

# Middle Atlantic States Mycology Conference 2015 MASMC Program

## Friday, April 10

**5-9 pm: reception for early arrivals**

Vilgalys' home, 2920 Hope Valley Rd., Durham,  
NC. Dinner and drinks provided.



## Saturday, April 11

**all events are located in French Family Science Center room  
2347. Parking is available in the Chemistry lot (corner Circuit Dr.  
& Towerview Dr, parking pass required)**

### 8:30-10:00 ORAL PRESENTATIONS

**Steven A. Higgins, Robert A. Sanford, Joanne Chee-Sanford,  
Christopher Schadt, and Frank E. Löffler.** Univ. Tenn. and ORNL.  
Growth and denitrification potential of a *Guehomyces pullulans* soil  
isolate

**Khalid. M Hameed, Kevin Le, Gregory Bonito, Hui-Ling (Sunny)  
Liao and Rytas Vilgalys**

Duke University and Michigan State Univ.

Ecological aspects of chitinase activity of *Mortierella elongata* (PMI#  
93) and its prospects on plant protection and nutrition in soil

**Yufeng Fang and Brett M. Tyler.** Virginia Tech and Oregon State  
University.

Deciphering nuclear localization signals in *Phytophthora sojae*

**Emma Harrower.** Department of Ecology and Evolutionary Biology,  
University of Tennessee

Transcriptomics and Functional Differences in *Cortinarius* species

### 10:30-11:00 MORNING COFFEE BREAK

### 11:00-12:00 ORAL PRESENTATIONS

**Jessica M. Vélez, Cyd E. Hamilton, Khalid Hameed, Nancy Engle, Gregory Bonito, Timothy J. Tschaplinski, Jessy L. Labbé, Rytas Vilgalys, Christopher W. Schadt** Oak Ridge National Laboratory, Duke University, and Michigan State University

A radial growth rate comparison and competitive inhibition study of an *Atractiella* species: characterization of a little-known fungal root endophyte

**Jan M. Pettersson, J. Frampton, J. Rönnerberg, D. Shew, D. M. Benson and M. A. Cubeta.** North Carolina State University.

A Survey of Phytophthora Species in Fraser fir Christmas Tree Plantations in the Southern Appalachians

**Tatsiana Shymanovich, Nikki D. Charlton, Carolyn A. Young, Ashleigh Musso, Nadja B. Cech, Stanley H. Faeth** University of North Carolina at Greensboro and The Samuel Robert Noble Foundation

Distribution and description of *Epichloë* endophytes from *Poa alsodes* along a latitudinal gradient

## **12-2 pm: GROUP PHOTO FOLLOWED BY LUNCH AND POSTERS**

**H. Van T. Cotter, Carol Ann McCormick, Shanna Oberreiter, Liane Salgado**

University of North Carolina Herbarium (NCU). Chapel Hill, North Carolina

NCU Fungal Herbarium – a Mother Lode of Types

**Jessie Uehling, Gregory Bonito, Khalid Hameed, Jessy Labbé, Dale Pelletier, Tim Tschaplinski, Amy Schaefer, Chris Schadt, Francis Martin, Rytas Vilgalys**

Duke University, Oak Ridge National Laboratory, University of Washington, Institut National de la Recherche Agronomique  
Genomic analyses of *Mortierella elongata* and associated bacterial endosymbiont (Candidatus *Glomeribacter* sp.)

**Kathryn Hanser, Bryan R. Cody, and Marc A. Cubeta.** North Carolina State University

Focus Stack Imaging of Herbarium Collections of Wood Decay  
Macrofungi:  
A Demonstration of Proof of Concept

**Ko-Hsuan Chen, Hui-Ling Liao, A. Elizabeth Arnold, Francois Lutzoni**

Duke University and Univ. of Arizona

Using metatranscriptomics to characterize functional shifts in endophytic fungi at plant senescence: Are endophytic fungi latent saprotrophs?

**Brianna L. Hoge, Marc A. Cubeta, and David F. Ritchie.** North Carolina State University.

Characterization of *Colletotrichum* species on apple in North Carolina utilizing fruit symptoms and colony morphology.

**Posy E. Busby, Kabir G. Peay, and George Newcombe**

Duke University, Stanford University, and University of Idaho,  
Common molds modify plant disease.

**Maria Gabriela Mariduen-Zavala, Mirian Villavicencio, Fernando Espinoza, and Juan Manuel Cevallos-Cevallos.** Centro de

Investigaciones Biotecnológicas del Ecuador

Morphological and molecular diversity of *Moniliophthora roreri* and *Moniliophthora perniciosa* in Ecuador

**Juan Manuel Cevallos-Cevallos and Maria Gabriela Mariduen-Zavala**

Centro de Investigaciones Biotecnológicas del Ecuador

Metabolomics and fungicide resistance: Insights into the *Phytophthora infestans* and *Mycosphaerella fijiensis* models

## **2:30-4:00 AFTERNOON ORAL PRESENTATIONS**

**Marisol Sánchez-García and Brandon Matheny.** University of Tennessee

Diversification in the Tricholomatoid clade

**Inga M. Meadows, Julia L. Kerrigan, Joan L. Walker.** Clemson University and USFS Southern Research Station, Clemson, SC  
Burn times influence smut distribution in longleaf pine savannas

