Title: Ectomycorrhizal fungi associated with Ozark chinquapin

Summary: Ozark chinquapin (*Castanea ozarkensis*), like all members of the Fagaceae, is ectomycorrhizal. The proposed project would generate the first body of data on the ectomycorrhizal fungi associated with Ozark chinquapin in northwest Arkansas. Root-tips would be collected from selected individual trees, placed in C-tab buffer and returned to the laboratory, where DNA would be extracted from these root-tips (using a standard DNA extraction kit), amplified and then sequenced. The sequences obtained will be cleaned up using the appropriate software and then compared with sequences of macrofungi available on the GenBank web site to identify the species of fungi involved.

Principal investigator: Dr. Frederick Paillet, Department of Geosciences, University of Arkansas, Fayetteville, Arkansas 72701

Research assistants: Dr. Steven L. Stephenson (research professor), Dr. Mourad Ben Hassine Ben Ali (visiting Fulbright Scholar) and Donald Nelsen (graduate student), Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701

Duration of project: 12 months (Nov. 1, 2015 to Oct. 31, 2016)

Total amount requested: $2,000

Short-term goals: To generate a body of data on the ectomycorrhizal fungi associated with Ozark chinquapin in northwest Arkansas. The majority of the funding requested in this proposal would to be used only for meeting this single short-term goal. However, if possible, a limited amount of data would be obtained from the European chestnut (*Castanea sativa*).

Long-term goals: (1) to compare the assemblages of ectomycorrhizal fungi associated with Ozark chinquapin with the assemblages associated with American chestnut (*Castanea dentata*) and Allegheny chinquapin (*Castanea pumila*), (2) to compare these data with the assemblages of ectomycorrhizal fungi associated with other ectomycorrhizal-forming trees (e.g., various species of *Quercus*) in the forests of northwest Arkansas and (3) to compare the data from the three species of *Castanea* in the Eastern and Central United States with data similar data for European chestnut.

Narrative: See next page

Timeline: Nov. 1 to Nov. 30, 2015 (collect root-tips), Dec. 1, 2015 to March 15, 2016 (extract, amplify and sequence DNA), March 15 to May 15, 2016 (collect additional set of root-tips and analyze sequences from first set of root-tips), May 16 to Oct. 31, 2016 (process these additional root-tips and analyze additional sequences) and Nov. 1 to Nov. 30, 2016 (assemble data and prepare report/manuscript for publication).

How results will be measured and reported: In brief, the data will be collected and processed in the manner described in the narrative. Later, one (or more) manuscripts will be prepared for publication.

How and when funds would be spent:

- Mileage (personal vehicle) to visit a series of study areas in northwest Arkansas (approximately 1,000 miles @ $0.42 per mile) = $400 (to be spent in the fall of 2015 and the spring of 2016)
- DNA extraction kits (2 @ $400) = $800 (to be spent in the fall of 2015)
- Miscellaneous laboratory supplies (e.g., C-tab buffer, plastic sample tubes, etc.) = $300 (to be spent in the fall of 2015)
- Sequencing costs = $500 (to be spent throughout the period of the project)

NARRATIVE

It is now known that the vast majority of vascular plants, including all forest trees, establish symbiotic relationships (called mycorrhizal associations) with certain soil-inhabiting fungi. These fungi are critical for the survival of the trees, since they play a significant role in the uptake of nutrients and water by the root systems of the trees in question. There are two primary types of mycorrhizal associations—endomycorrhizal and ectomycorrhizal (Stephenson 2010). Fungi involved in the latter produce many of the familiar "mushrooms" that appear on the forest floor in late summer and early fall (Binion et al. 2011). Examples of trees that form ectomycorrhizal associations include all members of the family Fagaceae. In the forests of northwest Arkansas, this family contains the oaks, American beech and Ozark chinquapin (*Castanea ozarkensis*). Recent study of chinquapin in northwest Arkansas showed that Ozark chinquapin expresses ecological characteristics in growth form and size that are distinctly different from those of Allegheny chinquapin and American chestnut (Paillet & Cerney 2012), and these differences could be partly reflected in unique mycorrhizal associations.

In brief, the primary objective of the proposed project would be to characterize the taxonomic assemblage of ectomycorrhizal fungi associated with Ozark chinquapin in the forests of northwest Arkansas. We are not aware of any previous study that has attempted to examine the ectomycorrhizal associates of Ozark chinquapin. In fact, there have been few studies of ectomycorrhizal fungi anywhere in this region of the United States. The cherty residual loam that developed on Mississippian limestone where Ozark chinquapin is often found is distinctly different from most soils associated with species of *Castanea* in the Appalachians, and the ancient DNA lineage inferred for the species (Shaw et al. 2012) may indicate that unique

ectomycorrhizal associations could have developed in northwest Arkansas. The proposed study definitely would yield one of the most comprehensive bodies of data on ectomycorrhizal fungi available for any species of tree in the Central United States. Among the questions to be addressed are (1) whether or not there is evidence of any unique species of ectomycorrhizal fungi associated with Ozark chinquapin (i.e., species that are found only on the latter), (2) how biodiverse is the assemblage of ectomycorrhizal fungi associated with Ozark chinquapin, (3) what are the dominant species of ectomycorrhizal fungi associated with Ozark chinquapin, and (4) how similar are the species of ectomycorrhizal fungi associated with Ozark chinquapin and various species of oak that co-occur in the same forest communities. The data to be obtained would be of value in any efforts to reestablish Ozark chinquapin in areas where the species has been eliminated or if there was a need to establish Ozark chinquapin seedlings in a nursery setting.

