw (2), M. Bartolo (1), P. Nachappa (2), (1) Colorado State University, CO, USA; (2) Colorado State University, Fort Collins, CO, USA

The proximity between plants can influence the likelihood of detection and/or susceptibility of a given plant to insect herbivores. This has been called “associational susceptibility” or “shared doom”. There are several examples of plants being subjected to greater herbivory

when spatially associated with heterospecific neighbors. Our research discovered an example of associational susceptibility of pepper plants to pea aphids and pea aphid-vectored *Alfalfa Mosaic Virus* (AMV) infection from nearby alfalfa fields. AMV causes disease in over 50 plant families including economically important crops such as potato and soybean, among others. The virus can be transmitted mechanically, through seed and is vectored by at least 15 species of aphids. In a test plot of peppers located at the Arkansas Valley Research Station in Rocky Ford, Colorado, we observed virus symptoms including leaf deformation and mosaic on several pepper varieties. We also found a large population of pea aphids in alfalfa fields to the east and west border of the test plots. ELISA and PCR were carried out to detect AMV in leaf tissues from the pepper varieties, alfalfa and aphids and positive results were obtained from all samples. To our knowledge this is first report of associational susceptibility of a plant host to a pest and pathogen, and should be a consideration in management practices for pepper producers in the southwestern US, as well as for producers growing any crops around alfalfa fields.

Friend or enemy? Understanding the effects *Claviceps purpurea* has on its host

S. WYKA (1), K. Broders (2), V. Nalam (1), (1) Colorado State University, Fort Collins, CO, USA; (2) Smithsonian Tropical Research Institute, PANAMA

The genus *Claviceps* are strict ovarian parasites of Poaceae. Some species, such as *C. purpurea* are defensive mutualists or weak pathogens which can infect 400+ species while others, like *C. africana* are aggressive pathogens and have a very restrictive host range. The genus *Claviceps* represents an excellent system to study the evolution of fungal species that share identical infection patterns, but vary in aggressiveness and host range. The goal of this project was to understand the effects *C. purpurea* has on its host and its evolution through greenhouse inoculations, field surveys, and genomic analysis. Greenhouse inoculation experiments of *C. purpurea* on barley revealed no significant differences of plant health parameters between infection severity of 0%, 5%, 15% or 25%. Furthermore, phylogenomic analysis of 54 *Claviceps* genomes using 2,722 BUSCO orthologs showed strong support for recent evidence of cryptic species, within the genus and divergence of clade *Claviceps*, that have overlapping host range. This suggests that host specialization might not be a factor for the divergence of cryptic species. Clusters of functionally redundant secreted metabolites and effectors were found in clade *Claviceps*, but absent in other clades with reduced host range. These results, along with research from collaborators suggests that creativity of secondary metabolite production may be responsible for the success of *C. purpurea* and its cosmopolitan distribution and vast host range.

Quantification and characterization of bacteria associated with Russian wheat aphid feeding in co-fed and isolated populations

J. SHIPP (1), E. K. Luna (1), P. Trivedi (1), K. L. Otto (1), J. Corwin (1), A. Iyer-Pascuzzi (2), D. Caldwell (2), J. E. Leach (1), (1) Colorado State University, Fort Collins, CO, USA; (2) Purdue University, West Lafayette, IN, USA

As the human population grows, the demand for wheat will continue to increase. Unfortunately, infestations by the Russian wheat aphid (RWA) can decrease wheat yields by 30%. Bacteria associated with RWA enhance aphid virulence to wheat, but it is not known which members of the aphid microbiome are involved. Our goal is to identify which bacteria are delivered during feeding and which members of that community contribute to aphid virulence. Our strategy is to study the microbiomes of diet (feeding cells) and leaves after aphid feeding. We first established two distinct aphid populations, one that was co-fed with its siblings and mother (dirty), and the other that was started from a single nymph isolated from its mother at birth and raised on plants that were previously untouched by aphids (clean). Dilution plating of whole aphids demonstrated more bacterial colonies in the dirty aphid population as compared to the clean aphid population (2.6×10^7 CFU/ml vs 9.2×10^4 CFU/ml, respectively). To determine if bacteria could be detected in leaf tissue and feeding cell solution after feeding with dirty or clean aphid populations, we extracted DNA and amplified the taxonomically-informative bacterial 16S gene. Amplicons were detected in all biological samples showing bacterial presence in the diet and leaves after feeding with both aphid populations. Sequencing is underway to identify which bacterial taxa are delivered to the plant by feeding and which enhance aphid virulence.