**Hailee Korotkin, K. McFarland, P.B. Matheny.** Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville  
Bringing fungal-bryophyte associations to light: studies of the *Rickenella-Dicranum* association

**Lauren Czaplicki, Rytas Vilgalys and Claudia Gunsch**  
Duke University  
Correlating Contaminant Profiles to Fungal Communities at the Atlantic Wood Industries Superfund Site

**Megan Miller and Marc A. Cubeta.** North Carolina State University  
Thermal Inactivation of the Causal Agent of Boxwood Blight, *Calonectria pseudonaviculata*

**Brian Looney, Jessy Labbé and P. Brandon Matheny.** University of Tennessee and Oak Ridge National Laboratory  
Are decomposition pathways maintained in a family of ectomycorrhizal fungi?

**4:00 pm                    MASMC BUSINESS MEETING**

**6 pm DINNER followed by MASMC lecture by Prof. Cathy Cripps, Montana State University, Bozeman:  
“Rocky Mountain Highs and Arctic Lows: Diversity, ecology, and systematics of ectomycorrhizal fungi above and beyond treeline”**

## **Sunday, April 12**

**8:30 am-12:00 pm                    Morel Foray in Duke Forest, followed by a tailgate lunch (provided) before guests depart. (Directions to the foray site will be passed out to guests during Saturday’s meetings.)**

## Welcome 2015 MASMC Speaker

### Professor Cathy Cripps

Department of Plant  
Sciences and Plant  
Pathology, Montana State  
University, Bozeman, MT  
59717, USA



### "Rocky Mountain Highs and Arctic Lows: Ectomycorrhizal fungi above and beyond treeline"

The windswept arctic tundra together with disjunct alpine areas on mountain tops comprise a cold-dominated biome that covers 8% of the earth's land. A limited diversity of ectomycorrhizal fungi (EMF) survive by clinging to roots of dwarf and shrub willows, low bog birch, *Dryas*, and *Kobresia*. Sporocarp surveys in Greenland, Iceland, Svalbard, Scandinavia, Alps, Pyrenees, and the Carpathian mountains suggest a common set of EMF species across this broad landscape. Some of the same EMF species appear to be present in the alpine zone of the southern and central Rocky Mountains, hundreds of miles from the arctic. Recent molecular phylogenetic studies are beginning to confirm conspecificity of Rocky Mountain taxa (in *Inocybe* and *Lactarius*) with European type specimens and collections from other Arctic and alpine habitats; additional studies are underway. Distributions reflect plant host range, glaciation and limiting abiotic factors. Climate change has brought shrub encroachment which likely will alter EMF communities and ultimately overall microbial functioning in Arctic and alpine systems. Lessons from the alpine and on cold-blooded mycologists will punctuate the research presentation.

## 2015 MASMC ABSTRACTS (in no particular order!)

### **Growth and denitrification potential of a *Guehomyces pullulans* soil isolate**

Steven A. Higgins, Robert A. Sanford, Joanne Chee-Sanford, Christopher Schadt, and Frank E. Löffler

The nitrogen (N) cycle is Earth's most disrupted biogeochemical cycle. Human technology has altered the N cycle, resulting in increased soil N inputs (e.g., fertilizers) and microbial activity responsible for greenhouse gas release from soils. Specifically, denitrification, the formation of gaseous products from nitrate or nitrite, is implicated in increased nitrous oxide (N<sub>2</sub>O) release from soils. Although the prevailing dogma suggests *Bacteria* dominate N<sub>2</sub>O production, recent evidence implicates *Fungi* in substantial contributions to soil N<sub>2</sub>O emission, especially in agricultural ecosystems with high N input. Efforts to cultivate denitrifying fungi from agricultural soils in the Midwest Cornbelt resulted in the isolation of a dimorphic yeast strain putatively identified as *Guehomyces pullulans*, a fungus previously observed to be abundant in agricultural soils and capable of degrading lignin. After growing *G. pullulans* in nitrite-amended anoxic mineral salts medium for 20 days, micromolar concentrations of N<sub>2</sub>O were detected in the headspace of the cultivation vessels. Degenerate oligonucleotide primers previously designed to target *p450nor* genes, encoding the enzyme responsible for N<sub>2</sub>O production in fungi, were unable to amplify this gene from *G. pullulans*. A lack of molecular tools to comprehensively target fungal denitrifiers highlights the importance of cultivation-based studies to identify the role of fungi in N cycling. Further efforts to characterize the mechanism of denitrification in this fungus are ongoing. These findings identify a previously unknown role for *G. pullulans* in N cycling and may offer a potential link between carbon (e.g., lignin) and nitrogen cycling in soil fungi. Current efforts also aim at optimizing growth of *G. pullulans* under anoxic conditions.

