



2022 Pacific Division Meeting Abstracts

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Assessing fitness cost in *Penicillium expansum* isolates with resistance to multiple fungicides

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Penicillium expansum is the main causal agent of blue mold (BM) on apple and pear, the most important disease of pome fruit in the Pacific Northwest (PNW). BM is primarily controlled through sanitation and application of fungicides at harvest. To better understand the control threat posed by the evolution of fungicide resistance in *P. expansum*, pathogenic fitness was assessed in *P. expansum* isolates with multiple resistance phenotypes to thiabendazole (TBZ), pyrimethanil (PYR), and fludioxonil (FDL), the three most commonly used fungicides approved for postharvest application in the PNW. Isolate fitness was assessed *in vitro* using several growth media and *in vivo* on detached Fuji apples. *In vitro* fitness assays for conidial germination, sporulation, mycelial growth, response to osmotic stress and reactive oxygen species, and resistance stability were conducted at 20° and 1°C. *In vivo* assays for virulence, sporulation, and resistance stability were performed at 1°C in standard atmospheric conditions. Preliminary *in vitro* trials at 1°C indicate that many dual- and triple-resistant isolates have reduced conidial germination, and triple-resistant isolates showed susceptibility to osmotic stress. In preliminary *in vivo* assays, resistant isolates showed no penalty to virulence when compared with sensitive isolates. Understanding fitness penalties present in resistant populations of *P. expansum* will help growers make informed decisions on fungicide application at harvest.

Harnessing soil microbiomes for camelina growth in low nitrogen inputs

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Camelina is an oilseed crop that could potentially be grown as a biofuel feedstock in rotation with dryland wheat systems if economic barriers such as nitrogen demands could be reduced. Microbiomes are known to impact plant growth and nutrient uptake, but knowledge of camelina microbiome is scarce. This study evaluated the microbiome of camelina grown in soil collected from 33 locations across four climate zones in eastern Washington, USA. We found that camelina roots had significantly lower bacterial and fungal alpha diversities compared to bulk and rhizosphere soils. Bacterial alpha diversity was highest in bulk soil from the high precipitation zone and lowest in the low precipitation zone, but fungal alpha diversity was consistent across climate zones. A core microbiome was characterized. The most abundant taxa were from the bacterial genus *Sphingomonas* and the fungal genera *Alternaria* and *Mortierella*. The core also contained several putative plant-growth-promoting, stress response and nitrogen cycling rhizobacteria, including *Rhizobium*, *Caulobacter*, *Streptomyces*, *Nitrospira* and *Bradyrhizobium*. In parallel with cultivation-independent methods, over 3000 bacterial colonies were isolated from the rhizosphere and 51 unique genera were identified through sequencing 920 colonies. These bacteria will be used in co-culture with camelina to further investigate the impacts of bacteria on camelina growth under low nitrogen conditions, disease pressure and drought stress.

Summary of late-season strawberry soilborne pathogens in the Watsonville-Salinas growing district

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There are four major soilborne pathogens of strawberry production in California, but their distribution and prevalence in the Watsonville-Salinas production district is unknown. To fill this knowledge gap, 74 symptomatic strawberry plant samples were collected from 69 fields in the Watsonville-Salinas growing district between 11 August and 15 October 2021. Field samples consisted of eight plants exhibiting moderate to severe plant collapse. Crown tissue from each plant was excised and pooled for recombinase polymerase amplification (RPA) to detect *Macrophomina phaseolina*, *Fusarium oxysporum* f. sp. *fragariae*, *Verticillium dahliae*, and *Phytophthora* spp. Root, petiole and crown tissue from plant samples in which no pathogens were detected by RPA was plated on semi-selective media to verify the absence of the four pathogens and screen for other pathogenic fungi. At least one of the four pathogens was detected in 55 of the 74 samples (74.3%). *Fusarium oxysporum* f. sp. *fragariae* was detected in 23 samples (31.1%), *M. phaseolina* in 22 samples (29.7%), *Phytophthora* spp. in 18 samples (24.3%), and *V. dahliae* in 16 samples (22.0%), demonstrating that all four of the major soilborne pathogens are prevalent in this growing district. No strong associations were found between the pathogens and growing practices.

