# 44th Mid-Atlantic States Mycology Conference (MASMC)

**April 14-16, 2023**

**Bowie and Beltsville, Maryland**

The Mid-Atlantic States Mycology Conference is a scientific meeting bringing together mycologists from the greater Mid-Atlantic Region. MASMC began in 1979 with participating mycology labs from Virginia Tech, University of Maryland, Howard University, and Towson State University. Each spring, MASMC provides scientists studying fungi with an opportunity to present their latest research findings, meet new colleagues, and develop and strengthen scientific collaborations. MASMC provides undergraduates, graduate students, and postdocs a unique opportunity to present their mycological research findings in a less formal venue than at a national or international scientific meeting.

This year's MASMC is co-hosted by the Department of Natural Sciences at Bowie State University (Bowie, MD) and the USDA ARS Mycology & Nematology Genetic Diversity & Biology Laboratory (Beltsville, MD).

## DR. LAFAYETTE FREDERICK

MASMC 2023 is dedicated (*in memoriam*) to the late Dr. Lafayette Frederick. Dr. Frederick (or "Fred" as he liked to be called by colleagues and friends) was an American plant pathologist, mycologist, and specialist in myxomycete ecology and systematics (https://en.wikipedia.org/wiki/Lafayette_Frederick). He was born in Bog Dog, Mississippi on March 9th, 1923 and passed away December 29th, 2018 (aged 95). This year would have marked his 100th birthday! His legacy includes training many generations of underrepresented minorities and The Lafayette Frederick Underrepresented Minorities Scholarship given by the Association of Southeastern Biologists. He was one of the founders of MASMC back in 1979 when he was at Howard University. There is a very long and detailed biography with interviews at https://www.thehistorymakers.org/biography/lafayette-frederick. Bowie State University, as well as Howard University, are Historically Black Colleges and Universities (HBCU) and it was long overdue that we dedicated this event to one of the very few black mycologists in the history of the United States.

*Source: Wikipedia*

## Locations of the meeting

Talks and Posters: Bowie State University (Ballroom at the Student Center)

Social events: Catalina Salgado's home

Foray: Patuxent Research Refuge, South Tract

## Bowie State University

How to get to BSU? https://bowiestate.edu/admissions-and-aid/visit-us/

Address: 14000 Jericho Park Road, Bowie, MD 20715

A map of the campus and an interactive version are available.

*The talks and posters will be held in the **Ballroom of the Student Center (20).** The entrance to the Ballroom area is indicated by the red arrow. The entrances to the campus are indicated by the purple arrows. Most likely, you will be parking in lots L, M, N, or O (red circles).*

Parking: See map above. **Lot N is the closest.**

## Catalina Salgado's home

History:

Catalina's home was once owned by the mycologist Marie Leonore Farr. "*Dr. Marie L. Farr, known as Lennie, contributed to a systematic understanding of two major unrelated groups of organisms: slime molds and tropical fly speck and black mildew ascomycetes. Most of her professional career was spent as a research scientist at the United States Department of Agriculture (USDA) Mycology Laboratory in Beltsville, Maryland* [now the Mycology & Nematology Genetic Diversity & Biology Laboratory]*, an organization in which Lennie was one in a long and continuing line of accomplished women*." (Source: A.Y. Rossman. 2021. In memoriam: Marie Leonore ("Lennie") Farr, 6 September 1927–13 May 2014 First Woman President of MSA. *Mycologia* 113: 509-511, DOI: 10.1080/00275514.2020.1836901).

Address: 4601 Wicomico Avenue, Beltsville, MD 20705.

## Patuxent Research Refuge, South Tract entrance

Patuxent Research Refuge was established in 1936 by executive order of President Franklin D. Roosevelt, and is the only wildlife refuge established to support wildlife research.

Address: Patuxent Wildlife Refuge, South Tract, National Wildlife Visitor Center, 10901 Scarlet Tanager Loop, Laurel, MD 20708

A map of the South Tract trails can be found here: https://www.fws.gov/media/south-tract-trail-map

iNaturalist MASMC 2023 Fungi of Patuxent Research Refuge:

We have created a project on iNaturalist (https://www.inaturalist.org/projects/masmc-2023-fungi-of-patuxent-research-refuge) where we can post our observations and identifications. Please join the project before the foray if you want to contribute.

## Who to contact?

Talks, posters, Bowie State University, social activities: Priscila Chaverri, pchaverri@bowiestate.edu, Mobile 301-891-9365

Foray and social activities: Catalina Salgado, catalina.salgado@usda.gov, Mobile 301-256-8762

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## PROGRAM SCHEDULE (April 14-16, 2023)

### FRIDAY APRIL 14TH

Location: Catalina Salgado's home. 4601 Wicomico Avenue, Beltsville, MD 20705

6-8 pm: **Welcome Reception**. Drinks and heavy hors d'oeuvres will be served.