The methods used in the proposed project would involve making several collecting trips during the fall of 2015 and spring of 2016 to places where populations of Ozark chinquapin are known to occur. These would include Hobbs State Park, Pea Ridge National Military Park and the Buffalo National River. On each trip, root-tips would be collected from selected individuals of Ozark chinquapin, placed in C-tab buffer and returned to the laboratory, where DNA would be extracted from these root-tips (using a standard DNA extraction kit), amplified and then sequenced. The sequences obtained would be cleaned up using the appropriate software and then compared with sequences of macrofungi available on the GenBank web site and to similar sequences being generated by a graduate student (Donald Nelsen) at the University of Arkansas. Donald is studying the ectomycorrhizal associates of some of the major species of trees (e.g., white oak and red oak) that consistently co-occur with Ozark chinquapin in the forests of

northwest Arkansas as part of his Ph.D. research. The proposed project is also likely to involve Dr. Mourad Ben Hassine Ben Ali, a mycologist from Tunisia who will be spending the 2015-2016 academic year at the University of Arkansas as a Fulbright Senior Scholar. Mourad has considerable background working with ectomycorrhizal fungi. It also should be noted that since Donald Nelsen is currently carrying out research in a laboratory in the Netherlands, he should have the opportunity (pre-project) to collect some samples of root-tips from European chestnut.

The DNA extraction/amplification/sequencing approach has been used successfully in numerous other studies, including one study of American chestnut (*Castanea dentata*) in western Wisconsin (Palmer et al. 2008). The same DNA marker (the internal transcribed spacer region) used in this earlier study will be used in the proposed study. Don is also using this same approach in his Ph.D. research, so the methods outlined in this proposal already have been tested and found to be work successfully.

It should be noted that the project should yield a considerable body of new data that are likely to form the basis for at least one paper to be published in a peer-reviewed journal in addition to adding to what is known about the fungal component of forest ecosystems of the Central United States.

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2002-2007, Research Professor of Earth Sciences, University of Maine, Orono, ME. Graduate student supervision and watershed research at the Miner Experimental Forest, Plattsburgh, NY

1983-2002, Chief, Borehole Geophysics Research Project, U. S. Geological Survey, Water Resources Division, Denver, CO. Directing research in application of borehole measurements in ground water, paleoclimate, and general geology studies. Long term field studies in Hubbard Brook Experimental Forest, Big Cypress National Preserve, and Hawaiian Volcanoes National Park.

1982-1983, Visiting Scientist, Earth Resources Laboratory, Massachusetts Institute of Technology, Cambridge, MA. Conducted field modeling studies in geophysics, and field work related to the paleoecology of chestnut in Massachusetts.

1978-1982, Hydrogeologist/Geophysicist, U. S. Geological Survey, Water Resources Division, Denver, CO. Conducted research in borehole geophysics related to aquifer characterization and basic geology, including past climates at the Nevada Test Site.

1976-1978, Assistant Professor, Department of Geology, Wright State University, Dayton,OH. Responsible for undergraduate and graduate course in hydrogeology, and graduate student supervision in ground water studies.

Environment, ecology, and watershed studies:

More than a decade of involvement with inter-disciplinary studies at the Hubbard Brook Experimental Forest, White Mountains National Forest, NH

Chestnut ecology and paleoecology studies at the Harvard Forest, Petersham, MA

Environmental and ground water studies in Great Cypress National Preserve, FL and surrounding areas.

Reconstruction of Pleistocene climate in the Great basin using geomorphology, geochemistry, and packrat midden analysis

Chestnut ecology publications:

Paillet, F. L., 1982, The ecological significance of American Chestnut (Castanea dentata (Marsh.)Borkh.) in the Holocene forests of Connecticut: *Torrey Botanical Club Bulletin*, v. 109, no. 4, p. 457-473.

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Professional Preparation:

Lynchburg College (Lynchburg, Virginia)	Major: Biology	B.S. (*cum laude*)	1968
Virginia Polytechnic Institute and State University	Major: Botany	M.S.	1970
Virginia Polytechnic Institute and State University	Major: Botany	Ph.D.	1977

Appointments:

2014 – Fulbright Specialist Award, Hanoi National University of Education (Hanoi, Vietnam)
2003 - Research Professor, Department of Biological Sciences, University of Arkansas
2002 - William Evans Visiting Fellow, University of Otago (New Zealand)
1995 to 2002 - Visiting Professor, Division of Forestry, West Virginia University (summers)
1998 - Visiting Scientist, Manaaki Whenua Landcare Research (New Zealand)
1995 - Visiting Scientist, Australian Antarctic Division (Australian National Antarctic Research Expedition to Macquarie Island)
1987 - Fulbright Visiting Scholar, Himachal Pradesh University (Shimla, India)
1976 to 2003 - Professor, Fairmont State College (Fairmont, West Virginia)

Five publications most relevant to this proposal:

Stephenson, S. L. 1974. Ecological composition of some former oak-chestnut communities in western Virginia. Castanea 39:278-286.
Stephenson, S. L. 1986. Changes in a former chestnut-dominated forest after a half century of succession. American Midland Naturalist 116:173-179.
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Stephenson, S. L. 2010. The Kingdom Fungi: The Biology of Mushrooms, Molds, and Lichens. Timber Press, Portland, Oregon. 272 pp.

Expertise as Related to the Proposed Project:

Stephenson studied the successional dynamics of former chestnut-dominated forests for both his M.S. and Ph.D. degrees and has carried out research on the upland forests of the Central Appalachians for more than 40 years. He teaches Forest Ecology and Plant Ecology at the University of Arkansas.