A disease survey of industrial hemp grown outdoors in Oregon and Washington

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Hemp (*Cannabis sativa* L.) grown in OR and WA for flower, seed, and fiber is valued at \$249 million. Growers face production challenges due to plant pathogens. An assessment of pathogen impact on hemp production has not been conducted previously for the Pacific Northwest (PNW). In 2021, a two-year survey was initiated to determine disease occurrence in PNW hemp crops. Fields were evaluated one to three times during the growing season; 32 fields from OR and 11 fields from WA were surveyed. One or two 100-plant transects were evaluated, depending on field size. Each plant was visually examined for signs or symptoms of bacterial, fungal, oomycete, or virus-like diseases. Representative samples of suspect disease tissue were collected for pathogen identification by morphological characteristics or genetic typing. Plant-parasitic nematode population densities were also measured from soil samples collected in autumn from 9 OR and 9 WA fields. Virus-like diseases were suspected in 75% of OR and 100% of WA fields. Curtoviruses have been confirmed via PCR of coat protein from 54% and 91% of suspect OR and WA fields, respectively. Plant parasitic nematodes were generally found in low levels, though *Pratylenchus* spp. were found in 58% of sampled fields. Preliminary results from this survey indicate the presence of multiple pathogens that may impact hemp and other crops in the PNW landscape. This work will generate foundational data of yield-limiting diseases in PNW hemp.

Efficacy of sulfur and biological fungicide regimes on management of grape powdery mildew

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During the 2021 growing season, micronized sulfur and biological fungicides were applied during critical periods for grape powdery mildew (GPM) management on Pinot noir (PN) and Pinot gris (PG) vines while tank mixes of biological fungicides with a low rate of sulfur were tested on Chardonnay (CH) vines. In PN and PG trials, biological fungicides were evaluated by application only during the critical period of flowering (PG), or before and after flowering (PN) with 5.6kg/ha (5lb/Acre) sulfur applied outside of those timeframes. In the CH trial tank mixes of biological fungicides with 2.8 kg/ha (2.5lb/Acre) sulfur were compared to using 2.8 kg/ha sulfur alone. All fungicide treatments within each individual trial had similar GPM leaf incidence as measured by area under disease progress curve. Fungicide regimes in PN and PG trials resulted in 6-10% and 13-19% cluster severity, respectively, with non-treated controls resulting in 75% severity. In the CH trial, average cluster severity ranged from 8%-20% compared with 27% for the 2.8 kg/ha (2.5lb/A) sulfur control. These data suggest that applying biological fungicides before GPM is established in a vineyard early in the season and using known efficacious fungicides at bloom, in this case sulfur, was the most effective strategy at managing GPM cluster infections implemented in the trials. Further examination of this strategy with other products as well as other tank mix regimes will be investigated in 2022.

Exploring the epiphytic colonization capability of *Xanthomonas hortorum* pv. *carotae* on non-carrot crops

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Carrot (*Daucus carota* subsp. *sativus*) seed is an economically significant crop grown in central Oregon. One of the challenges facing carrot seed production is the management of *Xanthomonas hortorum* pv. *carotae* (*Xhc*), the causal pathogen of bacterial blight of carrot.

Xhc is an epiphytic pathogen that has been isolated from carrot leaves, stems, flowers, and seed. Field sampling in 2021 showed that viable *Xhc* can be recovered from umbelliferous and non-umbelliferous crops grown near carrot fields. To test *Xhc*'s ability to colonize non-carrot hosts, eight crops commonly grown in central Oregon were selected for a greenhouse trial. Carrot, parsley root, curly parsley, alfalfa, Kentucky bluegrass, roughstalk bluegrass, soft white winter wheat, and mint were grown and inoculated with *Xhc* bacteria (3.52×10^7 CFU total). Plants were destructively sampled at 1, 7, 14, and 27 days post-inoculation (p.i.) with five replications per timepoint. No plants of any crop showed visible symptoms of disease. *Xhc* was quantified via qPCR and dilution plating assays. At 27 days p.i., statistically similar amounts of *Xhc* were recovered from all crops except mint and alfalfa ($P = 0.05$). This suggests that *Xhc* can asymptotically colonize other crops in addition to carrot plants. The geographical proximity of these crops to carrot seed fields and their overlapping or perennial growing seasons may allow other non-carrot crops to serve as reservoirs for *Xhc* in carrot seed cropping systems.

