lik[1], Neil Gudmestad[1], Binod Pandey[1], Fereshteh Shahoveisi[1], Sarah Budde-Rodriguez[2], Ohud Alam[1], Julie Pasche[1] 1) North Dakota State University; 2) BioSafe Systems.

Recent literature reported that brown leaf spot of potato in the Pacific Northwest US is caused by several small-spored *Alternaria* spp (SSA). Our laboratory has collected putative *A. alternata sensu lato* isolates from several potato producing states in the US for almost 20 years. An NCBI BLAST nucleotide match from *OPA1-3* gene sequences from a sub-set of two hundred isolates identified three species of SSA as *A. alternata sensu stricto*, *A. tenuissima* and *A. arborescens*. These three species are similar morphologically, displaying overlapping and variable characteristics, and have indistinguishable disease symptomatology. A phylogenetic characterization of sixty-nine SSA isolates were performed using four gene sequences, *OPA1-3*, *Alt a1*, *ITS* and *TEF*. The phylogenetic analysis based on *ITS*, and *TEF* revealed no diversity among the SSA isolates. The *Alt a1* analysis classified the SSA isolates into two major groups, with no distinction between *A. alternata sensu stricto* and *A. tenuissima*. The phylogenetic analysis based on *OPA1-3* gene sequences classified the SSA isolates in three distinct groups as *A. alternata sensu stricto*, *A. tenuissima* and *A. arborescens*. A multiplex real-time PCR was developed based on SNPs identified in the *OPA1-3* gene to distinguish isolates of *A. alternata* (E=100%, R^2 value=0.97), *A. tenuissima* (E=104%, R^2 value=0.98) and *A. arborescens* (E=102%, R^2 value=0.98). Multiplex real-time PCR differentiation was 99% successful when compared to sequences from the *OPA* gene, moreover closely-related phytopathogenic fungi were not amplified. The multiplex real-time PCR performed on three-hundred and four SSA isolates indicate that all three species were found in association with brown leaf spot of potato in the US and Canada. In both populations, *A. alternata* represented greater than 60% of all SSA isolates recovered. Isolates of all three species were found as early as 2000 in the US; however, the frequency of *A. alternata* isolates has decreased recently. Among US isolates collected in 2000, 2003, 2011, 2013 and 2014, the frequency of *A. alternata* ranged from 75% to 93%. In 2017, 2018 and 2019 that decreased to 47% to 61%. It is unclear if the shift in SSA species associated with brown leaf spot will affect disease severity or management but differences in sensitivity to foliar fungicides is being investigated. The use of the multiplex PCR assays will facilitate accurate characterization of the potato brown leaf spot pathogen complex in the future.

565F ***Fusarium*** **in Nebraska Corn** *Yuchu Ma*[1], Heather Hallen-Adams[1] 1) University of Nebraska-Lincoln.

Members of the *Fusarium sambucinum* (especially *F. graminearum*) and *Fusarium fujikuroi* species complexes are among the most common and economically important pathogens infecting corn in Nebraska. These fungi can produce trichothecene and fumonisin mycotoxins, respectively, causing harm to human and animal health. A total of 61 whole plant corn samples have been randomly collected from 21 countries in Nebraska. Two samples each for ear, stalk and root from each plant were placed on Fusarium Selective Media with pentachloronitrobenzene for 10 days incubation and isolates with *Fusarium* colony characteristics were transferred on to potato dextrose agar and incubated for 10-14 days. DNA was extracted and evaluated by PCR amplification reaction of the eukaryotic translation elongation factor 1 α (EF-1α), followed by Sanger sequencing and blastn. Besides *Fusarium graminearum*, we have found multiple species of both the *Fusarium sambucinum* and *Fusarium fujikuroi* species complexes and *Fusarium oxysporum*, as well as some additional species. This data is being compared with our previous characterization of *Fusarium* species on Nebraska wheat, especially in samples from areas where both corn and wheat are grown. Additionally, assays for deoxynivalenol and fumonisin B1 are being conducted.

566W Genetic diversity of *Fusarium oxysporum* f. sp. *vasinfectum* California race 4 isolates and Alabama field isolates *Miranda Otero*[1], Ambika Pokhrel[1], Seungyeon Seo[1], Laura Wendell[1], Jeffrey J. Coleman[1] 1) Auburn University, Auburn, Alabama.

