



# MASMC 2021

*Dedicated to Dr. W. C. Coker*

## **2021 Mid-Atlantic States Mycology Conference**

### **Abstract Book**

Issued 15 April 2021

Abstracts for oral presentations are first followed by abstracts for poster presentations. Within each category, abstracts are sorted by the last name of the first author. Names of presenters and co-presenters are underlined.

In recognition of Dr. William C. Coker to whom MASMOC 2021 is dedicated, two abstracts about his mycological taxa are reprinted and included after the MASMOC 2021 abstracts.

MASMOC 2021 will be held virtually through the University of North Carolina at Chapel Hill on April 17-18, 2021.

# Oral Presentation Abstracts

## Modeling in yeast how rDNA introns slow growth and increase desiccation tolerance in lichens

Daniele Armaleo<sup>1</sup>, Lilly Chiou<sup>2</sup>

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### Abstract

Lichens are desiccation tolerant symbioses between specialized fungi (mycobionts) and unicellular phototrophs. We test whether slow growth and extreme desiccation tolerance in lichens are driven by the unusual introns present in the nuclear ribosomal DNA of their mycobionts. Self-splicing introns are found in the rDNA of several eukaryotic microorganisms, but most introns populating lichen rDNA are unable to self-splice, being either degenerate group I introns lacking the sequences needed for catalysis, or spliceosomal introns ectopically present in rDNA. Using CRISPR, we introduced a spliceosomal intron from the rDNA of the lichen fungus *Cladonia grayi* into all nuclear rDNA copies of the yeast *Saccharomyces cerevisiae*, which lacks rDNA introns. Three intron-bearing mutants were constructed with the intron inserted either in the SSU repeats, the LSU repeats, or in both. The mutants removed the introns correctly but had half the rDNA genes of the parent strain, grew 4.4 to 6 times slower, and were 40 to 1700 times more desiccation tolerant depending on intron position and number. Intracellular trehalose, a disaccharide implicated in desiccation tolerance, was detected but not at levels compatible with the observed resistance. Extrapolating from yeast to lichen mycobionts we propose that the unique requirement for a splicing machinery by lichen rDNA introns slows down intron splicing and ribosomal assembly. This effect, and the distinctive roles played by group I vs. spliceosomal rDNA introns, partly modify the regulatory patterns of the fungal Environmental Stress Response, leading to the twin lichen phenotypes of slow growth and desiccation tolerance.

# Identification of *Cytospora* species isolated from cankers in peach, in South Carolina.

Stephen Baker, Julia Kerrigan

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## Abstract

*Cytospora* species are the causal agent of Cytospora canker on a wide range of tree hosts, including those of economic importance such as peach (*Prunus persica*). These fungi enter their host indirectly via wounds and lead to the formation of necrotic cankers. If left untreated, dieback symptoms will begin, leading to loss of major limbs and yield in fruit. A survey of twig blight in the major peach growing regions of South Carolina reported finding *Cytospora* for the first time within the state. In attempt to identify the species, it was found that previous methods of identifying the causal species have relied on morphological features, but due to overlap in the size and shape of key characteristics, this has proven to be problematic in making species level identifications. Subsequent efforts to identify the species found that, when grown in culture, isolates displayed highly variable morphology and sequencing of the internal transcribed (ITS) region found the isolates to be the same species. However, it has been found that the ITS region is not reliable for *Cytospora* identification at the species level. Therefore, the goal of this study is to provide a detailed assessment of the species from Cytospora canker on peach, and their relatedness, using morphological characteristics in combination with genetic sequences of four additional loci: actin (ACT), beta tubulin (TUB), calmodium (CAL), and translation elongation factor 1-alpha (TEF).

# Evaluation of resistance to *Calonectria pseudonaviculata* among five boxwood cultivars

Caleb Bollenbacher, Marc Cubeta, H. Van T. Cotter

North Carolina State University, Raleigh, USA

## Abstract

*Calonectria pseudonaviculata* (Cps) is a fungal pathogen of boxwood (*Buxus* sp.) and causal agent of boxwood blight disease. Symptoms include necrotic lesions on leaves, black streaking cankers on stems, and defoliation. Boxwoods constitute 15% of sales of ornamental broadleaf evergreens in the United States, representing an annual revenue of \$126 M. The main objective of this research is to determine symptom expression and spore production of Cps on detached leaves of boxwood cultivars exhibiting a range of susceptibility. Branches will be collected from each cultivar and leaves will be removed, disinfested in 70% EtOH for one minute, rinsed in sterile water and placed on moist filter paper in a 100-mm diameter plastic petri dish (10 leaves per plate). Each leaf will be inoculated by placing one 5  $\mu$ l drop of a  $1 \times 10^5$  spore suspension containing a mixture of four Cps isolates on the abaxial side. Leaves treated with 5  $\mu$ l of sterile distilled water will serve as the uninoculated. There will be four replicates of each cultivar and inoculation treatment arranged in a randomized complete block design. Plates will be incubated at 24 C and leaves will be assessed every day for 2 weeks for disease incidence and severity. The number of days required for symptom development (incubation period) and spore production (latent period) on each leaf will be determined. After incubation, leaves of each cultivar and inoculation treatment will be rinsed with sterile water to collect spores for microscopic examination and quantification of spore production.