Plant-parasitic nematodes associated with hop (*Humulus lupulus*) in the Pacific Northwest

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In 2021, the United States is the world's largest producer of hop valued at \$663 million. The Pacific Northwest (PNW) accounts for 98% of the US hop production. Despite the agricultural importance of hop, there is little information about the occurrence, distribution, and densities of plant-parasitic nematodes (PPN) in hop. In fall 2021, a three-year survey was initiated in the PNW to identify the PPN associated with hop. In the first year, 28 hop yards were sampled in WA. Seven different PPN were identified. Second stage juveniles (J2) of *Heterodera humuli*, were present in 75% of samples with an average of 47 J2/250g of soil. *Heterodera humuli* cysts were found in 98% of samples with a maximum density of 550 cysts/100g of dried soil. Other PPN commonly found in hop yards were dagger (*Xiphinema* sp.) and stunt nematodes with >50% occurrence. Three populations of stunt nematodes (one with males and two with no males) were selected for DNA extraction and sequencing of the *ITS1-5.8S-ITS2* region. The phylogenetic analysis placed the populations without males in a clade with *Tylenchorhynchus clarus* (99.8% identity with KJ461575). Meanwhile, the population with males was placed in a clade with *Bitylenchus hispaniensis* (91% identity with MZ725020). Our preliminary results indicate that a diversity of PPN are widely prevalent in WA hop yards. Future research should focus on determining the pathogenicity of PPN to hop and the development of management strategies.

Understanding Tobacco rattle virus epidemiology in potato through basic and applied assays

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Tobacco rattle virus (genus *Tobravirus*) is transmitted to potato (*Solanum tuberosum*) by stubby root nematodes of *Trichodorus* and *Paratrichodorus* spp., causing internal tuber necrosis known as corky ringspot disease that results in economic losses for growers. The continuing spread of *Tobacco rattle virus* and the nematode vector to fields across the U.S. and the reduced availability of nematicides for control of the vector has made management of this disease costly. Improving the overall understanding of corky ringspot disease epidemiology can aid in future development of novel management strategies. Using applied and basic assays, movement of the virus *in planta* and through seed tubers was assessed, as well as the expression of disease symptoms in plants grown under field conditions with heavy nematode/vector presence and in a greenhouse assay under mock-nematicide conditions. These trials determined that *Tobacco rattle virus* does not move systemically through a potato root system and that elimination of the pathogen and its vector from soil early in the season will reduce corky ringspot disease symptom development in tubers. Results also indicated that planting seed that does not have corky ringspot disease symptoms is important to prevent quality losses in daughter tuber production, even in the absence of the vector. Overall, these results suggest that growers can decrease economic losses by planting clean seed and by adequate early-season pathogen/vector control.

First report of *Podosphaera macularis* occurring on hemp in Washington State

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Hemp (*Cannabis sativa* L.) and hop (*Humulus lupulus* L.) are the two economically important crops in Cannabaceae. Hop acreage is concentrated in the Pacific Northwest (PNW), where powdery mildew incited by *Podosphaera macularis* has occurred since 1997. *Golovinomyces ambrosiae* has been reported to cause powdery mildew on hemp. During 2020, a hemp field on an Oregon State University farm was infected with *P. macularis*. In 2021, hemp plants in 32 fields located primarily in the Willamette Valley (OR) and Yakima Valley (WA) where hops are also produced were surveyed shortly after transplanting as well as mid- and late-season for powdery mildew incidence. Ten leaves were examined on each plant in two 100-plant transects. Molecular characterization of nine powdery mildew leaf samples from each field on each date was done with 28S rRNA-based assays. By the end of the growing season, powdery mildew was detected in 12 of 21 fields in OR and in one of the 11 sites in WA. Based on molecular typing, *G. ambrosiae* occurred in all affected fields while *P. macularis* co-occurred in several fields, including one site in the Yakima Valley. This is the first report of *P. macularis* on hemp in WA. Although hops are harvested before hemp, cultivation of *Cannabis* and *Humulus* in close proximity to each other may allow exchange of *P. macularis* inoculum between the crops, possibly infecting hop regrowth in the fall. This could portend potential development of novel genotypes by *P. macularis*.

