FOREST MYCOLOGY RESEARCH A NATIONAL RESOURCE

J.A. Glaeser[1], K.K. Nakasone[1], D.J. Lodge[1], B. Ortiz-Santana[1], and D.L. Lindner[1]

EXECUTIVE SUMMARY

- The Center for Forest Mycology Research (CFMR), U.S. Forest Service, Northern Research Station, Madison, WI, is home to the world's largest collection of wood-inhabiting fungi.
- These collections constitute a library of the fungal kingdom that is used by researchers thoughout the world.
- The CFMR collections have many practical uses that have improved the lives of Americans directly and indirectly over the past century in many ways.
- The CFMR and its collections contribute to tools to: identify and manage devastating fungal diseases of trees and wildlife, understand and maintain endangered wildlife populations that rely on fungi, provide fungal cultures to identify important pharmaceuticals and biotechnological processes, and develop sustainable forest management guidelines for bioenergy harvests from forests.

The Center for Forest Mycology Research (CFMR), U.S. Forest Service, Northern Research Station, Madison, WI, is home to the largest collection of wood-inhabiting fungi in the world. The culture collection includes approximately 20,000 living cultures of 1,800 species of fungi. The associated herbarium contains approximately 50,000 dried specimens of 4,200 species with many specimens dating back to the early 1900s. These collections constitute a library of the fungal kingdom that is used by researchers worldwide to classify, identify, and develop genetic profiles of wood-inhabiting fungi. Fungal identification is a difficult process, and these collections of living and dried organisms serve as reference standards for describing species, determining how much variability occurs within species, the extent of interbreeding among populations and species, and providing information on species' ecology and distribution. Mycologists have historically used macroscopic and microscopic features for fungal identification, augmented by culture morphology for wood-inhabiting fungi. Recently, molecular techniques involving DNA sequencing have become important tools aiding fungal identification (Glaeser and Linder 2011), but collection-based reference standards and DNA sequences based on those reference collections are still necessary. Whenever a new fungas is encountered, it is formally described based on a “type specimen.” A type is a reference specimen used as a standard to identify a species, often decades after it was originally described. The CFMR collection contains approximately 230 type specimens of important forest pathogens and decay fungi.

The CFMR collections have many practical uses that have directly and indirectly improved the lives of Americans over the past century. The identification of fungi associated with hardwood and conifer diseases was essential for the early forest pathologists of the 20th century (Davidson and Campbell 1943; Davidson et al. 1942). Working with forest managers, they developed management plans that reduced losses from disease and decay in forests being grown exclusively for timber production, During WWII, defects in lumber caused by decay fungi were identifed, evaluated and cataloged so that defective wood was not used in the manufacture of wooden ships and airplanes for the war effort (Davidson et al. 1947). Early work on decay fungi identified species that destroyed buildings, telephone poles, railroad ties, mine-timbers, and other forest products, leading to efforts to develop wood preservatives that prevent fungal decay (Duncan and Lombard 1965; Zabel et al. 1985). Current wood preservation evaluation tests continue to use CFMR cultures as standards for testing new, environmentally friendly preservative treatments (AWPA 2012). CFMR cultures have also been used to develop bioremediation

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protocols to break down toxic waste (Lamar et al. 1993; Lamar et al. 1994) and for biopulping (Kirk et al. 1993), a process for paper pulp production that could potentially reduce the amount of toxic chlorinated hydrocarbons in the effluent.

Identification and detection of current disease and decay fungi and potential threats from invasive species are essential to preserve and manage American forests even as the goal of management has expanded from timber production and watershed protection. The U.S. National Forests now supply many benefits to a variety of customers, including recreation, wildlife management, carbon sequestration, and purification of air and water. The mission and users of the CFMR collections have changed as well.

Fungi are now known to be an important food source for small mammals, which in turn are food for larger predators. Understanding the fungal composition in a forest stand allows wildlife biologists to estimate and better manage wildlife populations. One example of this is the preferential consumption of underground fungi, commonly known as "truffles," by flying squirrels in the Pacific Northwest. Flying squirrels foraging for truffles on the ground are vulnerable to predation by spotted owls and are the owls' primary food source. Understanding and maintaining truffle populations has therefore been identified as a critical aspect of to protect and manage spotted owl habitat (Waters et al. 2000). Another example of the importance of fungi to wildlife populations is the relationship between heart-rot decay fungi and cavity-nesting birds, such as the endangered Red Cockaded Woodpecker. CFMR scientists are currently working with ornithologists at Virginia Tech to identify and enumerate the succession of decay fungi necessary for nest cavity formation (Jusino 2011), expanding upon earlier work (Conner et al. 1976). Nest cavity formation greatly affects the reproductive success and population distribution of the bird – parameters that must be factored into management plans of southern forests.

