Investigating the decomposition by of disease-resistant transgenic American chestnut (*Castanea dentata*) leaf litter and the colonization of the litter by ectomycorrhizal fungi

Summary

In order to introduce transgenic American chestnut trees into forests, policy requires that we ensure the ecological functions of the transgenic trees are equivalent to those of wild type American chestnut trees. This study is interested in the decomposition of leaf litter and the mycorrhizal fungi colonizing leaf litter *in situ*. This study will involve placing litterbags in the field directly under seedlings of the same type, just above the mineral layer of soil for a period of 5 months. The bags will then be brought back to the lab so the number of species in each bag can be recorded and the mass can be recorded.

Principal Investigator(s) and Institutional Affiliation(s)

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Duration of project
Approximately 7 months from starting date in June 2014

Total amount requested
$1,900

Short and long-term goals of the project

The short-term goal of the project is to verify that two types of transgenic American chestnut leaf litter are colonized by the same number of ectomycorrhizal fungi species as wild-type American chestnut during a 5 month decomposition period, and to verify that all types of leaf litter decompose at similar rates.

The long-term goal of the project is to determine whether transgenic American chestnut trees are ecologically equivalent to wild-type American chestnut trees, and therefore able to be out-planted into forests. Also, the success of this project could promote the utilization of genetic engineering for other conservation or restoration projects.

Narrative

Much research is being done to determine whether transgenic American chestnut trees function ecologically the same as wild-type American chestnut trees. Many of these projects are investigating things such as growth rate, light intensity, and other above-ground measurements. What I am interested in is not so easily apparent– how do the transgenic trees measure up underground? Looking at the decomposition of the leaf litter and the colonization of that litter by ectomycorrhizal fungi is one way to get at this half of the tree's ecology.

The first objective of this project is to investigate the rate of foliar decomposition. Leaf litter decomposition, also known as mass loss, provides the primary source of mineral nitrogen for biological activity in most terrestrial ecosystems. Nitrogen does not have a regular, significant geologic input and therefore, is commonly a limiting factor in plant growth. The breakdown of nitrogen from leaf litter provides a significant amount of this key resource, which increases ecosystem productivity. For leaf litter to be completely broken down in most systems, years to decades must pass for the process of decomposition to be completed. (Parton et al. 2007). The decomposition of leaf litter involves two sets of processes that occur simultaneously. One set of processes involves the mineralization and humification of lignin, cellulose, and other compounds by a variety of microorganisms. The second set of processes involves the leaching downward of soluble compounds into the soil, where carbon and nitrogen are then mineralized or immobilized. These two sets of processes are controlled by abiotic factors, such as climate, and by biotic factors, such as soil organisms, and the chemical composition of the leaf litter (Coûteaux et al. 1995).

When green leaves senesce, many of their physiological characteristics remain, which is responsible for the strong correlation between green leaf tissue chemistry and the chemical composition of leaf litter (Aerts 1996). The carbon and nutrient chemistry and stoichiometry of leaf litter can then have a strong effect on the activity and abundance of decomposers in the soil. This difference in decomposer abundance and activity can lead to different types of leaf litter having different rates of decomposition (Taylor et al., 1989). Cornwell et. al (2008) demonstrated that plant functional type can have an effect on decomposition rates. Their meta-data analysis showed that leaf litter from woody-deciduous species decomposed 60% faster than litter from woody evergreen species. This is why it is imperative to measure the rates of decomposition in our transgenic American chestnut trees and compare the data to the decomposition rate we record for the wild-type American chestnut trees.

A portion of leaf litter decomposition that is often overlooked in research is the breakdown of foliage and the transportation of its nutrients by mycorrhizal fungi associated with nearby trees. Mycorrhizal fungi are found throughout natural and man-made systems. In the last ten or so years, it has been made evident that mycorrhizal fungi play an important role in facilitating the breakdown of organic material so that the nutrients can be taken up by the fungi and given to the host plant. The distinction between decomposers and mycorrhizal fungi is becoming increasingly blurred. It has been noted that ectomycorrhizal (ECM) fungi are capable of producing extracellular lytic enzymes that allow them to obtain carbon, phosphorus, and nitrogen from organic compounds (Dighton 1991). A recent publication by Bödecker et al. (2014) hypothesized that when supported by carbon from their host plants, certain groups of ECM fungi can use oxidative enzymes to decompose organic matter in order to mobilize nitrogen for themselves and their hosts. These fungi act as decomposers in order to forage for organic nitrogen in the absence of more easily available, inorganic nitrogen sources. Bödecker et al. (2014) were interested in the enzyme MnP, which is known to play a part in litter decomposition, and found a significant association between Mn-peroxidase activity and the occurrence of *Cortinarius* DNA in humus samples. This association indicates that members of this genus make an important contribution to overall Mn-peroxidase activity in boreal forest soils (Bödecker et al. 2014).

Comparing the ECM fungal colonization on transgenic litter to wild-type litter is important because ECM fungi have demonstrated specificity for leaf litter. The leaf litter of some plants and the nutrients released during decomposition influence the ECM fungal community found on adjacent hosts. Aponte et al. (2010) examined the ECM fungal community of two co-occurring Mediterranean oaks, and of the 69 OTUs (operational taxonomic units) recovered, only 13 were found on both oaks; 29 were exclusive to *Quercus canariensis* and 27 only on *Q. suber*. They also found that calcium content, which was highest under the winter deciduous *Q. canariensis*, was the most important variable to explain the differences in the ECM fungal communities. Morris et al. (2009) also suggested that differences in litter quality affected host preferences between two *Quercus* species in a tropical cloud forest in southern Mexico. It is for this reason that we are interested in noting any differences between ECM fungi colonization of wild type and transgenic American chestnut leaf litter.