### SATURDAY APRIL 15TH

Location: Ballroom at the Student Center, Bowie State University, 14000 Jericho Park Road, Bowie, MD 20715.

Abstracts for talks and posters are after the schedule.

8:30 am: Registration

9 am: Welcome (including a few words from Dr. Carl B. Goodman, Provost and Vice President for Academic Affairs, Bowie State University)

9:30 am: '**Some are sent and others just went': The impact of Dr. L. Frederick's development of six generations of underrepresented minority scientists**. Speaker: Dr. Chinyere A. Knight, Department of Biology, Tuskegee University

10:00 am: Coffee break

10:30 am: Keynote talk. **Invasion biology of mycorrhizal and saprophytic fungi linked to exotic forestry**. Speaker: Dr. Rytas Vigalys, Biology Department, Duke University

11:30 am – 2 pm: Poster session and lunch

2 pm: **The enemy of my enemy is my friend: Characterization of *Sphaerellopsis*, a mycoparasite of rust fungi**. Paula A. Gomez-Zapata, Samira Fatemi, Cristhian O. Ruiz-Castro, M. Catherine Aime.

2:20 pm: **Biogeography of *Sporobolomyces lactucae* is correlated with lettuce-producing regions**. Samira Fatemi, M. Catherine Aime

2:40 pm: **Biodiversity of *Ascocoryne*: New species from the Midwestern United States and Guyana**. Jeffery K. Stallman, Danny Haelewaters, M. Catherine Aime

3 pm: **Mycelial growth among different Isolates of *Tuber canaliculatum* in vitro.** Judith O. Enemudo, Nkese S. Udombang, Melvin Mensah-Bonsu, and Omon S. Isikhuemhen.

3:20 pm: Coffee break

3:50 pm: **Mining the decomposition biome of Spotted Lanternfly for entomopathogens**. Daniel Taratut, Barrie E. Overton, Joseph Calabrese

4:10 pm: **Effects of inorganic salts on mycelia growth and development in *Fusarium solani* and *F. oxysporum***. Nkese S. Udombang, Judith Enemudo, Melvin Mensah-Bonsu, Felicia N. Anike and Omon S. Isikhuemhen

4:30 pm: **Bioconversion of corn stover to food and feed: *Pleurotus ostreatus* mushroom yield corn stover substrate.** Brandon Essick, Uchenna Anele, Nathan Holt, Nkese S. Udombang, Roman Whitaker, Elbert Stephen Whitehead II, Breana Davis, Hammond Attigah, Omon S. Isikhuemhen

6 pm: **Dinner and social at Catalina's home**! Dinner and drinks will be served.

## SUNDAY APRIL 16TH

Location; Patuxent Research Refuge, South Tract, National Wildlife Visitor Center, 10901 Scarlet Tanager Loop, Laurel, MD 20708

9 am: **Foray at Patuxent Research Refuge**. Lunch box and drinks will be provided.

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## ABSTRACTS - TALKS

*Abstracts appear in the order in which they were presented in the schedule.*

### The enemy of my enemy is my friend: Characterization of *Sphaerellopsis*, a mycoparasite of rust fungi

Paula A. Gomez-Zapata[1*], Samira Fatemi[1], Cristhian O. Ruiz-Castro[1], M. Catherine Aime[1]. [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. *pgomezza@purdue.edu

Species of the genus *Sphaerellopsis* (Leptosphaeriaceae, Ascomycota) are commonly known as mycoparasites of rust fungi with promising potential as biological control agents for rust diseases. However, the scarce characterization studies on this genus restrict their potential applications. Here, we unravel the biogeography, host-specificity, and species diversity of *Sphaerellopsis* and elucidate the early mycoparasitic events of *Sphaerellopsis macroconidialis* against rust fungi. Of the 5,621 randomly screened rust specimens for the presence of *Sphaerellopsis* at the Arthur Fungarium, we found 199 confirmed as *Sphaerellopsis* members through multi-locus phylogenetic analyses and morphological observations. Five *Sphaerellopsis* species were recovered, infecting 122 rust species in 18 genera from 34 countries. One of these is a new species, *Sphaerellopsis melampsorinearum*. Our results also showed that *Sphaerellopsis* species do not have any preference for specific host rusts. Four of the five *Sphaerellopsis* species in this study showed a cosmopolitan distribution, and one, *Sphaerellopsis hakeae*, appears restricted to Australia. Finally, we confirm the antagonistic strategy of *S. macroconidialis* against rust fungi through in-vitro interaction tests with urediniospores of the southern rust of corn, *Puccinia polysora*. Hyphae of *S. macroconidialis* grew along the germinative tubes of *P. polysora* by coiling around them. After 12 days of co-cultivation, the germinative tubes of *P. polysora* lost turgor, and a few appressorium-like structures were observed on some urediniospores. The results of this study contribute some advances in characterizing the fungal genus *Sphaerellopsis* and understanding its ecological interactions with rust fungi.