Survey of *Cytospora* spp. in peach, cherry, and apple orchards in Colorado

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Cytospora canker causes high mortality in pome and stone fruits on the Western Slopes of Colorado. Surveys of peach, cherry, and apple orchards were conducted to investigate the occurrence and diversity of *Cytospora* species associated with canker damage across hosts. In this preliminary research, 3 peach, 1 cherry, and 1 apple block were surveyed. Samples were collected from the trunk and/or larger scaffolds using a knife to excavate 2-3 centimeters of tissue. Small limbs and first-year growth were collected using pruning shears. The wound was then treated with Vitaseal to protect the exposed tissue. All samples were cultured on ½ potato dextrose agar media for two days and emerging fungal colonies were hyphal tip transferred to new plates for morphological characterization. DNA was extracted from fungal hyphal was then and the ITS (internal transcribed spacer) region and EF1a (elongation factor 1 22 alpha) were sequenced for species identification. We identified *C. plurivora*, *C. parasitica*, *C. chrysosperma*, and *C. globosa*, and no individual *Cytospora* spp. was present in all hosts, potentially indicating host preference. These findings help us develop a better understanding of *Cytospora* diversity in Western Slope pome and stone fruit orchards and lay a foundation to further investigate the epidemiology of *Cytospora* pathogens in this region.

Compost amendments affect plant performance and soil quality following two years of application in a commercial crown gall-diseased vineyard

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Crown gall is an economically devastating grapevine disease that is most severe in cold-climate viticulture regions. This disease, induced by *Allorhizobium vitis*, is characterized by tumor growth that leads to reductions in grapevine performance and crop quality. Soil-borne *A. vitis* infection is augmented by the co-presence of root-feeding nematodes, increasing the number of root wounds through which *A. vitis* can establish systemic infection. In previous studies, compost application reduced populations of pathogenic nematodes in soil. It was hypothesized that compost amendments would reduce soil-borne *A. vitis* infection by reducing root-feeding nematode populations. Three composts were applied in-row in a vineyard infested with both *A. vitis* and pathogenic nematodes in spring 2019, 2020, and 2021. Bulk soil was collected twice per year for *A. vitis* and pathogenic nematode (cyst, dagger, lesion, pin, and ring) quantification. Plant performance, crop and soil quality, and disease severity were assessed each year. After two years of application, soil quality parameters improved, and bud fruitfulness increased compared to untreated controls. Nematode populations varied over two years and dagger nematode numbers were lower in one compost treatment compared to controls. No differences in *A. vitis* abundance or disease severity were observed. These results suggest that soil health and plant performance may improve in crown gall-diseased vineyards with repeated compost use.

Management of *Botryosphaeria*/*Phomopsis* canker and blight diseases of walnut in San Joaquin Co., California

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In the last few years, *Botryosphaeria* and *Phomopsis* canker and blight diseases of walnut have been observed increasingly in almost all walnut growing regions in California. Despite several management practices, recent field surveys in San Joaquin Co. showed Diaporthaceae fungi to be the most prevalent fungal pathogen isolated from diseased walnut samples while *Botryosphaeriaceae* fungi were only found occasionally. Following a two-year spore trapping study we confirmed the correlation between fungal spore releases and precipitation events, first season irrigation, and shredding of infected prunings in the orchard. Molecular identification revealed the presence of three new species of *Diaporthe* that were found in both spore traps and diseased samples. Pathogenicity of these new species was assessed using spore suspensions and mycelium plugs inoculations in 3-year-old branches in the orchard. Fruits were also inoculated in the laboratory using mycelium plugs. Results showed that the three species tested were pathogenic and infect both shoots and fruits of walnut. We conducted a spray program starting at bloom time and verified its effectiveness in significantly reducing blighted fruit. Protection of pruning wound trials were also conducted, and thiophanate methyl (Topsin M) was the most effective application in protecting pruning wounds from infection. A biological, *Trichoderma*-based product, showed promising results but provided lower disease control than Topsin M.