### **Transcriptomics and Functional Differences in *Cortinarius* species**

Emma Harrower

Univ. Tennessee, Knoxville, TN

How do multiple species of the same genus co-exist in the same environment? Are species functionally redundant, or is niche-partitioning present? The ectomycorrhizal mushroom genus *Cortinarius* shows great morphological and genetic diversity in forests around the world. Are they just as functionally diverse? I present a proposal to obtain 10 *Cortinarius* genomes and 10 *Cortinarius* transcriptomes from fruiting bodies found in a monodominant Fraser Fir (*Abies fraseri*) stand in the Great Smoky Mountains National Park. I will examine the similarities and differences in transcriptomes between species. I hypothesize that species will differ in the gene family, number of copies and the sequence similarity of genes involved in the carbon, nitrogen and phosphorous cycles.

### **Diversification in the Tricholomatoid clade**

Marisol Sánchez-García and Brandon Matheny

Department of Ecology and Evolutionary Biology, University of Tennessee

The Tricholomatoid clade is one of several major clades of Agaricales, the largest order of mushroom-forming fungi. Members of this clade exhibit variation in nutritional modes, including mycoparasites, saprotrophs, termite-associates, and ectomycorrhizal (ECM) symbionts. The Tricholomatoid clade represents an ideal group for the study of evolution of the ECM habit because it contains a variation of trophic modes and several independent origins of the ECM association. Here, we evaluate whether the ECM mode is a key innovation that promoted diversification within the Tricholomatoid clade. In order to detect the effect of switches from saprotrophic to ECM mode on diversification rates

and to evaluate whether these switches represent an evolutionary key innovation, we implemented the binary state speciation and extinction model (BiSSE). Additionally, we performed a Bayesian Analysis of Macroevolutionary Mixtures (BAMM) to identify shifts in diversification rates. We will present evidence to accept or reject the hypothesis that ECM mode represents a key innovation that promoted diversification in the Tricholomatoid clade.

### **Distribution and description of *Epichloë* endophytes from *Poa alsodes* along a latitudinal gradient**

Tatsiana Shymanovich<sup>1</sup>, Nikki D. Charlton<sup>2</sup>, Carolyn A. Young<sup>2</sup>, Ashleigh Musso<sup>3</sup>, Nadja B. Cech<sup>3</sup>, Stanley H. Faeth<sup>1</sup>

I.M.A. *Epichloë* endophytes

<sup>1</sup> - Department of Biology, University of North Carolina at Greensboro, Greensboro, NC

<sup>2</sup> - The Samuel Robert Noble Foundation, Ardmore, OK

<sup>3</sup> - Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC

Most plants have microbial symbionts, which may affect host performance and fitness. Epichloid endophytes are fungal microbial symbionts of many cool-season grasses. These species are known to have effects on their host performance due to altered resistance to drought, mineral, or herbivory stressors. *Poa alsodes* is native eastern North American woodland perennial grass harboring unknown *Epichloë* spp. Twenty three natural populations were sampled across latitudes starting from the southern edge of distribution range located in the NC state and northward to NY state. Genetic screening separated samples in two groups varying by mating type and alkaloid genes profiles. Cloning and indirect sequencing of *tefA* and *tubB* genes allowed phylogenetic analysis, which revealed the origin of a widely distributed endophyte as a hybrid of *E. amaryllans* and *E. typhina*. This endophyte will be named and described. Another species was revealed as *E. schardlii*, previously known from a different grass host. Genetic analyses predicted production of N-acetylnorloline alkaloid by the undescribed species, and peramine production by both species. Chemical analyses are not yet complete, but only N-acetylnorloline was detected to date from the plants with the undescribed *E. sp.* Peramine was not detected from plants with either infection. Sequencing of this gene from *E. sp.* already revealed deletion mutation in at least one allele. The majority of *Poa alsodes* populations had very high *Epichloë* spp. infection frequency (90-100%). The lowest infection rate (26%) was observed at a high elevation in the Great Smoky Mountains National Park. Interestingly, *E. schardlii* was limited to Pennsylvania, while *E. sp.* was widely distributed among all collection sites. Studies on effects of these endophytes on insect herbivory and plant performance are in progress.

### **Bringing fungal-bryophyte associations to light: studies of the *Rickenella-Dicranum* association**

Hailee Korotkin, K. McFarland, P.B. Matheny

Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville  
Fungal-bryophyte interactions widely exist across the fungal tree of life, but in many cases have not been sufficiently studied. The mushroom-forming fungus, *Rickenella fibula* (Hymenochaetales), is known to associate with the moss, *Dicranum scoparium*, as well as many other mosses. *Rickenella fibula* has been observed fruiting on non-chlorophyllous stems of *D. scoparium*. However, the nature of the relationship has not been clearly established. Our objectives are to use morphological, stable isotope, and genomic data to clarify the type of interactions between *R. fibula* and *D. scoparium* and

to determine what type of relationship is occurring. We hypothesize the relationship is commensal, parasitic, or mutualistic. The following predictions can be made based on the hypotheses: (1) if commensal, the fungus is gaining nutrients from the moss, but the moss is not being harmed in the process; (2) if parasitic, the fungus is gaining nutrients at a cost from the moss; (3) if mutualistic, the fungus is gaining nutrients and benefitting the moss. Currently, in vitro cultures of *D. scoparium* are in the gametophyte stage and are almost large and hardy enough for *R. fibula* introduction in order to observe where fungal hyphae will penetrate within the moss via transmission electron microscopy (TEM). Collections of naturally occurring *D. scoparium* are currently being processed for TEM to be compared to the in vitro cultures. Several fungal extractions on naturally occurring mosses have been performed as a precursory look into which parts of the moss the fungus exists. Furthermore, a JGI nomination for *R. fibula* has been accepted in order to analyze the genome that will allow us to better characterize the association through specific gene screening.