### Biogeography of *Sporobolomyces lactucae* is correlated with lettuce-producing regions

Samira Fatemi[1*], M. Catherine Aime[1]. [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. *sfatemi@purdue.edu

Despite the paucity of accessible water and nutrients relative to other parts of a plant biome, the phylloplane hosts a diverse community of microorganisms. Though bacteria are most numerous on the phylloplane, fungi are important members of the microbiome as plant growth promoters, nutrient recyclers, and occasionally plant pathogens. Basidiomycetous red yeasts are a conspicuous component of the phylloplane fungal community. For many economically important crops however, our understanding of the presence of red yeasts is limited. This is particularly true for romaine lettuce, a widely consumed vegetable crop often implicated in foodborne human pathogen outbreaks. Additionally, much about the ecology of red yeasts is not fully understood. Here we aim to determine the biogeography of *Sporobolomyces lactucae* (Microbotryomycetes, Pucciniomycotina), a dominant fungal component of the phylloplane of

California-grown romaine lettuce. We collected lettuce (*Lactuca sativa*) samples originating from California and Arizona, the two major lettuce growing states in the US, as well as samples originating from Ohio, Indiana, Hawaiʻi, and Alabama. Yeasts were isolated using the ballistospore drop culture method and maintained on potato dextrose agar. The internal transcribed spacer (ITS) region was amplified via colony PCR and sequenced to determine yeast identities. Preliminary results indicate a presence of *S. lactucae* in the lettuce growing regions of California and Arizona. Frequently isolated from other growing regions was *Sporobolomyces shibatanus*. Fungi from the genera *Bullera*, *Hannaella,* and *Filobasidium* (Tremellomycetes, Agaricomycotina) were also detected. These results are congruent with the literature regarding the presence of the red yeasts of Basidiomycota. The correlation of *S. lactucae* with romaine lettuce grown in the top producing areas of the US has implications for the spread of microbes through our food system, because lettuce produced in California and Arizona are shipped to all 50 states and internationally as well.

### Biodiversity of *Ascocoryne*: New species from the Midwestern United States and Guyana

Jeffery K. Stallman[1*], Danny Haelewaters[2], M. Catherine Aime[1]. [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN. [2]Department of Biology, University of Ghent, Ghent, Belgium. *jstallma@purdue.edu

The genus *Ascocoryne* (Helotiales, Gelatinodiscaceae) contains eight species that are primarily thought to be endophytes of living trees and are often recognizable by their purplish, gelatinous apothecia, giving them the common name, "purple jelly drops." Some also have macroscopic, distinctive asexual structures. Despite being relatively conspicuous for a discomycete and producing volatile organic compounds (VOCs) that have been investigated for their potential for use in biofuels, species differentiation in the genus can be confusing. Work on a putatively new *Ascocoryne* species collected from Guyana led us to sample locally in the Midwestern, USA and request additional herbarium specimens to increase sampling from tropical locations. While work is ongoing, we report that species diversity in the genus *Ascocoryne* is at least double what is currently known and characteristics of the living spores (vital taxonomy) appear to separate the genus into two broad groups. Important taxonomic characters in *Ascocoryne*, and an updated nrITS phylogeny of the genus and six-locus phylogeny of the broader Gelatinodiscaceae are presented.

### Mycelial growth among different Isolates of *Tuber canaliculatum in vitro*

Judith O. Enemudo*, Nkese S. Udombang, Melvin Mensah-Bonsu, and Omon S. Isikhuemhen**.** Mushroom Biology and Fungal Biotechnology Laboratory, Department of Natural Resources and Environmental Design, North Carolina Agricultural and Technical State University, Greensboro, North Carolina. *joenemudo@aggies.ncat.edu

*Tuber canaliculatum* like other mycorrhizal forming relatives grows in a mutualistic association with the roots of trees like oak, chestnut, pecan, spruce, and pine, and forms truffles. It is the only black truffle that is native to the US that has very high commercial value. However, it is difficult to grow this truffle commercially, hence it is scarce and very expensive ($3800/lb). The major problem is that spore mass inoculation commonly used in seedling inoculation will be

expensive for generating seedlings to cultivate *T. canaliculatum*. It is known that the mycelia of truffles grow very slowly compared to other commercially cultivated fungi, but can mycelia be used as an inoculant for seedling production? The objective of this study is to determine variations in mycelia growth among different isolates of *T. canaliculatum*. A modified PDA medium was used to cultivate six different strains of *T. canaliculatum* (00, 01, 02, 03, 04, and 05), each strain had five replicates agar plates. After 30 days of incubation, mycelia growth in the different strains was measured. When subjected to ANOVA analysis, mycelia diameter data revealed significant differences between mycelial growths in the different strains tested ($P < 0.001$). Strain 04 had the fasted growth (3.11± 0.275) followed by strain 05 (3.08 ± 0.737). Strains 01 and 02 had the same mycelia growth (2.6 ± 1.286 and (2.6 ± 0.125 respectively). Strain 03 had the slowest growth (1.6 ± 0.132). Strain 00 had no growth. In conclusion, strains 04 and 05 had the best mycelia growth and could be used for further studies on the domestication and cultivation of *T. canaliculatum*.