## **Mycelial mysteries and hope from hyphae**

Amy Gladfelter

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

### **Abstract**

We study how large mycelial fungal cells are organized in time and space. In our studies, we have discovered that large hyphal cells create smaller functional zones and sense their shape using many of the same processes relevant to neurons. These processes involve cellular components condensing like dew in different parts of the cell. I will discuss how these processes may enable fungi to adapt to different temperatures and how this may be relevant to resilience under climate change.

## **Culturing *Pseudogymnoascus* from post-wildfire soils of the Great Smoky Mountains**

Zane Smith, Abigail Rea-Ireland, Karen Hughes

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### **Abstract**

*Pseudogymnoascus* is a globally-distributed, diverse genus of soil fungi within Ascomycota, the diversity of which has scarcely been explored. However, the impact of *Pseudogymnoascus destructans*, causal agent of White-nose Syndrome which has killed over 6.5 million bats in the US alone, demonstrates the need for further investigation of native, congeneric diversity. Following the 2016 Great Smoky Mountain wildfires, soils were collected monthly for two years and sequenced via next-gen MiSeq sequencing. Preliminary data revealed incidence of undescribed *Pseudogymnoascus* within several burned areas, yet none within unburned sites of similar strata. This data has thus prompted an inquiry into the diversity of *Pseudogymnoascus* from burn-sites in order to compare the fire-responsive taxa to known members of the genus. As proof of concept, a pilot study was conducted on frozen, post-burn soil samples two years post-collection, in which *Pseudogymnoascus* was successfully cultured and identified through rDNA sequencing (ITS1F and ITS4R primers). Sequenced isolates have been confirmed to conform to Clade G in the *Pseudogymnoascus roseus* complex—the same phylogenetic clade in which the pyrophilic isolates were found. This study thus seeks to reconcile historical taxonomy with modern genetic approaches through additional sequencing of RPB2 (primers RPB2-7cF, and RPB2-11aR) and TEF1 (primers EF1-983F, EF1-2218R), establishing a multigene phylogeny to identify isolates from the Great Smoky Mountains National Park in a modern context and elucidate morphological characters for species identification.

## Comparative genome evolution of *Populus* root endophytes

Yi-Hong Ke<sup>1</sup>, Brian Looney<sup>1</sup>, Alejandro Rojas-Flechas<sup>2</sup>, Jake Nash<sup>1</sup>, Khalid Hameed<sup>1</sup>, Christopher Schadt<sup>3</sup>, Jose-Eduardo Marques Galvez<sup>4</sup>, Francis Martin<sup>4</sup>, Jessy Labbé<sup>3</sup>, Daniel Jacobson<sup>3</sup>, Claire Veneault-Fourrey<sup>4</sup>, Igor Grigoriev<sup>5</sup>, Kerrie Barry<sup>5</sup>, Rytas Vilgalys<sup>1</sup>

<sup>1</sup>Duke University, Durham, USA. <sup>2</sup>University of Arkansas, Fayetteville, USA. <sup>3</sup>Oak Ridge National Laboratory, Oak Ridge, USA. <sup>4</sup>INRAE Centre de Nancy, Champenoux, France. <sup>5</sup>Joint Genome Institute, Berkley, USA

### Abstract

Endophytic fungi have a strong influence on ecosystems by benefiting the health and survival of their host plants against biological and non-biological stresses. Fungal endophytes are polyphyletic and hyperdiverse, yet their common adaptations enabling endophytic lifestyle are largely unknown. We sequenced genomes of over 40 fungal taxa representing 17 unique lineages of endophytes associated with *Populus*. We compared the 17 endophyte lineages with their closest-related free-living saprobe species to identify common genomic features associated with endophytic symbiosis lifestyle. Among the genomic features we compared, endophytic fungi were observed to consistently have significantly greater genome size and gene count than their sibling taxa, including more CAZymes and more small secreted proteins. To correct bias from the phylogenetic structure, we used phylogenetic linear regression models and phylogenetically independent contrasts to compare genome evolution of each lineage with its nearest non-symbiotic relative. Results of multiple phylogenetically-corrected analyses suggest several mechanisms by which fungi have adapted to an endophytic lifestyle. To further differentiate the exact genes contributing to endophytic lifestyle, Wilcoxon signed-rank test, variable importance of random forest classification, and Weighted Correlation Network Analysis were applied to Pfam annotation to identify a set of proteins discriminating endophytes from their closely-related saprobes. Carboxymuconolactone decarboxylase family was consistently identified as key genes regardless of method used. These genes are likely to be the core genes that enable the endophytic lifestyle, and further investigations in other plant systems is required to verify their universality.