Certain fungi cause wildlife diseases that can significantly impact important animal populations. One of the most devastating examples of this in recent years is White Nose Syndrome of bats, caused by the fungus *Geomyces destructans.* Thought to be an invasive species, perhaps from Europe, this fungus has killed over 6 million bats since first observed in a cave near Albany, NY in 2006 (Blehert et al. 2009). CFMR scientists, in association with researchers from the U.S. Geological Survey and several universities, are using a newly acquired collection of cultures from cave soils and affected bats to design a sensitive molecular detection technique that can be used to screen for the pathogen in the environment and in affected animals. These researchers are also identifying and cataloging closely related, nonpathogenic species of *Geomyces*, previously unknown to science that may be able to out-compete the pathogen within the cave or on bat skin. These organisms could potentially be developed as biological control agents (Lindner et al. 2010; Lorch et al. 2013; Mueller et al., 2013).

In addition to harboring wildlife, many forests today are potential sources of renewable energy. Viewing forests as a source of bioenergy is becoming a vital component of forest management, but this type of management necessitates the removal of large amounts of biomass, either for burning directly or for the production of highly valued biofuels. Unfortunately, the long-term effects of removing large amounts of biomass on forest heath and forest sustainability are unknown. Woody biomass is broken down by fungi to create forest soil. The removal of large amounts of woody debris may result in nutrient deficiencies and losses in forest productivity. In addition, many wood-inhabiting fungi are considered endangered species and their presence and distribution can be used as an indicator of forest health. CFMR scientists are collaborating with university researchers on studies currently in place in the Upper Midwest and Rocky Mountain region to monitor fungal distribution changes over time under different management regimes with the goal of creating sustainable management strategies for biomass harvesting (Brazee et al. 2012). Previous studies have shown that statistically rigorous sampling of the long-lasting, wood- inhabiting fungi is an excellent tool for assessing fungal diversity and for evaluating forest management strategies and forest health (Lindner et al. 2006). Basic studies in fungal biosystematics conducted by CFMR scientists in which fungal species are defined and described are essential as identification tools needed for this type of assessment (Burdsall 1985; Burdsall and Banik 2001; Larsen and Cobb-Poulle 1990; Nakasone and Burdsall 1995; Nakasone 1997; Ortiz-Santana et al. 2007).

Fungi also have commercial value as sources of food and medicinal compounds; many human cultures thoughout the world have used fungi to improve human health for centuries. Secondary metabolites and polysaccharides from fungi display a wide range of biological activities, including antimicrobial activity against fungi, bacteria, and viruses. Many polyporoid wood decay fungi are known to have anti-inflammatory and antioxidant properties, produce compounds that can reduce the growth of cancerous tumors and stimulate the immune system, and have positive effects on cardiovascular health (Zjawiony 2004). Hundreds of cultures from the collection have been screened by pharmaceutical companies for potentially beneficial medicinal compounds that can be used to treat human diseases. CFMR scientists have also conducted collaborative research with Merck to determine factors that control microfungal diversity in tropical forests and have evaluated which of these fungi might be useful for production of pharmaceuticals (Polishook et al. 1996). CFMR personnel are currently working with researchers in the Department of Pharmacy of Concordia University Wisconsin, Milwaukee, WI, to identify cultures with potential biomedical properties. In addition to their health benefits, some fungi produce powerful toxins; mistaken identifications can be deadly. Commercial mushroom producers, as well as professional and amateur mushroom gatherers, often consult CFMR personnel for advice on fungal propagation and identification. Poison control centers and emergency room doctors contact CFMR researchers, sometimes in the middle of the night, when presented with potential poisonings.

Decayed and diseased trees are a source of danger and liability to homeowners, cities, National Parks, and National Forests. Arborists, foresters, and park personnel are being trained to identify fungi associated with hazard trees. Such assessments are needed to evaluate whether suspected trees can be left in place or need to be removed in order to protect human life and property (Glaeser and Smith 2010). Should a tree be removed if a certain type of fruiting body appears at its base or on its trunk? Does the fruiting body indicate of severe internal decay, suggestive of imminent failure, or a minor sapwood decay associated with wounding that the tree will be able to compartmentalize and remain otherwise healthy? Understanding decay patterns associated with fires and insect damage is also important for forest managers to plan forest restoration activities. Identifications based on culture collection and herbarium reference standards have been used to assess decay patterns in fire-killed western larch (Jackson and Bulaon 2004; Jackson and Bulaon 2005), Engelmann spruce and subalpine fir (Worrall and Nakasone 2009), and beetle mortality in Lutz spruce (Glaeser et al. 2009). The identity and roles of the fungi associated with and vectored by the walnut twig beetle in the development of Thousand Canker Disease of black walnut are also being assessed