Feeding location alters behavior and fecundity of soybean aphids

W. PITT, V. Nalam, P. Nachappa, Colorado State University, Fort Collins, CO, USA

Plant morphology can impact herbivore fecundity, survival, and distribution on the host plant. The soybean aphid (*Aphis glycines*) is one of the most significant pests of the soybean plant (*Glycine max*) around the world. Soybean aphids move to different locations on the soybean plant at different times of the year, resulting in a harder to target pest. Experiments were conducted to investigate the influence of feeding location on the behavior and fecundity of soybean aphids. Results showed that adult apterous (wingless) soybean aphid populations were significantly higher on the stem than on adaxial (upper) and abaxial (lower) leaf surfaces. Moreover, soybean aphids showed less salivation and continuous ingestion of phloem on stems of the soybean plant, suggesting an increase in host acceptance. It appears that feeding on stems as opposed to adaxial and abaxial leaf surfaces has beneficial effects for soybean aphids and results in higher populations, because of the ease of locating and feeding on phloem. Further analysis of nutrient levels in the phloem sap content in soybean stems and leaves is currently being conducted and will indicate where the most nutrient-rich areas are for aphids to feed. These findings could have important management implications for the soybean aphid.

First report of a new stem and crown rot disease of processing tomato in California caused by *Fusarium falciforme*

E. L. HELPIO, C. L. Swett, University of California, Davis, Davis, CA, USA

California produces over 90% of the countries processing tomatoes, with a net worth of \$1.1 billion. In 2017, *Fusarium falciforme* – a member of the *Fusarium solani* species complex (FSSC) - was recovered from processing tomatoes in three counties (13 sites) with stem and crown rot. As there was no record of any species in the FSSC causing stem and crown rot in California, we hypothesized that this was a new disease in the state. To test this hypothesis, Koch's postulates was conducted by inoculating three *Fusarium falciforme* isolates (one per county) into a stem wound in each of two processing tomato cultivars, H8504 and HM3887. Mock inoculations and two *Fusarium* species which are known not to cause stem rot in tomato served as negative controls. The trial was replicated with 24 plants in each of 5 blocks and repeated. Eight weeks post inoculation, *F. falciforme* isolates produced an average lesion length of 30-50 mm with a maximum stem lesion length of 105 mm; all isolates caused stem lesions significantly larger than controls (3-7 mm) in both cultivars ($P < 0.001$). There was significant variation in cultivar susceptibility in one isolate treatment (CS 109) ($P = 0.04$), indicating potential for variation in susceptibility. Based on these results, *Fusarium falciforme* is a new stem and crown rot pathogen of processing tomato in California. This information can be used to develop diagnostic tools and identify tolerant or resistant cultivars for control.

***Spongospora subterranea* detection in commercial peat-based potting mix**

A. CORDOVA, Y. Zeng, A. O. Charkowski, A. C. Fulladolsa, Colorado State University, Fort Collins, CO, USA

Spongospora subterranea (Ss) is a soil-borne pathogen of the Plasmodiophora family that infects solanaceous hosts. Ss causes powdery scab disease in potato, and symptoms include root galls and tuber surface ruptures. Certified seed potato production uses potting mix (PM) as a growth medium to produce clean seed in the greenhouse. However, powdery scab was recently reported in potato tubers grown in peat-based PM. qPCR is commonly used to detect Ss, but results are inconclusive for PM with low Ss levels. Our objective was to develop a reliable method to detect Ss in PM. PM samples were collected from two sources (1 & 2) and the presence of Ss was assessed using qPCR and a bioassay. The bioassay consisted of growing bait tomato plants in each PM sample, in Ss-free PM (negative control), and PM with Ss inoculum (positive control), for 4–6 weeks. Tomato roots were stained and observed under a light microscope for the presence of Ss. We observed Ss plasmodia and zoosporeangia in root hairs of bait plants grown in source 2 samples, but not in those grown in source 1 samples. Using qPCR, the number of sporosori per gram of PM (sp/g) was 0-12 and 0-18 sp/g for two samples from source 1 and 0-146 and 0-78 sp/g for two samples from source 2 (6 reps/sample). Our findings show that the bioassay is a reliable detection method for Ss and can be used to prevent Ss spread through peat-based PM.