## The John N. Couch *Coelomomyces* Collection at the Herbarium of the University of North Carolina at Chapel Hill

W. Wallace Martin<sup>1</sup>, H. Van T. Cotter<sup>2</sup>

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### Abstract

*Coelomomyces*, a genus of flagellated fungi in the Phylum Blastocladiomycota, includes obligate parasites, several of which been shown to exhibit an indirect life cycle that alternates between a microcrustacean host (typically a copepod) and a dipteran host (typically a mosquito). By the late 1950's *Coelomomyces* was regarded by the scientific community as a promising potential biological control agent against mosquitoes and from 1965-1985 the principal laboratory conducting research on *Coelomomyces* was that of Dr. John Couch at the University of North Carolina at Chapel Hill. During this time the Couch laboratory received collections of *Coelomomyces* from many collaborators, including Drs. H. C. Chapman (collections from Louisiana), M. O. T. Iyengar (India) and A. J. Walker (Africa). This presentation announces the recent "rediscovery" of these collections which have been in storage for the past 35 years and details a plan by which the Couch *Coelomomyces* collection will become incorporated into the UNC Herbarium as a resource for systematic study of the genus. The collection includes approximately 25 species of *Coelomomyces*, most of which are dried (non-fixed) collections of Indian species or formalin-fixed collections from the United States. The collection also includes several hundred prepared slides of unidentified species from Africa. Immediate goals include proper annotation of collections, identification of unknown species, use of light and electron microscopy to study morphological variation of the sporangium wall, and attempts to extract and sequence DNA from collections with the ultimate goal of producing a more complete molecular phylogeny of the genus.

# Muscarine Toxicosis in Dogs: A Literature Review

Julia Mays, H. Van T Cotter, Marc A Cubeta

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## Abstract

Muscarine is a cholinergic toxin produced by Agaricomycetes fungi that affects the peripheral nervous system of humans and other animals. Mushrooms of *Inocybe* and *Clitocybe* species contain the highest concentrations of muscarine at 0.1% to 1.6% per dry weight. Ingestion of muscarine-containing species can cause abdominal pain, bradycardia, diarrhea, lachrymation, miosis, salivation, and vomiting. In dogs, symptoms can exacerbate and result in collapse, depression, hypotension, hypothermia, and respiratory difficulty. Within the last two decades, reported cases of muscarine poisoning in dogs have increased in occurrence. In this review, we pulled cases of muscarine poisoning in dogs based on the ingestion of muscarine-containing species from 9 genera and *Amanita muscaria*. We examined 159 cases reported in Canada, Norway, South Korea, United States, and United Kingdom between the years 1994 and 2020 to synthesize the demographics, symptom expression, and treatment metadata. The objective was to examine the incidence of cases and identify patterns and gaps within the data. Species of *Inocybe*, *Amanita muscaria*, and *Clitocybe* were collectively implicated in over 75% of cases. Upper gastrointestinal (67.3%), secretory (50.9%), and lower gastrointestinal (45.9%) distress represented the most common symptoms. Supportive care was the most common treatment, but 62.9% of cases did not provide treatment information. Nearly half of cases did not provide an outcome, but of those that did the majority survived. From the metadata, we observed the most gaps in case demographics, treatments, and outcomes, which are key metrics understanding the occurrence, relative risk, and treatment efficacy of future muscarine toxicosis cases in dogs.

# History of Mycology at the University of North Carolina at Chapel Hill

Carol Ann McCormick

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## Abstract

The academic study of fungi at the University of North Carolina at Chapel Hill began with the founding of the Botany Department and the Herbarium (NCU) in 1908. Mycologist Dr. William Chambers Coker was the first Chair of the Botany Department, and his first graduate student, John Nathaniel Couch succeeded him in that post in 1945. Both were eminent mycologists who not only collected specimens for the Herbarium, but also received specimens from colleagues worldwide for study and determination. Between 1912 and 1997, about 400 graduate theses (M.A., M.S., and Ph.D.) were recorded in the Botany Department (or Biology Department, as Botany & Zoology merged in 1982); a quarter were on topics related to mycology. After a quiescent period which lasted from the 1990's until 2010, the mycological collections have seen a resurgence of activity thanks to a National Science Foundation grant to catalog NCU's mycological holdings. Specimens curated at NCU can be searched online at [mycoportal.org](http://mycoportal.org). NCU Associate Dr. H. Van T. Cotter and a group of undergraduate students and volunteers are once again adding specimens to NCU and using the older collections to address nomenclatural and taxonomic problems.

# The ancestral fungal cell cycle was rewired by a viral domain, or why we work with Chytrids.

Edgar Medina<sup>1</sup>, Kristyn Robinson<sup>2</sup>, Lillian Fritz-Laylin<sup>2</sup>, [Nicolas Buchler](#)<sup>3</sup>

<sup>1</sup>Duke University, Durham, USA. <sup>2</sup>University of Massachusetts, Amherst, USA. <sup>3</sup>North Carolina State University, Raleigh, USA

## Abstract

The mechanism and function of the G1/S regulatory network in animals and yeasts is conserved even though key regulators (transcription factors E2F and SBF, inhibitors Rb and Whi5) do not have a shared molecular ancestor. Our previous work indicated that SBF is a viral-derived gene that likely hijacked cell cycle control in the fungal ancestor. Early-diverging fungi, such as chytrids, have a hybrid G1/S regulatory network that contains both the ancestral E2F-Rb pathway and derived SBF-Whi5 fungal pathway. Most other fungi lost the ancestral pathway. Chytrids also retained ancestral features, such as motile cilia and crawling zoospores. Understanding how the hybrid G1/S regulatory network regulates the hybrid cell biology of chytrids will help address how lateral gene transfer can rewire essential regulatory networks in a eukaryote.