## Mining the Decomposition Biome of Spotted Lanternfly for Entomopathogens

Daniel Taratut*, Barrie E. Overton, Joseph Calabrese. Commonwealth University of Pennsylvania, Lock Haven, PA 17745. *dt71999@huskies.bloomu.edu

Spotted Lanternfly (SLF), *Lycorma delicatula*, is an invasive leafhopper that poses a significant threat to Pennsylvania's natural and cultivated systems. SLF hold the potential to dramatically alter the specialty crop industry in the state of Pennsylvania, making management tools that control insect pest populations necessary for the protection of the industry. The prevalence of native entomopathogens provides a variety of biocontrol management potential against this invasive pest. The objectives of this study have focused on (1) isolation of entomopathogens from obscure soil types using SLF cadavers as bait, (2) sequencing isolates using specific DNA barcode regions (ITS rDNA and 16S rDNA) for fungal and bacterial community analysis, (3) determining the suitability of species as entomopathogens based on a literature review, and (4) selecting best species candidates for efficacy studies on SLF egg masses and first instars hatch rate success. Baiting of entomopathogens from obscure soil types has yielded fungi and bacteria capable of successful internal colonization of adult SLF cadavers. All fungal isolations were conducted on DOC2-PDA selective medium and bacterial isolations on an experimental half-strength TSA medium with SLF cadavers as the primary carbon source. Ten (10) fungal isolates and 22 bacterial isolates have been isolated from various SLF life stages with sequences of the ITS rDNA and 16S rDNA barcode regions in progress, respectively. Results indicate a low fungal and bacterial diversity index based on Shannon Weaver calculations for all soil types with values between 0.26 and 1.1, and 0 for the negative control non-sediment substrate. SLF cadavers baited on Woodward cave sediment resulted in the highest bacterial morphotype diversity. Sabula railroad sediment gave the highest fungal morphotype diversity. In addition to baiting, several entomopathogenic fungi were observed parasitizing egg masses and determined to be *Cladosporium* sp. and *Fusarium* sp. based on microscopy analysis. These results have elucidated several forgotten entomopathogenic fungi that could be used as novel biopesticides to control SLF populations in a variety of natural and cultivated systems.

## Effects of Inorganic Salts on Mycelial Growth and Development in *Fusarium solani* and *F. oxysporum*

Nkese S. Udombang*, Judith Enemudo, Melvin Mensah-Bonsu, Felicia N. Anike and Omon S. Isikhuemhen. Mushroom Biology and Fungal Biotechnology Laboratory, Department of Natural Resources and Environmental Design, North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United State of America. *nsudombang@aggies.ncat.edu

The study investigated the effects of different inorganic salts ($NH_4NO_3$, $KNO_3$, and $KH_2PSO_4$) on the growth of *Fusarium solani* and *Fusarium oxysporum*. Agar medium containing salts at different concentrations (0.063, 0.125, 0.25, 0.5, 1, and 2 g/l) was inoculated with test fungi. Visual observations and measurements of colony diameter were recorded at intervals of 3 days. The results showed a significant difference in mycelia growth for both *F. solani* and *F. oxysporum* at all salt concentrations on day 3 compared to the control (P= < 0.0001). For *F. solani,* on day 9 there was no significant (P=0.005) difference in mycelia growth at any concentration tested, except the control. For $KNO_3$, all concentrations except 0.06 g/L for days 6 and 9 showed a significant difference in mycelia growth compared to the control (P=0.05). For $KH_2PO_4$, concentrations of 0.125, 1, and 2 g/L for days 6 and 9 showed a significant difference in mycelia growth compared to 0.063, 0.25, and 0.5 g/L (P=0.005). In *F. oxysporum,* concentrations of 0.063, 0.5, and 2 g/L showed significant differences in mycelia growth compared to 0.125, 0.25, 1 g/L, and control (P=0.005) for $NH_4NO_3$ on days 6 and 9. For $KNO_3$, the concentrations 0.063, 0.125, 1, and 2 g/L for days 6 and 9 showed a significant difference in mycelia growth compared to 0.25, 0.5 g/L, and control. For $KH_2PO_4$, concentrations of 0.063, 0.125, 0.25, and 0.5 g/L for days 6 and 9 showed a significant difference in mycelia growth compared to 1, 2 g/L, and control (P=0.05). In conclusion, different concentrations of these salts can enhance mycelia growth in test organisms. Further studies are aimed at understanding the dynamics of these salt concentrations on Fusarium disease manifestation and control in the field crops.