Fusarium oxysporum f. sp. *vasinfectum*, the causal agent of Fusarium wilt on cotton, can lead to leaf chlorosis, wilting, darkening of the vascular tissue, and plant death. Multiple genotypes of this soil fungal pathogen have existed in the United States for over one hundred years. However, a more virulent genotype (race 4) was initially found in California in 2001. Race 4 was previously restricted to India and can cause disease in the absence of nematodes which is a main management strategy for Fusarium wilt. In 2017, race 4 was identified in Texas and New Mexico and could potentially spread to the remaining cotton belt. Pulse-field gel electrophoresis and multi-locus sequencing were used to evaluate genetic diversity among California race 4 isolates and Alabama field isolates. Among California race 4 isolates, we observed a variation in the number and of size of small chromosomes which could be associated with host-specific virulence. Two housekeeping genes (*translation elongation factor 1 alpha* and *DNA-directed RNA polymerase II core subunit*) of 130 field isolates collected in Alabama from 2014 and 2016 were used to construct a phylogenetic tree. As in previous surveys, most Alabama isolates grouped with races 1, 2, and 6, and there were no isolates that grouped with races 3 and 5. Four isolates grouped with races 4 and 7 but were determined not to be California race 4 isolates due to the absence of the *Tf01* transposon insertion in the *PHO* gene. Unexpectedly, 20 different haplotype groups were recovered. Cotton virulence assays will be conducted to assess pathogenicity of representatives from the 20 haplotype groups and to compare virulence among California race 4 isolates.

567T Genetic diversity and pathogenicity of *Botryosphaeriaceae* and *Diaporthaceae* causing defects of hazelnut nuts from Italy. *Muhammad Waqas*[1,2], Vladimiro Guarnaccia[1,2], Davide Spadaro[1,2] 1) Centre of Competence for the Innovation in the Agro-environmental Sector-AGROINNOVA, University of Turin, Grugliasco, TO, Italy; 2) Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, TO, Italy.

Hazelnut (*Corylus avellana*) is considered an important nut crop worldwide and a rich source of vitamins, minerals, and plant proteins. Italy is the second-largest hazelnut producing country (110,000 t/year) after Turkey, on a surface of 81,000 ha. Major constraints for hazelnut production are members of *Botryosphaeriaceae* family and genus *Diaporthe* which are responsible for several defects (internal discoloration, necrosis, blemishes) in hazelnut kernel, and can reduce the hazelnut quality and yield by altering its kernel. In order to investigate the phenomenon of rotten hazelnuts and to identify the responsible agents, a survey was initiated during 2020. A total of 383 samples having the symptoms of black rot (incidence: 32%), mouldy rot (incidence: 41%) and necrosis (incidence: 27%) were collected from Piedmont, northern Italy. Fungal genomic DNA was extracted, and multi-locus phylogeny was performed based on combined partial genomic region ITS and the partial gene *tef-1α*. ITS and *tef-1α* sequences were obtained after PCR amplification using the primers ITS1/ITS4 and EF1-728F/EF1-986R, sequencing and phylogenetic analysis for species identification. Pathogenicity tests were performed on ripening hazelnuts 'Tonda Gentile del Piemonte'. Three nuts per isolate, and per three replicates were surface disinfected with 1% NaClO. A piece of shell (5 mm diameter) from nuts was removed with a sterile cork borer and inoculated with mycelium plug cut

from 7 days old PDA colony. Research results revealed that isolates represent 6 species of *Diaporthe* (*D. eres*, *D. rudis*, *D. novem*, *D. oncostoma*, *D. ravennica*, *D. foeniculina*) and 3 species of *Botryosphaeriaceae* (*Botryosphaeria dothidea*, *Diplodia seriata* and *Neofusicoccum parvum*). Overall incidence of *Diaporthe* spp., *B. dothidea*, *D. seriata* and *N. parvum* were 39%, 20%, 15% and 5%, respectively. Pathogenicity results revealed that all these species are pathogenic to the tested cultivar. All the hazelnut kernels showed abundant development of pycnidia with different disease index. Additionally, isolates from *B. dothidea*, *D. seriata* and *N. parvum* produced black lesions with softening pulp. *D. eres* was more virulent compared with the other *Diaporthe* spp. *N. parvum* was the most pathogenic species among *Botryosphaeriaceae.* The present study improves our understanding of the species associated with hazelnut defects and provides useful information for effective management of the nut disease.

568F The Systematics of North American *Rhizopogon* Using Modern Molecular Techniques *Thelmalyn Montenegro*[1], Emeline Pano[1], Alija Mujic[1] 1) California State University, Fresno, Fresno.

Rhizopogon is a genus of truffle-forming fungi that forms mutualistic relationships with Pinaceae trees, the family of pine trees, which are critical to the healthy function of coniferous forests. These mutualistic relationships are termed ectomycorrhizae (ECM), and they are important because fungi protect plant roots from pathogens, directly exchange nutrients with plants, and facilitate environmental nutrient cycling. *Rhizopogon* species possess reduced morphology, or loss of distinguishing morphological features over evolutionary time, compared with other fungi, and traditional identification methods based upon morphology have failed to accurately describe the true species diversity of the genus. The purpose of this study is to investigate the diversity of *Rhizopogon* species across North America, with a particular focus on the Pacific Northwest geographic region. The results of this research have implications for future systematic studies of many fungal genera and provide valuable information for federal land-use managers where sensitive or rare species of *Rhizopogon* are found. Previous work has used only morphological characters to assess evolutionary relationships within *Rhizopogon* and generated many species-level classifications which may be a misestimation of true species diversity in the genus. Previous molecular phylogenetic analysis of the genus established 5 subgeneric levels in genus *Rhizopogon*, and this work seeks to refine these taxonomic delimitations and expand sampling of type specimens to further clarify true species diversity. Accomplishing these tasks are the primary goals of this study. This work achieves a many-fold increase in holotype sequence data compared with previous studies by using modern enzyme technologies and refined DNA extraction protocols. Here we infer multigene phylogenies incorporating *Rhizopogon* holotype sequence data, using maximum likelihood and Bayesian analyses, and significantly revise species hypotheses and systematic relationships of North American *Rhizopogon* species.