Climate models show that the composition of North American forests will change due to the influence of climate change. Forest managers are using these projections to anticipate future forest health issues and what future North American forests will look like. The impact of climate change on the activities of wood decay fungi has been assessed by CFMR researchers (Kliejunas et al. 2009) and suggests that decay rates will probably decrease as water becomes more limiting. This can lead to increased damage from fire as decomposition rates slow and the amount of flammable slash and woody debris increases on the forest floor. In areas where moisture is not limiting, increased temperatures will likely increase decay rates, resulting in less accumulation of fuel. Decomposition of wood by brown-rot fungi, which degrade the cellulose and hemicellulose of wood cell walls but leave behind the carbon-rich lignin, sequester carbon on the forest floor and in mineral soils. Brown rotted logs dominate the organic layer in northern conifer forests, which is critical for maintaining soil fertility and forest productivity (Jurgensen et al. 1997). In interior northwestern forests, brown rotted logs are critical for forest regeneration in clearcuts, serving as nurse logs for seedlings because they retain moisture and preserve ectomycorrhizal fungal inoculum (Harvey et al. 1980; Jurgensen et al. 1997). As the lignin is slowly converted into humic acids, it augments mineral-associated carbon in forest soils. Northern conifer forests may eventually be replaced by hardwoods in response to climate change; this may reduce the input of conifer-associated brown rotted wood and thus decrease the amount of carbon sequestration, lowering soil fertility and forest productivity. CFMR has provided cultures of decay fungi to a forest restoration company in Colorado to test for accelerated decomposition of woody debris in the forest to decrease fire hazard and sequester forest carbon. Evolutionary studies of the largest group of brown-rot fungi, known as the Antrodia clade, are also in progress.

Guarding the Nation's forests against potentially invasive species is one of the most important aspects of Forest Service research in which CFMR plays a critical role. In the past, devastating diseases, such as Dutch elm disease and chestnut blight, have decimated North American forests and landscapes, causing massive mortality of some of the most beloved tree species. CFMR scientists are involved in preventing the introduction of potentially invasive pathogens through the production of Pest Risk Assessments resulting from their participation in the Wood Importation and Pest Risk Assessment Mitigation Team (WIPRAMET). Insect and fungal risks that have been evaluated include the proposed import to the U.S. of logs from Siberia (USDA FS 1991), Chile (USDA FS 1993), New Zealand (USDA FS 1992) and Mexico (Tkacz et al. 1998), and of both logs and wood chips from Australia (Kliejunas et al. 2003; Kliejuanas et al. 2006) and South America (Kliejunas et al. 2001). WIPRAMET is currently producing a risk assessment that will evaluate the risk to native Hawaiian tree species from insects and fungal pathogens. Several different pathways are being assessed, including the import of ornamentals, "hitchhikers," and various forest products to the islands. CFMR was directly involved in analyzing fungi from wood chips imported from Chile. The vast majority of these fungi were harmless molds that can grow rapidly on wood and out-compete the growth of any potential pathogens, although some sapstain fungi were detected (Glaeser and Burdsall 2008). Forest pathogens occurring in subtropical forests that could invade the U.S. in response to climate change have also been identified (Banik et al. 2012; Lodge et al. 2010).

CFMR collections serve the public in more indirect ways by providing source material to scientists throughout the world as they unravel the mysteries of Kingdom Fungi. In a typical year, CFMR sends 700 – 800 fungal cultures to research laboratories in North America, Europe, Asia, and Latin and South America. Between 1999–2009, specimens, cultures and DNA sequences from CFMR collections resulted in over 2,602 citations in scientific journals (http://www.nrs.fs.fed.us/ units/ foresthealth/ local-resources/docs/CFMR_spec_cult_cit.xls). This is a very conservative estimate since much of the material is used without proper accreditation. Over 100 CFMR cultures were sequenced in a recent publication on the evolution of white-rot fungi in the genus *Trametes*; this study resulted in a major redefinition of this important genus (Justo and Hibbett 2011). This study is one in a series of National Science Foundation-funded projects on the evolution of white- and brown-rot fungi that are using CFMR cultures as a major source of material (Hibbett and Justo 2012). The application of DNA sequences obtained from CFMR cultures was e also used in a widely publicized study published in *Science* to explore the evolution of enzymatic lignin decomposition. The authors concluded that the evolution of white-rot fungi corresponded with the sharp decrease in the rate of coal formation at the end of the Carboniferous period (Floudas et al. 2012). The improved understanding of lignin degradation mechanisms gained through the sequencing of CFMR cultures will aid industrial microbiologists in developing more efficient processes for converting woody biomass into biofuels.