### **Ecological aspects of chitinase activity of *Mortierella elongata* (PMI# 93) and its prospects on plant protection and nutrition in soil**

Hameed<sup>1</sup>, Khalid. M., Kevin<sup>1</sup> Le, Gregory Bonito<sup>2</sup> Hui-Ling (Sunny) Liao<sup>1</sup> and Rytas Vilgalys<sup>1</sup>, 1-Department of Biological Sciences, Duke university, 2- Department of Plant, Soil and Microbial Sciences, MSU.

*Mortierella* species are common soil inhabitant fungi and common endophytes isolated from almost all plant parts. The isolate NCPO7/05, PMI # 93 (Plant Microbe Interface culture list) was isolated from the root system of *Populus* tree at the Atkins River, NC, US. This particular isolate; PMI# 93 (*Mortierella elongata*) showed significant growth enhancement and plant protection when incorporated in the rooting medium of *Populus*, Pine (*Pinus taeda*), Oak (*Quercus* spp.) seedlings and Corn (*Zea mays*) plants under greenhouse conditions. The inoculum of PMI# 93 and other endophytes used in this work were established on millet seeds and used in 5% v/v incorporated in the rooting medium. Control treatments included rooting medium only and rooting medium and non-inoculated millet. The isolate PMI # 93 showed chitinase activity on colloidal chitin and raw powder chitin on agar plates and in liquid culture. Their chitinase activities was evaluated on time sequence that revealed that it begins after 24-43 hrs and reach its maximum 91-115 hours of incubation at room temperature in liquid cultures. The chitinase activity envisaged itself in term of change in the pH of the medium from acidic to basic due to release of the release of N-actyleglucoseamine monomers of the chitin substrate. Such chitinolytic activity of *M. elongata* may contribute to soil nutrients cycle through hydrolyzing the chitin in soil and making the N-actyleglucoseamine monomers source of nitrogen to plant roots. The chitinase-mediated metabolic networks are putatively involved in the plant growth promotions that have been observed.

Keywords; *Mortierella elongata*, chitin, chitinase, *Populus*, *Quercus*, *Pinus*, *Zea mays*

### **Deciphering nuclear localization signals in *Phytophthora sojae***

Yufeng Fang<sup>1, 2</sup>, Brett M. Tyler<sup>1, 2</sup>. Interdisciplinary Ph.D. program in Genetics, Bioinformatics & Computational Biology, Virginia Tech, Blacksburg, VA 24061; 2. Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331) Nuclear localization signals (NLSs) are amino acid sequences that direct proteins from the cytoplasm into the nucleus in eukaryotic cells, which play an important role in regulating nucleocytoplasmic transport. However, sequences that determine nuclear localization have not been defined in oomycetes, a fungus-like taxon that belongs to the kingdom Stramenopila. In this study we have investigated NLS functioning in *Phytophthora sojae*, a model species of oomycetes that infects soybeans. Using



confocal microscopy, we found that two well-studied NLSs, classical NLS (cNLS; from large SV40 T antigen) and proline-tyrosine NLS (PY-NLS; from the acidic M9 domain of hnRNP A1), as well as their derivatives cannot direct fluorescent proteins, such as GFP, into the nuclei of *P. sojae* transformants. Surprisingly, we also found that in highly conserved nuclear-localized proteins, such as ribosomal proteins and core histones, NLSs required for nuclear import in human or yeast, did not function in *P. sojae* even though the sequence was conserved in the *P. sojae* orthologs. Those results suggested that *P. sojae* may use a non-canonical mechanism for nuclear cargo transport. To identify functional NLSs in *P. sojae*, we experimentally examined 20 *P. sojae* nuclear-localized proteins, and defined in detail the NLS in three of those proteins. We found that *P. sojae* NLSs have several unique characteristics: (1) *P. sojae* NLSs are composed of several sub-sequences dispersed across the protein sequence. (2) NLS subsequences can either work individually when present in multiple copies or several subsequences can work collectively to direct proteins into the nucleus. (3) A *P. sojae* NLS was defined as three clusters of four positively charged amino acids with a minimum spacing. When the non-canonical *P. sojae* NLSs were tested in Arabidopsis and mammalian cells, they directed nucleolar localization, suggesting that the *P. sojae* NLSs may have evolved from nucleolar localization signals. We discuss the possible significance of this unusual arrangement.