569W Genomic diversification of the specialized parasite of the fungus-growing ant symbiosis *Kirsten Gotting*[1], Daniel May[1], Jeffrey Sosa-Calvo[2], Lily Khadempour[3], Charlotte Francoeur[1], Margaret Thairu[1], Shelby Sandstrom[1], Caitlin Carlson[1], Marc Chevrette[1], Monica Pupo[4], Tim Bugni[1], Ted Schultz[2], J. Spencer Johnston[5], Cameron Currie[1] 1) University of Wisconsin-Madison, Madison, Wisconsin; 2) Smithsonian Institution, Washington, DC; 3) Rutgers University, Newark, New Jersey; 4) University of São Paulo, Ribeirão Preto, Brazil; 5) Texas A&M University, College Station, Texas.

Fungi shape the diversity of life. Characterizing the evolution of fungi is critical to understanding symbiotic associations across kingdoms. In this study, we investigate the genomic and metabolomic diversity of the genus *Escovopsis*, a specialized parasite of fungus-growing ant gardens. Based on 25 high-quality draft genomes, we show that *Escovopsis* forms a monophyletic group arising from a mycoparasitic fungal ancestor 61.82 million years ago (Mya). Across the evolutionary history of fungus-growing ants, the dates of origin of most clades of *Escovopsis* correspond to the dates of origin of the fungus-growing ants whose gardens they parasitize. We reveal that genome reduction is a consistent feature across the genus *Escovopsis*, largely occurring in coding regions, specifically in the form of gene loss and reductions in copy numbers of genes. All functional gene categories had reduced copy numbers, but antimicrobial resistance and pathogenic virulence genes maintained functional diversity. Biosynthetic gene clusters contribute to differences among *Escovopsis* spp., and a similar diversity is also present in metabolomes of sister taxa in the Hypocreaceae. Taken together, our results indicate that *Escovopsis* spp. evolved unique genomic repertoires to specialize on the fungus-growing ant-microbe symbiosis. This genomic evolution represents an example of a eukaryotic genus evolving a reduced genomic toolkit while maintaining ancient host associations.

570T Interrogating the poplar fungal microbiome interactions using meta-transcriptomics and constructed communities *Jake Nash*[1], Keaton Tremble[3], Brian Looney[1], Corbin Bryan[1], Khalid Hameed[1], Yi-Hong Ke[1], Melissa Cregger[2], Nicholas Dove[2], Christopher Schadt[2], Rytas Vilgalys[2] 1) Duke University, Durham, NC; 2) Oak Ridge National Laboratory, Oak Ridge, TN; 3) The University of Utah, Salt Lake City, UT.

Poplar trees (genus *Populus*) are host to a diverse root fungal microbiome including ectomycorrhizal, arbuscular mycorrhizal, and endophytic fungi. These fungi perform services for the plant host including growth promotion, nutrient acquisition, protection from pathogens, and conferral of abiotic stress tolerance. Meta-transcriptomics can provide large amounts of data on the function and taxonomic composition of the poplar root fungal microbiome. We developed an RNA-seq method using a synthetic spike-in standard curve that allows for the calculation of absolute abundances of fungal transcripts on poplar roots. We implemented a bioinformatics workflow that provides taxonomic and functional annotations of assembled fungal contigs from meta-transcriptomic data. These methods were applied to an ecosystem-scale time-series field experiment to document taxonomic and functional shifts of the poplar fungal microbiome in response to a historic drought in the semi-arid American West during the summer of 2021. We identified transcripts from a previously isolated dark septate endophyte in the genus *Hyaloscypha* as a highly active root colonizer across our field sites. Dark septate endophytes are a functionally diverse group of root associates that have been described as either mutualists, commensalists, or latent pathogens. We conducted further work to understand the characteristics of the *Populus-Hyaloscypha* association. *In* vitro inoculations with this fungus demonstrated compatibility with both *Pinus* and *Populus*, and suggested that it engages in antagonistic interactions with arbuscular mycorrhizal fungi during plant host colonization. We were also able to establish simplified constructed communities with this fungus and three common ectomycorrhizal fungi, ranging in diversity from one to four species. These constructed communities will allow us to identify interactions between fungi during root colonization and evaluate the effects of fungal diversity on plant performance and nutrient uptake. Future work will also 1) dissect the molecular mechanisms of the antagonistic interaction with arbuscular mycorrhizal fungi, 2)