We identified the soil chytrid *Spizellomyces punctatus* as a model system for uncovering mechanisms driving cell cycle regulation and cell motility because it is fast-growing, displays crawling and swimming motility, and has both the E2F-Rb and SBF-Whi5 pathways. We adapted *Agrobacterium*-mediated transformation to generate stable genetic transformation of *Spizellomyces*. By coupling live-cell imaging with fluorescent tagging of both histone and cytoskeletal components, we characterized the cell biology of *Spizellomyces* throughout its life cycle. We show that nuclear divisions are highly synchronous, that actin is localized to the leading edge of crawling zoospores, and the formation of transient perinuclear actin shells coincides with the timing of mitotic cycling. These tools will allow us to test hypotheses about the evolution and regulation of the cell cycle.

## **NCU Herbarium Tour**

Dan Meyers

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### **Abstract**

A walkthrough of the NCU Herbarium that will display the breadth of collections held at the herbarium, some specimens of note, show what makes for a good mycological herbarium specimen (and label!), and includes an overview of some of the mycological work historically done at NCU, with a particular focus on the work of Dr. William Chambers Coker, to whom MASMC 2021 is dedicated. The fungal section of the NCU Herbarium contains a high type density, with over 3% of the over 31,000 specimens being typic material, or specimens that are associated with the publication of a new taxon, which has been the focus of the NCU Fungal Type Project, which is currently in its final stages. Fungal groups particularly well represented in the types held at NCU are boletes, clavarioid fungi, gasteromycetes, hydroid fungi, rusts, and septobasidia. Since 2014 more than 1,000 collections including over 100 types have been loaned by NCU to mycologists around the world. Additionally, the tour will touch on some of the other recent mycological work going on at the herbarium, including accessioning collections to the fungal herbarium database MyCoPortal, some taxonomic and sequencing work, the Funga of the North Carolina Botanical Gardens, and a recent state record collected by our very own Corbin Bryan!

# Populus root-associated fungal communities undergo strong and host-specific successional turnover

Jake Nash<sup>1</sup>, Brian Looney<sup>2</sup>, Andrew Bleich<sup>1</sup>, Khalid Hameed<sup>1</sup>, Rytas Vilgalys<sup>1</sup>

<sup>1</sup>Duke University, Durham, USA. <sup>2</sup>Clark University, Worcester, USA

## Abstract

*Populus* trees associate with diverse communities of arbuscular mycorrhizal, ectomycorrhizal, and endophytic fungi. These guilds of root-associated fungi are thought to temporally segregate with arbuscular mycorrhizal fungi predominating during early growth, later yielding to ectomycorrhizal fungi as the plant matures. However, it is not well understood 1) how temporal patterns of fungal colonization vary between dual-mycorrhizal plant species or 2) how individual fungal species vary in their temporal colonization patterns. To answer these questions, we grew two species of *Populus* – *P. tremuloides* and *P. trichocarpa* – in microcosms inoculated with field soils from mature *Populus* stands and comprehensively characterized the root-associated fungi at seven and 12 months using long-read RNA sequencing of transcribed fungal 28S RNA. At 7 months, both species of *Populus* were dominated by endophytic fungi. By 12 months, colonization by endophytes sharply declined in both species, with *P. trichocarpa* becoming extensively colonized by arbuscular mycorrhizal fungi and *P. tremuloides* becoming dominated by ectomycorrhizal fungi with very sparse arbuscular mycorrhizal colonization. Prominent ectomycorrhizal taxa included *Hebeloma*, *Tuber*, *Cenococcum*, *Ceratobasidium*, *Sebacina*, *Wilcoxina*, and *Hyaloscypha*. Arbuscular mycorrhizal fungal taxa included *Glomus*, *Rhizophagus*, *Funneliformis*, and *Claroideoglossum*. Despite the general trend of increasing dominance by ectomycorrhizal fungi on *P. tremuloides*, certain ectomycorrhizal fungi declined in abundance by 12 months, including *Cenococcum* and members of the Helotiales. Our findings also show that even closely related dual-mycorrhizal plants can vary widely in their temporal patterns of fungal colonization. Further work will characterize changes in plant-fungal signaling through time by measuring small secreted proteins and lipochitooligosaccharides.