#### **Genomic analyses of *Mortierella elongata* and associated bacterial endosymbiont (*Candidatus Glomeribacter* sp.)**

Jessie Uehling<sup>1</sup>, Gregory Bonito<sup>1</sup>, Khalid Hameed<sup>1</sup>, Jessy Labbé<sup>2</sup>, Dale Pelletier<sup>2</sup>, Tim Tschaplinski<sup>2</sup>, Amy Schaefer<sup>3</sup>, Chris Schadt<sup>2</sup>, Francis Martin<sup>4</sup>, Rytas Vilgalys<sup>1</sup>

Associations: Duke University<sup>1</sup>, Oak Ridge National Laboratory<sup>2</sup>, University of Washington<sup>3</sup>, Institut National de la Recherche Agronomique<sup>4</sup>

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Recently efforts to understand *Populus* microbiome dynamics have yielded cultures of many plant-beneficial fungi, including several *Mortierella elongata* (*Mortierellomycotina*) isolates. *M. elongata* associates with diverse rhizosphere bacteria that alter growth and function of their fungal associates. Some bacteria are free-living around root tips and hyphae while others are confined to living inside of fungal hyphal cells. The ecological strategies of these bacteria living inside of fungal cells fall along a spectrum from facultative to obligate endosymbiosis. While bacterial endosymbiont presence and identity can be established with next generation sequencing, especially in obligate symbionts these systems are challenging as conditions for growth of the bacteria are unknown and may be unachievable. Experimental tractability can be gained by clearing bacterial associates from fungi with antibiotics, and analyzing comparative growth and metabolite accumulation of cleared and uncleared fungal strains. Our research goal is to use multiple -omics approaches to analyze interaction dynamics between *M. elongata* and its endosymbiotic bacterial associates to answer the following questions; **1. What effect on fungal health and functioning do these bacteria impart?** **2. What factors dictate assembly and function of hyphal endosymbiont communities in the *Populus* rhizosphere?** We used 454 pyrosequencing, antibiotic passaging, radial growth assays, and gas chromatography coupled with tandem mass spectrometry to address these questions in pairs of cleared and uncleared *M. elongata* isolates. Identifying functional attributes of the fungal microbiome and rhizosphere microbial interactions will ultimately enable study of communities with maximal synergistic effects on plant growth, stress tolerance, and fitness.

### **Metabolomics and fungicide resistance: Insights into the *Phytophthora infestans* and *Mycosphaerella fijiensis* models**

Juan Manuel Cevallos-Cevallos, Maria Gabriela Mariduen-Zavala

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*Phytophthora infestans* and *Mycosphaerella fijiensis* are the most important pathogens of potato and banana respectively. Disease control has traditionally been achieved by application of fungicides but both pathogens have developed resistance to chemical treatments and new fungicides need to be frequently formulated. The mechanisms of fungicide resistance in *P. infestans* and *M. fijiensis* are poorly understood. Isolates of both pathogens were obtained from 7 regions of Ecuador where bananas or potatoes are produced. Isolates were characterized for metalaxyl and thiabendazole sensitivity using carrot agar and PDA, respectively. Fungicide doses of 0, 0.5, 1, 5, 10, 50, 100 or 500 µg/ml were tested and LD50 were estimated using nonlinear regression. Fungal isolates grown in solid media with and without fungicide were submitted to metabolite extraction using methanol, chloroform and water as well as detection using GC-MS. A total of 351 metabolites were characterized for each pathogen and multivariate data analyses revealed a distinct metabolite profile of isolates resistant to metalaxyl or thiabendazole when compared to sensitive isolates. Metabolic routes associated with butanodioic acid, L-sorbose, reibonic acid, gluconic acid, Fructofuranose, Fructose, Galactinol and melobiose were significantly upregulated in resistant isolates. Results provide a deeper understanding on fungal resistance to fungicides.

### **Correlating Contaminant Profiles to Fungal Communities at the Atlantic Wood Industries Superfund Site**

**Lauren Czapliski** ([lmc58@duke.edu](mailto:lmc58@duke.edu)), Rytas Vilgalys ([fungi@duke.edu](mailto:fungi@duke.edu)), and Claudia Gunsch ([ckgunsch@duke.edu](mailto:ckgunsch@duke.edu)) (Duke University, Durham NC, USA)

The widespread use of creosote as a wood preservative has led to contamination of many soils with heavy polycyclic aromatic hydrocarbons (PAHs). Remediating heavy PAH contamination in soil is challenging due to physical and chemical properties associated with the compounds. These properties limit sustainable treatment options such as bacterial bioremediation. This work aims to expand bioremediation to utilize fungi, as many studies have demonstrated fungal enzymes to degrade heavy polycyclic aromatic hydrocarbons and other contaminants. The current pilot study uses amplicon sequencing on Illumina's MiSeq platform to survey the fungal community within a gradient of contamination. The contaminated site consists of a 46-acre parcel of industrialized waterfront in Portsmouth, VA- the Atlantic Wood Industries Superfund Site. Wood preservation activities contaminated this site from 1926-1992 with over 30 different PAHs. PAH concentrations were determined using gas chromatography combined with electron impact mass spectrometry. The gradient of total PAH concentrations was found to span 40 µg/g to 96,000 µg/g soil. Efforts are currently underway to characterize soil nutrients as well. Fungal genera possessing enzyme activity were identified among the community within the contaminated soil. Largely, the contaminated soils have fungal genera belonging to Ascomycota and Basidiomycota with a few belonging to Zygomycota. ANOSIM and NMDS display relationships between PAHs and fungal community similarities. Further statistical analyses are currently underway to identify specific fungal genera found under specific PAH contamination. This pilot project established a method to link contaminant profiles to fungal community

membership. Results from this project feed into a targeted biostimulation approach and may inform future site investigations at other PAH contaminated sites.