Primer design for improved detection of *Pectobacterium* species

M. REYNOSO, A. C. Fulladolsa, A. O. Charkowski, Colorado State University, Fort Collins, CO, USA

Several species and subspecies of the plant pathogenic genus *Pectobacterium* have recently been discovered, reclassified, renamed, or elevated to species. *Pectobacterium* species cause blackleg of potato and soft rot on a broad range of hosts. Currently available primers do not distinguish among species. For example, primers routinely used for *P. carotovorum* detection also amplify *P. parmentieri* DNA. Moreover, there are no PCR-based diagnostic methods for multiple newly described *Pectobacterium* species. The aim of the study is to improve and develop conventional PCR detection methods for several species of *Pectobacterium* by designing highly specific primers. The Uniqprimer pipeline was used to align complete genomes, isolate distinct sequences in target strains, and design species-specific primers for *P. parmentieri*, *P. brasiliense*, *P. odoriferum* and *P. carotovorum* detection. These primer sets are being evaluated for specificity and sensitivity to improve diagnostics and epidemiological studies of this genus.

Acid soils in PNW wheat production and impacts on the soil microbiome

C. Yin (1), D. C. Schlatter (2), D. Kroese (3), T. C. Paulitz (4), C. HAGERTY (3), (1) Dept. of Plant Pathology, Washington State University, Pullman, WA, USA; (2) USDA-ARS, Wheat Health, Genetics and Quality Research Unit, Pullman, WA, USA; (3) Oregon State University, Pendleton, OR, USA; (4) USDA ARS, Wheat Health, Genetics and Quality Research Unit, Pullman, WA, USA

Soil acidity is an emerging issue in the \$2B wheat industry of the PNW. The process of nitrification is a bacterial mediated process that converts ammonium to nitrate but also acidifies the soil. Our recent survey of the PNW wheat production region indicates 73% of surveyed fields are below pH 5.5, the threshold for optimal wheat production. Acidic soil can have a negative impact on wheat yields, favor some yield limiting plant pathogens such as *Cephalosporium*, and can lead to aluminum toxicity. However, the influence of soil acidity on the soil microbial community is less understood. We established winter wheat plots in two rainfall zones with pH ranging above and below the critical threshold of pH 5.5 and conducted fungal and bacterial microbiome analysis. Preliminary results indicate bacterial soil communities may be more strongly influenced by soil pH than fungal soil communities. Liming significantly increased the relative abundance of some bacterial families, including *Pseudomonadaceae*, *Opitutaceae*, and *Flavobacteriaceae*, while decreasing others, such as the *Bradyrhizobiaceae*, though this effect was often seen only at the 0-3 inch depths. Among fungal families, liming significantly reduced the relative abundances of *Teratosphaeriaceae*, *Hypocreaceae*, and *Piskurozymaceae*, though these patterns often depended on location and soil depth. Results suggest that liming may reshape soil communities, primarily impacting bacteria, in ways that may influence plant health.

Genomic analyses of methyl-accepting chemotaxis proteins of animal and plant pathogens within the order ‘*Enterobacteriales*’

A. S. B. NASARUDDIN, A. O. Charkowski, Colorado State University, Fort Collins, CO, USA

Many bacterial members in the order ‘*Enterobacteriales*’ are plant pathogens that can cause significant losses on a wide variety of hosts, jeopardizing global food security. For instance, soft rot bacteria, which include *Dickeya dianthicola*, cause at least \$40 million of potato losses each year in the United States. There are also human/animal pathogens in this order such as *Escherichia coli* and *Salmonella typhimurium* that cause life-threatening diseases. A genomic comparison among 307 sequenced strains revealed that plant pathogenic ‘*Enterobacteriales*’ contain a higher number of methyl-accepting chemotaxis protein genes (*mcp*) compared to the human/animal pathogens in this order. Methyl-accepting chemotaxis proteins (MCPs) play a role in chemotaxis, a mechanism by which bacterial cells move in response to chemical stimuli. We hypothesized that MCPs assist plant pathogens to find their host plants and survive in various environments. To test our hypothesis, we retrieved MCP sequences from representative human/animal and plant pathogens in this order and analyzed these protein sequences to determine their functional sections (domains) and section arrangements (architectures). *E. coli* MCPs have two distinct architectures, whereas *D. dianthicola* MCPs have twelve. Our initial findings suggest that the high variations in MCP architecture in plant pathogenic ‘*Enterobacteriales*’ are important for navigating their environments and host plants.