Botrytis cinerea varies in its sensitivity towards common fungicides used in pear orchards

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The fungal pathogen *Botrytis cinerea* causes gray mold in pear and has developed resistance to various fungicides. In this study, 133 *B. cinerea* isolates collected from pear orchards and packing houses in southern Oregon were screened against commonly used fungicides. *B. cinerea* isolates were tested against seven fungicides containing the following active ingredients: dicloran, iprodione, triflumizole, cyprodinil, fenhexamid, dodine, and polyoxin D zinc salt. Spore suspension of isolates (1×10^5 spores/ml) was added to

fungicide amended media and spore germination (%) and germ tube length were estimated for 50 spores per isolate. Based on germ tube length, isolates were classified as sensitive, moderately sensitive, moderately resistant, or resistant. Each isolate was also classified as one of these categories based on which category contained the highest percentage of spores. Dodine was the most effective fungicide with 98% of the tested isolates classified as sensitive or moderately sensitive. Fenhexamid also was effective with 94% of the tested isolates classified as sensitive or moderately sensitive. Dicloran, triflumizole, and polyoxin D zinc salt were the least effective with 29, 31, and 29% of the tested isolates classified as sensitive or moderately sensitive against each fungicide respectively. These findings show that *B. cinerea* varies in its sensitivity towards different fungicides that are commonly used in pear orchards across the Pacific North West.

Evaluation of US mini-core collection of peanut for resistance against *Athelia rolfsii*

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A necrotrophic soil-born fungus, *Athelia rolfsii* causes devastating stem rot disease on peanuts (*Arachis hypogea* L.). Developing and planting resistant peanut varieties has been considered a sustainable strategy to manage the disease, and robust phenotyping is critical for the efficient identification of resistant sources. Germplasm is a valuable source for required genes and has a diverse genetic base. Seventy-nine peanut accessions were evaluated for stem rot resistance in a growth chamber by artificial inoculation using the slurry method. Disease incidence and severity were determined every 3 days after fungal inoculation. Disease incidence and disease severity was rated on a scale of 0 to 4, with 0 = no disease symptoms, 1 = disease symptoms without visible fungal growth, 2 = disease symptoms with visible fungal outgrowth, 3 = partial wilting of the plant and 4 = complete wilting resulting in plant death. Initial evaluation from this study resulted in 32 accessions with zero disease symptoms; 14 accessions showing symptoms without visible fungal growth; 19 accessions showing symptoms with visible fungal growth and 14 accessions showing partial to complete wilting. We will be repeating this study to evaluate the stem rot based on phenotype data and modeling data for further utilization in breeding programs towards developing stem rot-resistant peanut cultivars with minimal compromise of yield, thus resulting in better economics for the farming community.

Efficacy of crop termination on managing *Verticillium* wilt in strawberry

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Verticillium wilt in strawberry causes plant wilt and collapse. The effect of crop termination on soil inoculum density and pathogen survival in crop tissue was evaluated during the 2020-21 growing season on California's central coast. Fumigation treatments included nontreated control, metam potassium (KPAM), and metam sodium (VAPAM). Subplots (30 × 1.6 m) containing 310 'Seascape' plants were treated with either KPAM or VAPAM. A total of 12 plots were terminated with KPAM, while 6 plots were terminated with VAPAM. Three control plots were left untreated. Pre-plant *Verticillium dahliae* soil inoculum density was 7.8 colony forming units (CFU)/g. Plots were terminated when plants were at 5% mortality and 45% symptom expression. Post crop termination inoculum densities were control (15.0 ± 2.7), KPAM (5.7 ± 0.4), and VAPAM (4.8 ± 0.5) CFU/g. Soil inoculum density significantly decreased in treated versus nontreated beds ($P < 0.001$), and the two fumigants achieved similar efficacy ($P = 0.73$). With all other predictors held constant, the log-odds of *Verticillium dahliae* survival were four times less in terminated crops than for unterminated crops. There was no significant difference in the likelihood of *Verticillium dahliae* survival in both crown and petiole ($P = 0.73$) in the unterminated crop. *Verticillium dahliae* was more likely to survive in the petiole ($P < 0.001$) in crops terminated with KPAM and VAPAM.