### **Using metatranscriptomics to characterize functional shifts in endophytic fungi at plant senescence: Are endophytic fungi latent saprotrophs?**

Ko-Hsuan Chen, Hui-Ling Liao, A. Elizabeth Arnold, Francois Lutzoni

Endophytic fungi live inside healthy plants but do not cause obvious symptoms. The same fungal species can often be isolated from living and senescent tissues of the same plant, consistent with the widespread hypothesis that many endophytic fungi are saprotrophs during part of their life cycle. However, these investigations are mostly based on culture-dependent or DNA-based environmental sampling methods. It is not known whether fungal strains detected in healthy and senescent plant tissues are functionally active under both conditions, and which genetic mechanisms are involved in this functional transition. To gain a better understanding of endophytism-saprotrophism transitions in situ, we sequenced the metatranscriptome of the broom moss *Dicranum scoparium*, and its microbiota, which were sampled from local forest sites in North Carolina. Three distinct layers (healthy, senescent, and dead tissue) can be recognized from the top to the base of the gametophytes of *D. scoparium*. We extracted total RNA from these three layers. Illumina Hi-Seq sequencing generated about 39 million qualified reads for each individual layer sample. By using NCBI GenBank and a customized database of the DoB project (Dimensions of Biodiversity; EndoBiodiversity.org), we were able to extract reads that mapped to the D1/D2 region of fungal ribosomal large subunit gene using Bowtie2. Through de novo assembly using Velvet, followed by BLASTN, we identified several fungi that are functionally active throughout the three gametophytic layers of *D. scoparium*, including: *Rickenella*, *Sistotrema*, *Cladophialophora*, *Epibryon* and *Mortierella*. We also found fungal taxa that were active only in the youngest, photosynthetic, layer (e.g., *Hyaloscypha*). We further compared gene expression patterns of fungi under various degrees of host senescence to determine the molecular mechanisms involved in transitions between saprotrophism and symbiotrophism. Our study sheds light on the potential for ecologically important functional shifts in host use.

### **Focus Stack Imaging of Herbarium Collections of Wood Decay Macrofungi: A Demonstration of Proof of Concept**

Kathryn Hanser, Bryan R. Cody, and Marc A. Cubeta

Dept. of Plant Pathology, Center for Integrated Fungal Research, North Carolina State University, Raleigh, NC

The digitization of fungal herbaria collections has been ongoing worldwide with public data access provided by IDigBio ([www.idigbio.org](http://www.idigbio.org)) and MyCoPortal ([mycoportal.org](http://mycoportal.org)) websites. To generate high quality, scientifically useful digital images, staff at the Larry F. Grand Mycological herbarium developed a protocol for photographing fungal specimens with controlled subject lighting using a digital SLR camera equipped with a macro lens and an electronically controlled shutter and rail system. This system was utilized to generate a series of images that were merged into a single image with an extended depth of field using focus-stacking software. Experimental parameters evaluated in this study included total specimen handling and software processing time, camera settings and image quality, with the ultimate goal to create a streamlined imaging procedure that can be performed by competent non-experts. Exposure, aperture and input file processing time were evaluated, and two methods for rail use (top-down and bottom-up) were applied depending on specimen size and field of view requirements. Sixteen specimens representing a range of taxa were used for initial

intensive methods testing, followed by application and method refinement using an additional 200 specimens. All images contained printed labels and field notes, a color checker, and metric rulers. Even newly-trained imaging technicians can generate excellent images in as little as 10 minutes, including specimen layout and simple image retouching. This provides a valuable photo documentation technique for fungal herbarium collections of woody macrofungi.

### **Burn times influence smut distribution in longleaf pine savannas**

Inga M. Meadows, Julia L. Kerrigan, Dept. Agricultural and Environmental Sciences, Clemson University, Clemson, SC