A new project related to development of industrial enzymes, including those used in biofuel processing and discovery of new pharmaceuticals, is the "1000 Fungal Genome" program, a Department of Energy-based initiative to develop total genome sequences of 1000 different fungi distributed throughout the fungal kingdom (Spatafora 2011). This effort will serve as a basis for future work in fungal genomics, physiology, and biochemistry and will lead to advances in medicine and bio-based industries as researchers unravel the mechanisms behind disease development and the production of fungal secondary metabolites, such as antibiotics. Cultures from the CFMR collection are particularly valued in this effort since many of them are in a haploid condition (N), containing only one copy of fungal DNA per cell instead of the more typical 2N or N+N state. The use of haploid cultures greatly facilitates data analyses and simplifies this complex research effort. CFMR scientists are also participating in the initial production of a proposed North American *Mycoflora* (Bruns 2011), a massive "wiki"-like on-line encyclopedia that will combine molecular data with other traditional identification tools – keys, pictures, ecological and morphological descriptions - to become a centralized location of fungal information for all macrofungi in North America. The CFMR collections are also included as a resource in the U.S. Culture

Collection Network (McCluskey 2012) − a 5-year National Science Foundation- funded project that will network all U.S. plant-associated microbial collections and develop standardized protocols for their management.

The CFMR collections are an important asset to science, the Forest Service, and the American public. The use and value of the collections have changed and increased since its establishment in the 1920s. These collections are repositories for the future, preserving the genetic heritage of the past and present, and need to he preserved. More information about the collections and a link to the searchable database can he found at http://www.fpl.fs.fed.us/research/centers/mycology/culture-collection.shtml.

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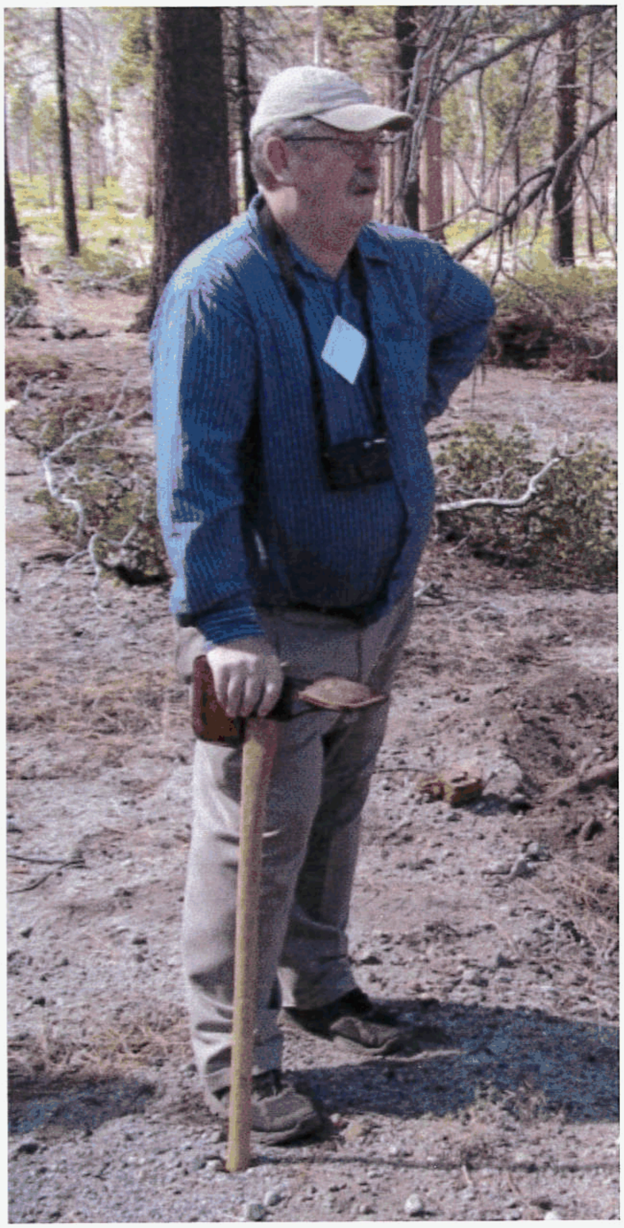

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John Browning
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