# How does atmospheric deposition affect fungal soil communities in an endangered spruce-fir ecosystem?

Chance Noffsinger, Brandon Matheny

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## Abstract

The spruce-fir ecosystem in the southern Appalachian Mountains is considered one of the most endangered in North America and the largest communities of spruce-fir are found in the Great Smoky Mountains National Park. Fraser fir (*Abies fraseri*) is only found in the southern Appalachians and has experienced massive decline due to the invasive woolly adelgid and atmospheric deposition. Spruce-fir forests improve watershed quality and provide important habitat for endemic species, some of which are poorly understood and may be at risk of extinction. Soil fungi play a dynamic role in the spruce-fir ecosystem and many mutualistic fungi are specifically tied to tree survival. The two major ecological guilds of fungi, biotrophs and saprotrophs, respond differently to the changing edaphic conditions caused by atmospheric deposition. The goal of this work is to understand how atmospheric deposition and other edaphic factors vary across the spruce-fir ecosystem and how these will affect fungal community diversity. This research has the following aims: to characterize the fungal community in spruce-fir forests using soil collections, to understand how edaphic factors like total N, total C, pH, and soil moisture are linked to changes in community composition for the major fungal guilds, and to perform a systematic analysis of a major mutualistic genus (*Russula*) in the spruce-fir ecosystem. This research will provide valuable baseline information on the fungal community present in the spruce-fir ecosystem and has the potential to identify regions where the soil fungal communities are at risk or contain rare species.

# The spindle mediates nuclear migration through the penetration peg of the rice blast fungus, *Magnaporthe oryzae*

Maribel Pfeifer, Chang Hyun Khang

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## Abstract

The blast fungus, *Magnaporthe oryzae*, causes severe destruction to rice and other crops worldwide. As the fungus infects rice, it develops unique cellular structures, such as the appressorium and a narrow penetration peg, to permit successful invasion of host rice cells. Yet fundamental knowledge about these cellular structures and how organelles, such as the nucleus, are positioned within them is still emerging. Here, we investigate the role of the mitotic spindle in mediating extreme nuclear migration through the penetration peg. We find that the spindle is involved in nuclear migration through the penetration peg, which permits successful development of the fungus within the first-invaded rice cell. Furthermore, regulated expression of conserved kinesin motor proteins, MoKin5 and MoKin14, is essential to form and maintain the spindle throughout this extreme nuclear migration event, as well as, properly nucleate the primary hypha. Overexpression of MoKin5 leads to formation of aberrant microtubule protrusions, which contributes to formation of nuclear fragments within the appressorium and primary hypha. Conversely, overexpression of MoKin14 causes the spindle to collapse leading to the formation of monopolar spindles. These results establish a model towards understanding the intricate subcellular dynamics of extreme nuclear migration through the penetration peg, a critical step in the establishment of rice blast disease.

# Antifungal effect of cold atmospheric plasma for treatment of equine fungal keratitis.

Darby Roberts<sup>1</sup>, Marc Cubeta<sup>2</sup>, Brian Gilger<sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, North Carolina State University, Raleigh, USA. <sup>2</sup>Department of Entomology and Plant Pathology, Center for Integrated Fungal Research, North Carolina State University, Raleigh, USA

## Abstract

Equine fungal keratitis (EFK) is a highly prominent infectious corneal disease across the Eastern USA. EFK causes severe pain and inflammation while frequently resulting in horse blindness. Challenges in treating EFK include low susceptibility and increasing resistance of the causal fungi *in vivo* organisms to currently approved topical treatments, presenting a need for improved treatment options. Recently, the use of cold atmospheric plasma (CAP) has gained attention in the medical community for its antimicrobial properties. CAP generates reactive oxygen (ROS) and nitrogen (RNS) species which have been demonstrated to inactivate a vast array of bacterial and fungal species, without risk of developing resistance. This study seeks to investigate the efficacy of CAP against two fungal species most commonly associated with EFK, *Aspergillus flavus* and *Fusarium keratoplasticum*. An *in vitro* assay will be performed to determine the relative susceptibility of *Fusarium keratoplasticum* and *Aspergillus flavus* to CAP generated using a dielectric barrier discharge (DBD) device. After determining initial susceptibility, an *ex vivo* porcine cornea assay will be used to determine CAP treatment response in a more biologically analogous model. Successful inactivation of the causal fungal organisms using CAP could greatly increase EFK treatment efficiency by reducing dependency on multi-dose, topical antifungal drugs.

## Gene family evolution among species in the Nectriaceae

Layne W. Rogers<sup>1</sup>, Marc A. Cubeta<sup>1</sup>, Nicholas LeBlanc<sup>2</sup>