Temporal changes in soil microbiome in response to soil fumigation in strawberry production systems

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Verticillium wilt of strawberry is an economically significant disease in California. This study aimed to elucidate the temporal changes of soil microbiome composition in response to fumigation using a highly susceptible cv. Portola. The study was conducted in a field naturally infested with *Verticillium dahliae*. Soil fumigant treatments were allyl isothiocyanate (AITC), chloropicrin (PIC), allyl isothiocyanate + chloropicrin (AITC+PIC), metam potassium (MP) and no-treatment control (CON). Treatments were replicated 6 times in a completely randomized design. Soil samples were obtained at pre-treatment, planting, one month after planting, peak fruit production and the end of the season (T5). DNA was extracted from soil and amplicon sequencing was conducted for bacteria and fungi with Illumina MiSeq platform. Fumigants affected diversity of bacterial and fungal communities over time. At T5, relative abundance of specific Planctomycetes, Proteobacteria, Ascomycota and Basidiomycota operational taxonomic units (OTUs) increased in the AITC treatment whereas specific Actinobacteria, Firmicutes, Proteobacteria OTUs decreased in the PIC and AITC+PIC treatments compared to CON. Relative to CON, certain OTUs of Ascomycota increased in the AITC+PIC treatment at T5. Relative abundance of specific Armatimonadetes, Bacteroidetes, Chloroflexi, Firmicutes, Proteobacteria, Ascomycota, Zygomycota and Chytridiomycota OTUs decreased in the MP treatment in comparison to CON at T5.

Characterizing species composition of *Neonectria* spp. of pome fruit in the Pacific Northwest

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Neonectria fruit rot (NFR) is a widespread disease of apple and pear in Washington (WA) and Oregon (OR) and it has been detected in 40% of lots surveyed in the US Pacific Northwest (PNW). NFR is caused by ascomycete fungi of the *Neonectria* genus., which also cause cankers on trees on which they overwinter and produce globose perithecia as they mature on the cankers. The causal species of tree cankers and NFR in the PNW have not yet been characterized. Here, we aimed to characterize the species of *Neonectria* that cause NFR on pome fruit in the PNW. Thus, 133 *Neonectria*-like isolates were isolates from decayed apples and pears collected from different warehouses between 2016 and 2018. Isolates were grown in potato dextrose broth (PDB) for 7 days at 22°C and DNA was extracted. The isolates were sequenced to identify species using β -*tubulin* and *translation elongation factor* (EF) DNA markers. Assembled nucleotide sequences were generated and Basic Local Alignment Search Tool (BLAST) search was carried out on NCBI. Preliminary results indicate that four *Neonectria* species, i.e., *N. neomascorpa*, *N. cinnabarrina* (*N. dematiosa*), *N. nigrescens* and *N. candida* are causing NFR and Nectria cankers in the PNW. Work is ongoing to characterize additional isolates, their virulence, and fungicide sensitivity to enhance *Neonectria* management on pomes.

Host range and genetic diversity of *Fusarium commune* among conifer nurseries of the contiguous U.S.A.

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Fusaria can be introduced into novel landscapes through latent infected nursery stock and cause seedling mortality. Molecular tools can rapidly identify and track these pathogens to mitigate novel pathogen introductions by informing both pathogen composition and pathogenicity of *Fusarium* spp. *Fusarium commune* is an understudied species that can be pathogenic to both crops and timber trees; however, host range and virulence mechanisms have not been elucidated. Our work has three objectives: 1) evaluate the diversity and occurrence of *Fusarium* spp. in conifer nurseries across the U.S.A. to identify host associations and geographic distributions among populations of pathogenic *Fusarium*, 2) evaluate host range of *F. commune* isolates collected from conifer hosts through virulence assays on both conifer and non-conifer hosts, and 3) use genomic tools to identify candidate virulence genes in host-pathogen interactions and design diagnostic primers for rapid identification and tracking. A total of 19 *Fusarium* spp. were collected from conifer nurseries in the U.S.A., including *F. commune*. For diagnostic primer development, unique genomic regions and virulence genes of two conifer-derived, *F. commune* isolates were compared with two non-conifer isolates and reference genomes. This work will help develop tools to quantify inoculum pressure in conifer nurseries, and identify factors/genomic regions involved in host specificity within *Fusarium*.