Joan L. Walker; USFS Southern Research Station, Clemson, SC

Longleaf pine savannas are unique to the Southeastern U.S., they are characterized by an open canopy dominated by longleaf pine (*Pinus palustris*) and a dense ground layer dominated by herbaceous species. A high frequency, low intensity surface fire regime both maintains and is facilitated by the classic longleaf pine forest structure. Once the predominant forest type in the southeast, longleaf pine ecosystems have been reduced to a fraction of their size due to land use changes, urbanization, and fire suppression. Longleaf pine and wiregrass bunchgrasses, *Aristida stricta* and *A. beyrichiana*, are keystone species and efforts are being made to preserve and propagate them, as well as other species in these disappearing ecosystems. Smut teliospores have been reported from ovaries of developing flowers of *Aristida stricta* and *A. beyrichiana*, and the effect of this pathogen on seed viability is a concern. The smut has been tentatively identified as *Langdonia confusa*, based on morphology and host. DNA sequencing is being performed to confirm the identity and compare sequences by host species and location. The effects of fire timing and soil factors on incidence of smut infection were also examined. We predicted that later burn dates would reduce smut infections. In 2014 85 sites (nested within 15 properties) were sampled; samples were distributed across the middle and south Atlantic coastal plain, 4 soil orders, and burning in every month from December 2013 through July 2014. At each site the presence of smut was tallied at 20-m intervals along transects, and culms were collected from 8 clumps. We quantified the presence of smut at site, clump, and culm levels. Both the burn month and soil order had significant effects (ANOVA;  $p < .05$ ) on stand smut abundance; the interaction was not significant. Clump and culm infection were correlated (Pearson;  $r = .53$ ;  $p < .01$ ) and showed patterns similar to the stand level. Results showed that sites burned in May-July had significantly higher rates than early burns, contrary to expectation. Reasons behind these results are under examination.

### **NCU Fungal Herbarium – a Mother Lode of Types**

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A project to identify and document all of the fungal type collections held in the NCU Fungal Herbarium is well under way and is being conducted in conjunction with creating computerized records of all NCU macrofungal collections in MyCoPortal. A master index to all fungal types along with pertinent information is being compiled. MyCoPortal records for the type collections are being annotated with type information and literature citations. All of the type collections are being moved and stored in a dedicated area of the herbarium separate from the general collections. Major groups of fungal types at

NCU include William C. Coker's taxa, John N. Couch's taxa and isotypes including taxa described by W. A. Murrill, C. H. Peck, C. L. Shear, A. H. Smith and others. Major taxa with many types at NCU include Boletes with >2000 collections including 138 types, Clavarioid Fungi with >2000 collections including 114 types, and Septobasidium with many types! Indexing of Septobasidium is in progress. The NCU Fungal Herbarium has a remarkably high type density with circa 3% of the collections being type collections. The project to identify and index all of the type fungal collections is ongoing with >750 type collections identified to date; many more are certain to be found.

### **A Survey of Phytophthora Species in Fraser fir Christmas Tree Plantations in the Southern Appalachians**

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Fraser fir (*Abies fraseri*) is the primary species cultivated by the Christmas tree industry in the Southern Appalachian Mountains and in North Carolina alone, revenue from Christmas tree sales exceeds \$US 100 million annually. A major problem facing the industry is *Phytophthora* root rot that causes significant mortality of Fraser fir. In surveys conducted in 1972 and from 1997 to 1998, *P. cinnamomi* was the pathogen most frequently recovered from diseased Fraser fir roots. Due to recent changes in production practices that involve increased importation of seedlings from other regions of the US, we hypothesized that the species composition of *Phytophthora* in Fraser fir production regions in North Carolina and surrounding states has changed since the original surveys. During the summer and autumn of 2014, a survey of Fraser fir Christmas tree plantations in the Southern Appalachians (NC, TN, and VA) was conducted. Symptomatic Fraser fir roots were collected in 103 commercial production fields distributed over 14 counties using a weighted sampling strategy based on Christmas tree acreage. *Phytophthora* was recovered from 82 sites and was present in 13 counties. Six species of *Phytophthora* were identified with *P. cinnamomi* accounting for 70% of the species recovered from 13 counties. Twenty-three percent of the isolates recovered were *P. cryptogea* and this species was found in eight counties. Four species, *P. citrophthora*, *P. europaea*, *P. pini* and *P. sansomeana*, not identified in the previous surveys were recovered from infected roots of Fraser fir in Ashe (*P. europaea*, *P. pini* and *P. sansomeana*), Alleghany (*P. citrophthora*), and Watauga counties (*P. europaea*). These counties are adjacent and are among the counties with the largest acreage of Christmas trees. In summary, *P. cinnamomi* was still the predominant species recovered from diseased Fraser fir, but *P. cryptogea* also now appears to be an important pathogen contributing to losses of Fraser fir Christmas tree production in the Southern Appalachian Mountains.

### **Morphological and molecular diversity of *Moniliophthora roreri* and *Moniliophthora perniciosa* in Ecuador**

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Cocoa trees are among the most important commodities in South America but the production of cocoa is being threatened by the pathogenic fungi *Moniliophthora roreri*

and *Moniliophthora perniciosa*, causal agents of frosty pot rot and witches broom diseases respectively. However, the pathogenicity, morphological and molecular diversity of the pathogens in Ecuador are unknown. Symptomatic samples of cocoa pods were collected from the 6 cocoa-producing regions of Ecuador and isolates of both pathogens were submitted to morphological, molecular, and pathogenic characterization. Morphological characteristics such as mycelial growth, edge shape, colony color and texture as well as type and size of conidia were recorded for each isolate. Pathogenicity of the isolates was tested by inoculation onto cocoa pods and symptoms were evaluated at 3-day intervals during 16 days prior to estimating the area under the disease progress curve. Molecular variation was assessed by direct sequencing of the ITS1, 5.8s, and ITS2 DNA regions as well as by RFLP analyses on the same regions. Results showed morphological and pathogenic homogeneity across isolates. The average polymorphism was 48.98%. and Shannon's diversity index was 0.21. Mycelial growth rate fitted a Gompertz model with parameters estimates that significantly correlated with mycelial color. Multiple discriminant analyses carried out on the RFLP patterns showed a 100% accurate grouping by both sampling province and mycelial color.