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### Abstract

The Ascomycota family Nectriaceae includes important human and plant pathogens as well as saprobes that mediate decomposition and nutrient cycling in soil. Estimating changes in gene family size during evolution is useful for determining species adaptation to pathogenic and saprobic lifestyles. Rapidly evolving gene families (REGFs) among 22 species in the Nectriaceae and two species from the Stachybotryaceae (*Stachybotrys chartarum* and *S. chlorohalonata*) were identified from predicted proteomes using the Computational Analysis of gene Family Evolution (CAFE) software tool. Of 24 species analyzed, 422 gene families were identified as rapidly evolving ( $p \leq 0.01$ ). Total REGFs ranged from one in *Pseudonectria buxi* to 178 in *Fusarium oxysporum*. Putative functional classes of REGFs were established by annotating protein sequences within each REGF using the Clusters of Orthologous Groups (COG) database for high-level functional annotation and the Pfam database for precise functional annotation. In total, 4468 protein sequences were assigned a COG and Pfam annotation and used to characterize 304 REGFs. The three most frequently observed COG categories for REGFs among all species were unknown function (101 gene families); secondary metabolism, biosynthesis and catabolism (40 gene families); and carbohydrate transport and metabolism (19 gene families). Within each of these three COG categories for REGFs, the most frequently observed Pfam annotations were heterokaryon incompatibility protein (PF06985.12), cytochrome p450 (PF00067.23), and major facilitator family (PF07690.17), respectively. The experimental approach and data analyses used in this study provide a framework for evaluating gene family evolution among pathogenic and saprobic species in the Nectriaceae.

# Using fluorescent probes and COMSTAT to assess cell viability in *Aspergillus niger* biofilms treated with antimicrobial agents

Aswathy Shailaja, Julia Kerrigan, Terri Bruce, Patrick Gerard

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## Abstract

Filamentous fungal biofilms are ubiquitous in the built environment but testing the efficacy of antimicrobials is typically based on laboratory protocols utilizing processed fungal cultures rather than biofilms. The purpose of this project was to visualize and quantify filamentous fungal biofilms treated with several common antimicrobial agents using a fluorescent probe staining method. This method allows for the visualization of live and dead cells with confocal laser scanning microscopy. *Aspergillus niger* biofilms, modeled to reflect those that form under drip flow, were created in a controlled reactor under low-shearing force on glass coupons. The samples were treated with different concentrations of standard bleach with sodium hypochlorite, Comet® disinfecting cleaner with sodium hypochlorite, clove bud essential oil containing phenyl terpenoid and control was not treated. Specimens were stained with a combination of SYTO9 and propidium iodide fluorescent dyes. The cell viability of biofilms was calculated from their biomass using Z-stack images and the computer program COMSTAT. The least square student's t-test was performed, significant difference was observed between the different treatment types and different concentrations. From our study, it was observed that Comet® disinfecting cleaner showed the highest antimicrobial efficiency while clove bud essential oil negatively impacted the development of *A. niger* biofilms instead of completely killing the biofilm cells.

# Fungal degradation of neonicotinoids for water treatment

Leah Weaver, Tarek Aziz

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## Abstract

The prevalence of neonicotinoid insecticide use across the country has resulted in their widespread presence in surface water, wastewater, and drinking water. These insecticides can negatively impact aquatic life at environmentally relevant concentrations, but traditional water and wastewater treatment methods do not successfully remove them. The white rot wood decay fungus *Phanerochaete chrysosporium* has been shown to co-metabolically degrade environmental contaminants of emerging concern, including neonicotinoids, due to their production of extracellular and intracellular enzymes. However, this process is not fully understood and has not been optimized for application in a water treatment setting. We are currently conducting laboratory experiments to examine the degradation of three neonicotinoids, imidacloprid, clothianidin, and thiamethoxam, by *P. chrysosporium* to determine the relative contributions of key metabolic pathways and to optimize degradation conditions for treatment design. Preliminary experiments are underway to examine the degradation rate of imidacloprid for two strains of *P. chrysosporium* sustained on imidacloprid-amended liquid nutrient medium. These experiments will inform future research using submerged wood chips as a growth medium in a flow-through filter reactor, potentially lowering the cost of the technology. The long-term goal of our research is to establish key parameters for the engineering design of a microorganism-based system, allowing us to develop an optimized flow-through pilot bioreactor to treat stormwater, surface water, and potentially wastewater for emerging contaminants.

# Poster Presentation Abstracts

## Phylogeny, morphology and growth characterization of *Lasiodiplodia theobromae* associated with dieback symptoms of cacao (*Theobroma cacao*) in the Philippines

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### Abstract

Botryosphaeriaceae fungi are known to have different ecological roles. Their ability to persist endophytically or become pathogenic when their hosts are under stress makes them challenging to manage. In a 2016-17 survey, the endophytic fungus *Lasiodiplodia theobromae* was isolated from cacao twigs exhibiting dieback symptoms sampled from 12 cacao-growing provinces in the Philippines. Phylogenetic analysis of 80 *L. theobromae* isolates from symptomatic twig samples using ITS and *tef1- $\alpha$*  loci revealed high intra-specific variation among isolates. To examine phenotypic variation, we selected 12 isolates based on their phylogenetic relationships to compare morphological characters and mycelial growth at different temperature and water activity levels. Isolates produced fast growing white mycelia on potato dextrose agar and became gray to black in three days. Optimum temperature for mycelial growth was 30°C with minimum growth at 15°C and reduced growth at 35°C. Spore germination was greater than 85% at 40°C and less than 10% at 15°C across all the isolates. Water activity at -3 MPa reduced mycelial growth and spore germination across all isolates with the lowest values observed at -5 MPa. Conidia were subovoid with broadly rounded apex initially hyaline and aseptate, eventually becoming dark brown and one-septate at 12 days after pycnidia formation. However, variation in the incubation period for pycnidia production and number of pycnidia produced on oatmeal agar under 12-hr light: dark incubation was observed. Our results show wide genetic variation but similar morphological and growth characters for cacao-associated *L. theobromae* and we are currently evaluating their disease-causing ability on cacao.