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## Bioconversion of corn stover to food and feed: *Pleurotus ostreatus* mushroom yield corn stover substrate

Brandon Essick[1*], Uchenna Anele[2], Nathan Holt[1], Nkese S. Udombang[1], Roman Whitaker[1], Elbert Stephen Whitehead II[1], Breana Davis[1], Hammond Attigah[1], Omon S. Isikhuemhen[1]. [1]Mushroom Biology and Fungal Biotech Lab, NRED, CAES, North Carolina A&T State University, Greensboro, NC 27411. [2]Department of Animal Science, CAES, North Carolina A&T State University, Greensboro, NC 27411. *bgessick@ncat.edu

Corn is a large commodity crop in the United States for centuries. It is a food crop for people but is also used to feed livestock. Corn stover (CS), a by-product of corn farming is largely a waste that is underutilized that is rich in lignocellulose like the wheat straw used in mushroom cultivation and animal feed. Our goal is to use CS to grow mushrooms and use the spent substrate in animal feeding trials. This report is focused on the mushroom yield from CS substrate. Dried CS was chopped (5-10 cm lengths), soaked in water overnight, drained of water overnight, and supplemented with wheat bran (WB). 114 bags of substrate consisting of CS 2.25kg and WB 0.250kg each were loaded into polypropylene bags, and sterilized at 121°C, 15 PSI for 1 h. Each bag was inoculated with *P. ostreatus* spawn (5%) and incubated at 25°C for 4 weeks before they were subjected to fruiting. The total yield from all the bags was 50.19 kg of

mushrooms; an average of 0.440 kg per bag. The resulting spent mushroom substrate is being studied in animal feeding trials.

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## ABSTRACTS - POSTERS

*Abstracts are not in a particular order*

### Unraveling the metabolite richness of underexploited Amaranth using Metabolomic approaches

Mariam Koroma[1]*, Anne Osano[1], Elizabeth Arnold[2], Eike Reich[3]. [1]Department of Natural Sciences, Bowie State University, Bowie, MD. [2]School of Plant Sciences, University of Arizona, Tuscan, AZ, [3]HPTLC Association, Dianastrasse 10, Rheinfelden CH4310, Switzerland. *Koromam0713@students.bowiestate.edu

The United Nations designated grain amaranth (*Amaranthus* spp.) as "The Future Crop." This plant has been overlooked and undervalued yet the Grain amaranths is a leafy vegetable with a lot of health-promoting properties and therefore cannot be overlooked at a time when gluten-free, protein-rich, high-fiber and high nutritional qualities are becoming attractive labels in supermarkets throughout the world. The plant is reported to have anticancer, antivirals, and antioxidants properties. Documentation on environmental effects on secondary metabolites profiles in amaranth is missing. Symbiotic endophytes associated with plants are known as resources of novel metabolites. Fungal endophytes of the Amaranthaceous family have received inadequate attention. We hypothesis that endophytes contribute to the synthesis of metabolites in the amaranth plant. The objective of this research was therefore to isolate the fungal endophytes from different tissues of grain amaranth and use metabolomics approaches to understand the role the endophytes play in metabolite synthesis. The further objective was to study the effect of the environment of metabolite synthesis in *Amaranthus.* Plant samples were collected from Africa and different states in the USA. The method of High-Performance Thin Layer Chromatography (HPTLC) used in metabolomics for the purpose of conducting an examination of metabolite components. The findings of the HPTLC analysis revealed the presence of flavonoids inside the plant leaves. There were primarily three types of fungi (Sordariomycetes, Dothideomycetes, and Eurotiomycetes). *Fusarium, Stemphylium, Epicoccum*, and *Aspergillus* were among the most prevalent genera, all of which are known to produce a wide variety of secondary metabolites. It is demonstrated that several of the most widespread fungus are already well-understood from other plants found naturally in the United States, but which have no recognized medical applications. This raises the question of how they affect the metabolome of their host and, therefore, their applications in traditional usage.