### **Thermal Inactivation of the Causal Agent of Boxwood Blight, *Calonectria pseudonaviculata***

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*Calonectria pseudonaviculata* (Cps) is a fungal, foliar pathogen causing boxwood blight on *Buxus sempervirens*, *Buxus harlandii*, and *Buxus microphylla*. Currently, the use of resistant cultivars is a commonly used practice to combat this disease. However, a method for insuring disease-free boxwood cuttings for both propagation and the holiday decoration industry needs to be developed. Our study on the thermal inactivation of *Calonectria pseudonaviculata* had two objectives; 1) to determine the thermal inactivation points of the Cps. conidia 2) to determine the ability of resistant boxwood cultivars to survive after exposure to hot water. To accomplish our first objective, Cps sporulation was induced on PDA plates. Spores were harvested and diluted to 10,000 spores/mL. The spore solution was then heated to five different temperatures for up to twenty minutes, and the ability of the spores to germinate on PDA plates was evaluated after 24 hours. Calculations will be performed to determine the thermal death kinetics, decimal reduction times (D-values), and rate constants. In order to accomplish our second objective, woody cuttings from seven different boxwood cultivars were placed in a circulating water bath at 45°C, 50°C, and 55°C for up to one hour. The cuttings were then dipped in rooting hormone and placed in soilless potting mix. After 1.5 months, the rooting ability of the cutting will be assessed using a scale. This is the first study to determine the thermal tolerance of Cps conidia and of different boxwood varieties for the purpose of developing a cultural control measure.

### **A radial growth rate comparison and competitive inhibition study of an *Atractiella* species: characterization of a little-known fungal root endophyte**

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Little is known about the growth characteristics and competitive interactions of members of the fungal order Atractiellales. However, recent studies have shown it to be one of the most common root associates of *Populus* tree species in the Eastern and Western US. To begin to address possible roles of the ubiquitous *Atractiella* sp., both growth rate in general and in response to competitive interactions were studied. Competitive performance of *Atractiella* was compared in response to a panel of *Populus* rhizosphere and endosphere isolates as well as in detail against the presence of two fungi: *Fusarium oxysporum* or *Leptosphaerulina chartarum*. We found *Atractiella* growth rates were generally slow; that all strains grew faster on potato dextrose agar compared with a minimal synthetic media formulation; and that the different *Atractiella* isolates varied significantly in growth rate. In competition experiments, *Atractiella* growth became arrested in the presence of either fungal species producing distinct zones of inhibition combined with significantly reduced growth rates of *Atractiella* and the competitor suggestive of reciprocal and negative intra-specific responses. Additional metabolomic analyses of the interacting species are ongoing to try to understand the chemical mechanisms of interaction and growth inhibition. Cumulatively, these results suggest competitive interactions of *Atractiella* may be an important trait contributing to the function of these organisms in the roots and rhizosphere of *Populus*.

#### **Common molds modify plant disease.**

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Microfungi regarded as common molds (e.g., *Alternaria*, *Cladosporium*) occur in plant leaves as non-pathogenic leaf endophytes. Some endophytes are known to decrease or increase disease severity in their host plants, serving as defense mutualists or pathogen enablers, respectively. However, the generality of endophytes in modifying leaf disease severity has been explored rarely, and has not been coupled with studies of their abundance and distribution in wild populations. We used next-generation DNA sequencing to characterize the fungal leaf microbiome of *Populus trichocarpa* in wild populations throughout the Pacific Northwest (USA) and to determine how the relative abundance of common leaf endophytes correlated with the severity of a major leaf rust disease of *P. trichocarpa*, *Melampsora*. We observed both positive and negative correlations between the relative abundance of common endophytes and rust severity. Using controlled inoculation experiment, we then confirmed that the endophytes modify rust disease severity in the predicted directions. Our results suggest that disease modification is a central function of common endophytes within the leaf microbiome of *P. trichocarpa* and that leaf endophytes help explain geographic variation in plant disease severity.

#### **Are decomposition pathways maintained in a family of ectomycorrhizal fungi?**

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Recent evidence suggests that some ECM fungi have retained the capability to degrade complex plant biopolymers, thus contributing to carbon cycling in new and unexpected ways. The family Russulaceae (Russulales) is one of the most widespread and species rich ECM lineages, and preliminary evidence suggests that members of this family have retained genes responsible for the degradation of the most recalcitrant class of plant biopolymers, lignin. Here I will present an overview of a project that seeks to utilize dense genome sampling within the family to investigate to what extent genes involved in

plant biopolymer degradation have been maintained within a single, diverse, ECM lineage. I will present progress and issues involved in sample preparation for genome sequencing, which is considered a major bottleneck towards genome-enabled mycology. Plans for utilizing genomic information for phylogenomic inference and building a phylogenetic “toolkit” for future systematics in the family will also be discussed.