# Elucidating the wheat seed mycobiome: a culture-based approach

Lindsey Becker, Marc Cubeta

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## Abstract

The occurrence of pathogenic fungal taxa associated with wheat (*Triticum aestivum* L.) seeds is well studied, but less is known about beneficial taxa of the wheat seed mycobiome. The goal of our research is to characterize wheat seed fungal endophyte and ectophyte diversity via a culture-based approach. We hypothesize that there will be greater alpha diversity of fungi on the exterior of the wheat seed (ectophytes) compared to fungi residing inside wheat seeds (endophytes). To isolate ectotrophic fungi, wheat seeds were immersed in sterile water and vortexed gently for 60 seconds. A 1:100 diluted suspension was transferred to Malt Yeast Extract Agar (MYEA) amended with streptomycin and tetracycline (50 mg/L each), and cyclosporin (4 mg/L). Preliminary experiments suggest that cyclosporin reduces individual fungal colony growth, thus preventing rapid overgrowth that can lead to lower diversity estimates for culture-based approaches. To isolate endophytic fungi, wheat seeds were sequentially submerged in 95% EtOH for 60 seconds, half-strength household bleach (3% active NaOCl) for 2 minutes, 95% EtOH for 30 seconds, sterile water for 30 seconds, followed by a final rinse in sterile water. Post surface sterilization, seeds were air dried, macerated, and sieved to obtain < 177  $\mu\text{m}$  fragments, followed by plating on amended MYEA media as described above. By utilizing these methods, we are developing a catalog of fungal taxa associated with the wheat seed exterior and interior. These cultures will be key members of a diversity panel to reveal the mycobiome associated with wheat seeds.

## Will the real *Fuscoboletinus weaverae* please stand up?

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### Abstract

*Fuscoboletinus weaverae* was described in 1965 by Smith and Shaffer from a collection associated with pines in Minnesota. Thereafter it was only collected once more, also at the same locality. This apparent rarity led to listing by Minnesota as endangered. In 1996, Kretzer *et al.* reported that the *F. weaverae* topotype ITS sequence matched *Suillus cf. brevipes*. In 2016, Nguyen *et al.* reported that the *F. weaverae* holotype ITS sequence matched sequences of the North American '*Suillus granulatus*' associated with *Pinus strobus*. Furthermore, they recognized *S. weaverae* as a species distinct from the European *S. granulatus*. However, *Fuscoboletinus weaverae* was described as having a spore print with red and purple hues and a veil, neither of which the *Pinus strobus* '*Suillus granulatus*' has. Both the holotype and topotype of *F. weaverae* were examined and resequenced which resolved these questions and supported Nguyen's position. The red and purple colors of the *F. weaverae* spore print are from glandular pigments not from the spores. The 'veil' is mycelium of a mycoparasite as postulated by Nguyen. Resequencing confirmed that the topotype is not conspecific with the holotype and that the holotype is indeed identical with the North American '*Suillus granulatus*' associated with *Pinus strobus*. Also requiring resolution were the proper authorities for the generic transfer to *Suillus*. By a matter of weeks, Engel & Klofac were first and thus the proper authorities are *Suillus weaverae* (A.H. Sm. & Shaffer) H. Engel & Klofac. And finally, '*F. weaverae*' is not rare after all.

## Mycological Survey of a Property in Western Johnston County, NC

John Gibbs

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### Abstract

Species surveys are an important tool for monitoring biodiversity and the health of the environment. A difference in the number of species or in the percentage of species per ecological role may indicate changes in the environment. As such, an initial benchmark measurement provides the necessary basis of comparison for future observations. In this project, a list of macrofungal species was compiled for a property in Willow Spring, North Carolina, for the year 2019, over the course of 51 weekly forays, to produce such a benchmark. A secondary objective was to research the ecological roles filled by these macrofungi. This project resulted in a list of 151 species of macrofungi representing three major ecological roles, which has since been used as the basis for continuing research on the property.

## The first species of *Lentinula* described from Africa: patterns of genetic divergence and historical biogeography in *Lentinula*

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### Abstract

The genus *Lentinula* (Agaricales) is a small lineage in Omphalotaceae with seven described species, including the shiitake mushroom (*L. edodes*), which is the most widely cultivated mushroom in the world. Species of *Lentinula* are distributed throughout Australasia, the neotropics, and the Gulf Coast and Caribbean regions of the Americas, but none have been described from Africa. Here, we describe the first species of *Lentinula* from Africa, *Lentinula madagasikarensis* sp. nov., from central Madagascar. This report constitutes a 4000-mile, trans-oceanic range extension for *Lentinula*. The new taxon is strikingly similar to *L. edodes*, but a multi-locus phylogenetic analysis places it as sister to the neotropical *L. aciculospora*. A combination of macro- and micromorphological characters clearly distinguish *L. madagasikarensis* from all other species of the genus. We will discuss the implications of this discovery for the geographic origin of *Lentinula*, as well as a peculiarly high rate of interspecific sequence divergence in the ITS region detected in the group.