### Diversity of endophytes in Sunn hemp (*Crotalaria junicea*)

Madison Marshall[1]*, Mary Simiyu[2], Anne Osano[1], Joseph Mafurah[2], Joshua Ogendo[2]. [1]Department of Natural Sciences, Bowie State University, Bowie 20715, Maryland, USA. [2]Department of Crops, Horticulture, and Soil Science, Egerton University, Njoro 20115, Kenya. *Madimarsh910@gmail.com

Sunn hemp, commonly known in Kenya as *Mito,* is a very important indigenous vegetable in Kenya. It also fixes nitrogen, good green manure, reduces soil erosion, conserve soil moisture, suppress weeds and nematodes, and recycle plant nutrients. It grows at fast rate in sub-tropical and tropical climates and its stem is used in industrial production of fiber. The crop is plagued with numerous biotic and abiotic stresses that can be solved by inoculation of endophytes. The objective of this study was to identify endophytes associated with sunn hemp and profile their functions.  The healthy vegetables were randomly collected from vegetable farmers in Vihiga count, Kenya. Bacterial and fungal endophytes were isolated on nutrient agar (NA), and Potato Dextrose Agar (PDA) media supplemented with antibiotic, respectively. Pikovskayas media was used to test for phosphorous solubilization efficiency (PSE) while Jensen's media was used to test nitrogen fixation. Total of 31 bacteria and 22 fungal endophytes were isolated from the plant.  *Aspergillus* spp*.* was the most dominant fungal endophyte isolated with the highest PSE of 11.84%. 58.83% of the bacterial endophytes fixed nitrogen while 1.87% fungal endophytes were able to solubilize phosphates. The endophytes found to enhance plant growth promoting traits will be recommended for inoculation on seed or incorporated in soil to improve crop productivity. More research will be done on the other endophytes to establish their role in terms of pest, disease or drought resistance.

## Isolating cold tolerant lichen-associated yeasts from *Candeleria concolor* and *Physcia* sp. from Northern Indiana

Ryan Doty[1*], Daniel B. Raudabaugh[1], M. Catherine Aime[1.] [1]Department of Plant Pathology, Purdue University, West Lafayette, IN, USA. *ryanpdoty@gmail.com

There are approximately 20,000 known lichen species which contribute ecologically in various ways such as food, shelter, and act as pioneer species. Lichens are a mutualistic association between algae and/or cyanobacteria, and are known to have associated mycobiomes. A recent study indicated many lichens contain a diverse pool of basidiomycetes yeast; however, these yeasts are presumptively mesophilic taxa due to the study's isolation strategies. Therefore, our research goal was to determine if there are cold tolerant basidiomycete yeasts in lichen thalli. For this study we focused on two commonly occuring lichens found around Purdue University (Northern Indiana); *Candelaria concolor and Physcia* sp. We collected 10 lichen samples at 3 different time points, for a total of 30 samples, in December 2022 and January 2023. Lichen thalli were placed into liquid media for two weeks. Next, 300**μ**L of supernatant was lawn plated onto 90 mm Petri plates containing potato dextrose agar and incubated for another two weeks prior to isolating colonies with unique morphology. All incubation steps were completed at 4℃ under 24 hour darkness, and all pure isolates were stored at 8 °C. Isolate identity was based on DNA (ITS1-5.8S-ITS2) sequence similarity using BLAST analysis against the NCBI database. To date, ninety-four isolates have been obtained and many of them have unique morphologies suggesting a diverse group of fungi. This research is important because these isolates may perform important functions for the lichens during winter months, and may represent novel fungal taxa.

## Mycorrhizal type mixtures promote neighborhood-level tree diversity

Kathryn N. Maley[1*], Gordon G. McNickle[1], M. Catherine Aime[1]. [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN. *maleyk@purdue.edu

Mycorrhizal fungi are the most widespread and ubiquitous fungal symbionts of plants, associating with 80-90% of land plant species. Most trees associate with either arbuscular mycorrhizal (AM) fungi, which live intracellularly, or ectomycorrhizal (ECM) fungi, which live extracellularly. These two mycorrhizal types have different effects on soil chemistry, host tree physiology, and ultimately forest community structure. Based on global trends, many fungal ecologists have adopted the ectomycorrhizal dominance hypothesis, which suggests that tree species richness decreases with increasing proportion of ectomycorrhizal trees. Alternatively, the mycorrhizal mixture hypothesis has been proposed, proclaiming that tree species richness is highest in approximately even mixtures of ECM and AM trees and decreases with dominance of either mycorrhizal type. Both hypotheses are supported by large datasets, but it remains unclear which hypothesis is more accurate at the neighborhood scale, or the level at which species directly interact. The purpose of this study is to determine the relationship between local tree species richness and mycorrhizal type proportion at the Ross Biological Reserve, a temperate deciduous hardwood forest in West Lafayette, Indiana that has been owned by Purdue University since 1949. Because the Ross Reserve is a mixed-mycorrhizal forest with a preexisting spatial grid system (40 $m^2$ plots), it provides an ideal opportunity to study this relationship at the neighborhood scale. Results show that neighborhood-level species richness is positively correlated ($P < 0.001$, F 1,103 = 21.35, Adjusted R2 = 0.19) with ECM tree proportion between the values of 0-60% ECM trees. Although the full spectrum of ECM trees was not represented by the Ross Reserve at the plot level, the results are consistent with the mycorrhizal mixture hypothesis rather than the ectomycorrhizal dominance hypothesis, as diversity is highest when there is approximately equal proportions of AM and ECM trees.

## Expanding accelerated identification of Pucciniales with DNA barcodes

Carlos A. López Manzano[1*], Mark Anderson[1], M. Catherine Aime[1]. [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. * clopezma@purdue.edu

The Pucciniales, known as rust fungi, are an order of obligate plant parasitic fungi, constituting the most species-rich group of plant pathogens, with more than 7,000 known species. Rust fungi have had a significant impact on agriculture, causing some of the earliest recognized diseases in crops. Identification of Pucciniales has a long history of challenges, as diverse and complex organisms. The great species diversity can be attributed to co-evolution with hosts, host jumping, and subsequent adaptation to new hosts. For rapid and accurate identification of rust fungi and monitoring of their outbreaks, DNA barcoding data are essential. This research contributes to the Rust Herbarium-based Universal Barcode BLAST (RustHUBB) database in order to provide rapid identifications. The methodology for this project was developed in the Aime Lab at Purdue University, where over 1600 samples of rust fungi stored at The Arthur Fungarium were utilized. These samples were excised for DNA extraction, which was amplified with the 28S region of ribosomal DNA using Rust2INV/LR6 primers and, nested with Rust28SF/LR5. The DNA concentrations used for PCR were 1, 5 and 10.5 µL, depending on

which provided correct amplification. PCR products were sequenced for further verification by BLAST against both the NCBI and the growing RustHUBB databases. More than 400 LSU barcodes were added in the last year from verified voucher material, bringing the total number of sequences in RustHUBB to over 3600. To date, these represent more than 1,170 species in 114 genera, with *Puccinia* and *Uromyces* having the highest number of barcodes (38% and 11% respectively). In addition, more than 80 DNA sequences belonging to currently undescribed species have been included. The creation of RustHUBB can serve as a valuable resource for the scientific community. Our goal is to eventually provide vouchered DNA barcodes for all described species of rust fungi.

## Preliminary Studies into the Phylogeny of Phakopsoraceae

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Rust fungi (Basidiomycetes, Puccinales) are a large and diverse order of obligate plant-parasitic pathogens. Currently, Puccinales is classified into 7 suborders and 18 families, but the family Phakopsoraceae remains difficult to resolve. Phakopsoraceae contains ca. 157 species in 8 genera of globally distributed rust fungi, some of which, such as Phakopsora pachyrhizi (Asian Soybean Rust), are extremely important agents of exotic invasive diseases.It has long been suspected that the family and the genus Phakopsora are polyphyletic; confusion as to the identity of type species for Phakopsora have restricted taxonomic treatments. In recent years, Phakopsora has been conserved with a neotype, and the family has been split into two families. Phakopsoraceae s.s. was classified in the suborder Raveneliineae while genera in the suborder Uredininae were classified Crossoporaceae. However, multiple genera in both families are currently polyphyletic, making them unsuitable as a systematically robust classifications. To provide a stable taxonomy for these fungi, this study sought to resolve the phylogeny of phakopsoroid fungi. To do this, sequences of ca. 105 species from the large ribosomal subunit (28S), small ribosomal subunit (18S), and the cytochrome c oxidase III (CO3) loci were used to propose a phylogeny based on Maximum Likelihood (ML) scores calculated using IQTree. All genera of Phakopsoraceae/Crossopsoraceae were represented except three (Monosporidium, Scalarispora, and Stomatisora) along with multiple asexual species currently classified in Aecidium, Malupa, and Uredo. Presently, results support recognizing several genera formerly synonymized with Phakopsora—such as Angiopsora, Arthuria, Bubakia—as distinct monophyletic genera that have specialized on different host families. These and other results will be discussed.

## Compiling a Checklist of the Fungi of North Carolina

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How many fungi occur in North Carolina? No one knows. Our estimate is circa 8k macrofungi and a total of circa 80k fungi. This is based on existing records and the scientific literature on mycobiota. We are compiling a list of the fungi that have been reported from North Carolina for the North Carolina Biodiversity Project (NCBP) (https://nc-biodiversity.com/). An initial list of NC Fungi was generated from the USDA records and the specimen records in MyCoPortal (https://www.mycoportal.org/portal/). This list consists of 10,353 taxa after partial removal of Myxomycete and Oomycete taxa. Common names have been added from the Global Biodiversity Information Facility (https://www.gbif.org/). Common names were found for 16% of the taxa. Current efforts editing the list include: Updating nomenclature and taxonomy including removing synonyms; Removing remaining lichenized fungi from the List as they are covered in a separate list of lichenized fungi prepared for NCBP by Gary Perlmutter; Removing remaining non-fungi from the List such as Bigyras, Myxomycetes. Oomycetes, etc. Myxomycetes are covered in a separate list prepared for the NCBP by Meriel Goodwin. After these edits are completed, the Checklist of NC fungi will be published on the NCBP Website ordered by division/order/specific and subspecific names along with authorities and common names. Ultimately it would be desirable to add county level distribution data, conservation rank and status (state/federal/global levels), and ecological (e.g. host/substrate) information.