# Friend or foe: exploring the diversity of fungi associated with strawberry production in Arkansas

Ashley Smith, Juan Moscoso, Scott Winters, Amanda McWhirt, Alejandro Rojas

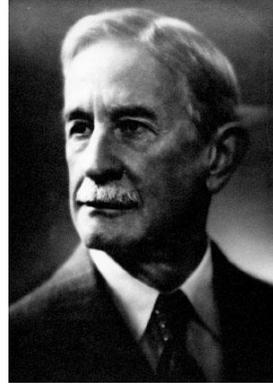
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## Abstract

Strawberry is one the most widely cultivated fruits in the US and it is a major berry crop in Arkansas. In the state, the predominate production system is annual plasticulture production, and the majority of berries produced are consumed locally. The plasticulture production system is used by growers who seek to increase yield, control weeds and reduce plant diseases among other benefits. However, the lack of soil fumigation or crop rotation before planting, as a result of the methyl bromide transition program, has created a gap in control that can lead soilborne pathogens. Most of these soilborne pathogens are fungi and oomycetes. A survey of the presence, intensity, and the species of disease-causing pathogens in Arkansas soils is needed. Therefore, our project seeks to combine efforts on first identifying the major disease-causing pathogen present in Arkansas strawberry production and to identify which practices favor reduced pathogen inoculum in the soil, such as crop rotation, fumigation or cover cropping. After one year of collection of soil and plant material, *Fusarium* spp. followed by the binucleate *Rhizoctonia* are the most common pathogens identified. From plugs are bare-root material, we have a diverse set of isolates, but interestingly, *Neopestalotiopsis rosae* has been identified, which is an emergent disease in this production system. By identifying the most significant threats in these systems we will be able to better recommend management strategies to control diseases and to promote practices that favor healthy soils and sustainable systems.

## Reprints - Poster Abstracts

In recognition of Dr. William C. Coker, we are reprinting these 2 abstracts about his mycological taxa.



Dr. William C. Coker

### Coker's *Amanita* taxa: 100 years later

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#### Abstract

Seven *Amanita* taxa are among the greater than 100 fungal species that Dr. William Chambers Coker described during his career at the University of North Carolina at Chapel Hill. Two *Amanita* species, *Amanita atkinsoniana* and *A. hygroskopica*, and three varieties, *A. rubescens* var. *alba*, *A. mappa* var. *lavendula*, and *A. spissa* var. *alba*, were published in "The Amanitas of the Eastern United States" (1917, J. Elisha Mitchell Sci. Soc. 33:1-88), as well as two new *Amanita* species, *A. roanokensis* and *A. gwyniana*, in "New or Noteworthy Basidiomycetes" (1927, J. Elisha Mitchell Sci. Soc. 43: 129-145). All of Coker's four *Amanita* species and one of his varieties (*A. rubescens* var. *alba*) remain accepted. A second variety has been elevated to the species level as *Amanita lavendula*. The third *Amanita* variety described by Coker is illegitimate taxonomically, but may still represent a new species or variety. The University of North Carolina at Chapel Hill Herbarium (NCU) boasts a modest *Amanita* collection of 650 collections representing 80 taxa. Included are 42 type specimens. Coker's *Amanita mappa* var. *lavendula* (now *A. lavendula*) is of special note given its lavender staining. In recent years, work with Coker's species resulted in the discovery of two additional lavender staining species of *Amanita* that have been given provisional names. Phylogenetic analysis of the LSU sequences of the three known lavender staining Amanitas and Amanitas featuring similar LSU sequences revealed that lavender staining Amanitas constitute a monophyletic clade.

## Coker's *Lactarius* taxa - 100 years later

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### Abstract

Dr. William Chambers Coker described over 100 new species of fungi during his career at the University of North Carolina at Chapel Hill. Included in these were seven species and two forms in the genus *Lactarius* published by Coker in "Lactarias of North Carolina" in 1918. Coker's seven *Lactarius* species have stood the test of time, six at the species level, now spread across three genera (*Lactarius*, *Lactifluus*, and *Multifurca*) and one at the variety level. As yet, his two forms have not been deemed suitable for taxonomic recognition. The *Lactarius sensu lato* collection of the University of North Carolina Herbarium (NCU) is the third largest in the southeastern United States, holding 456 preserved specimens representing 76 species. Of these specimens, 32 are type collections including types of Coker's taxa. Of particular note, the holotype of *Lactarius furcatus* Coker [now *Multifurca furcata* (Coker) Buyck & V. Hofstetter] has been rediscovered. NCU's *Lactarius* collection includes 48 Gertrude S. Burlingham collections from North Carolina and Vermont, 1907-1922, of which at least 5 are isotypes or topotypes of species of *Lactarius* that she described.