## Identification and biodiversity studies of mushroom species on the Bowie State University Campus using DNA barcoding

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Fungi are one of the most abundant species worldwide. A common species of this are mushrooms. Mushrooms are very similar in phenotype but are ample in species. This can be very problematic due to the fact that mushrooms are in an edible fungal group. While there are plentiful mushroom species worldwide, the focus of this study was Bowie State University in Bowie, Maryland. The purpose of this research was to determine the species of mushroom that grows on campus; also, to examine its biodiversity level. This was done by using DNA Barcoding. 63 mushroom samples were collected from different areas on campus and analyzed. The mushroom population of this area has never been analyzed beforehand. DNA barcoding is a scientific technique used to distinguish different organisms and categorize them in different species by using a short genetic marker. For fungi, the short genetic marker used are internal transcribed spacer regions (ITS). ITS helps to amplify different genes of the fungi, keeping it broad enough to know that the species is mushroom, but making it specific enough to possibly see the differences between organisms. DNA was extracted from each mushroom. Samples that were amplified via PCR underwent electrophoresis to verify successful amplification. Successful amplicons were sent to GeneWiz, LLC for sequencing. Sequences were uploaded to DNA Subway, a system used to analyze genetic relationships between species. Using the basic local alignment search tool (BLAST), mushroom sequences from the class were aligned with sequences in the DNA Subway database. From this analysis, it was found that out of about 63 samples analyzed, there were only 20 different mushroom species that were identified on the University's campus.

## Incorporating Experimental Mycology into Interdisciplinary STEM Curricula

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There is growing enthusiasm for fungi in popular culture influencing students at Tuskegee University (TU). This heightened interest offers creative approaches to transform curricula in an area important to many careers pathways. Launching an area of interdisciplinary research in mycology at TU will broaden training for undergraduate students and faculty. A major goal of TU is to establish interdisciplinary projects to serve students and faculty from the Colleges of Arts and Sciences (CAS), Agriculture, Environment and Nutritional Sciences (CAENS), Veterinary Medicine (COV) and the Engineering (COE). The development of a taxonomic identification workflow for fungi is critical to investigations in biodiversity, ecology, evolution, agriculture, engineering, and natural product discovery. Research projects and educational strategies will be established in mycology to promote innovative approaches to investigation and learning. Moreover, studies in mycology will be a niche to attract, prepare and retain students in STEM. The initial focus of the proposed research activities is to assess the diversity of fungi in Macon County, Alabama. **The specific objectives are to: 1) develop the first local comprehensive survey and inventory of mushrooms, 2) train a multidisciplinary research team of undergraduate and graduate students to collect, morpho-identify, DNA barcode and curate specimens, and 3) Isolate unique cultures to study the chemical diversity of their secondary metabolites and evaluate their therapeutic potential in antimicrobial/human health research.** Formative and summative assessments will be created to be more inclusive of diverse learners. By implementing inquiry based laboratory projects, citizen science using the iNaturalist mobile application and community engagement, students will become agents of change. These efforts will preserve the legacy of Dr. Lafayette Frederick’s activism, pedagogy and research; thereby building a diverse, collaborative STEM workforce to develop strategic partnerships and entities that will generate more solutions for antiracism, justice, diversity, equity and inclusion (AJDEI) in STEM leadership.

## Outreach activities to improve the visibility of mycology in South Carolina

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While fungi play essential roles in virtually all ecosystems, they have historically been underrepresented in science education and popular culture. To address the issue, the Fungal Biology Creative Inquiry (CI) at Clemson University is working to increase public awareness of fungi and their importance through outreach events and activities. On campus, our efforts include maintaining an interactive mushroom garden; edible mushrooms are cultivated and labeled for anyone to pick and enjoy. We also maintain a fungus-themed display case, focusing on a different mycological topic each semester, to generate interest in fungi for passersby. At the Middle Atlantic State Mycology Conference in 2022, a poster presentation on methods of outreach to improve the visibility of mycology in the state was presented. As a result, CI students were invited to present a display for Darwin Day at the North Carolina Museum of Natural Sciences, thanks to Dr. Marc Cubeta. By making information about fungal biology more accessible to our campus and community, we hope to continue to foster a greater understanding and appreciation of this diverse kingdom.

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# THANK YOU FOR PARTICIPATING!

# SEE YOU ALL NEXT YEAR!