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Timeline, showing start and completion dates for each goal

November, 2013
Collect senesced leaves from seedlings

May, 2014
Select the seedlings that will be used for placing the litterbags
Get litterbags ready for field

June -October, 2014
Place litterbags in field beside selected seedlings

November, 2014
Collect litterbags from field and sort hyphae into types

December-January, 2014
DNA sequencing on fungal hyphae from bags

How results will be measured and recorded

The rate of decomposition of the litter by fungi will be calculated by subtracting the final dry weight of the litter from the initial mass of the liter. The number of species of ectomycorrhizal fungi that colonize the litter will be sorted by RLFP typing and analyzed through DNA sequencing.

Breakdown of how funds will be spent

$700	Materials including 44 micron nylon mesh litterbags, heat sealer, markers for the field, identification tags, and solution for storing DNA
$1,000	RLFP typing and DNA sequencing
$200	Publication costs
$1,900	Total

Timeline for when funds will be needed

August, 2014 – cost of materials: $700
November, 2014 – cost of DNA sequencing: $1,000
February, 2014 – publication cost: $200

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Education

Ph.D., Silviculture/Forest Soils — 1985
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M.S., Silviculture/Forest Biometry — 1982
SUNY College of Environmental Science and Forestry, Syracuse, NY

B.S., Forest Biology — 1979
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A.A.S. Forest Technology — 1975
N.Y.S. Ranger School, Wanakena, NY

Professional Experience

<u>SUNY College of Environmental Science and Forestry, Syracuse, NY</u>

Director of the Division of Environmental Science	2010-present
Professor of Forest Soils	2003-present
Associate Professor	1995-2003
Director of Forest Soils Analytical Laboratory	1995-present
Post-Doctoral Research Associate	1985-1988

<u>Cooperative Forestry Research Unit, College of Forest Resources, University of Maine, Orono</u>

Associate Research Professor	1993-1995
Assistant Research Professor	1989-1992

Teaching within the past 5 years

- FOR 345 Introductory Soils
- FOR 535 Advanced Forest Soils
- FOR 446 Soil Genesis, Classification and Mapping
- FOR 635 Forest Soils and Their Analyses

Publications within the past 5 years

Pacaldo, R.S., T.A. Volk, and R.D. Briggs. 2013. Greenhouse gas potentials of shrub willow biomass crops based on below- and aboveground biomass inventory along a 19-year chronosequence. Bioenergy Research. 6(1):252-262.

Kroll, S.A., N.H Ringler, J. De las Heras, J.J. Goméz-Alday, A. Moratalla, and R.D. Briggs. 2012. Analysis of anthropogenic pressures in the Segura Watershed (SE Spain), with a focus on inter-basin transfer. Ecohydrol. published online: 20 SEP 2012 DOI: 10.1002/eco.1311

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Education

Ph.D., Plant Pathology 1997
University of California, Berkeley, California

M.A., with honors: Biology: Ecology and Systematic Biology 1992
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Single Subject Credential, Biology with a Supplementary Authorization in English 1987
Humboldt State University, Arcata, California

B.A. Biology 1986
Humboldt State University, Arcata, California

Professional Experience

Student awards committee, Mycological Society of America 2013-present

Program Committee, Mycological Society of America 2006 - 2010. Chair 2010

Associate Professor, Mycorrhizal Ecology 2007-present
SUNY College of Environmental Science and Forestry, Syracuse, NY

Faculty advisor for the Central New York Mycological Society 2001 – present

Assistant Professor, Mycorrhizal Ecology 2001-2007
SUNY College f Environmental Science and Forestry, Syracuse, NY

Research Associate, Mycology 1997-2001
Oregon State University, Corvallis, Oregon

Biology teacher 1987-1989
Logan High School, Union City, California

Teaching within the past 5 years

- EFB 320, General Ecology
- EFB 428/628, Mycorrhizal Ecology
- EFB 496/796, Advanced Mycology: Basidiomycetes

- EFB 797, Mycorrhizal Symbiosis
- The Origin of Species Seminar

Publications within the past 5 years

Dulmer KM, LeDuc SD, Horton TR (2014) Ectomycorrhizal inoculum potential of northeastern US forest soils for American chestnut restoration: results from field and laboratory bioassays. Mycorrhiza 24 (1), 65-74.

Horton TR, Hayward J, Tourtellot SG, Taylor DL (2013) Uncommon ectomycorrhizal networks: richness and distribution of Alnus- associating ectomycorrhizal fungal communities. New Phytologist 198: 978-980

Nuñez MA, Hayward J, Horton TR, Amico GC, Dimarco RD, Barrios-Garcia MN, Simberloff D. (2013) Exotic Mammals Disperse Exotic Fungi That Promote Invasion by Exotic Trees. *PLoS ONE* 8(6): e66832.

LeDuc SD, Lilleskov EA, Horton TR, Rothstein DE (2012) Ectomycorrhizal fungal succession coincides with shifts in organic nitrogen availability and canopy closure in post-wildfire jack pine forests. Oecologia: 10.1007/s00442-012-2471-0.

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Galante TE, Horton TR, Swaney D (2011) 95% of basidiospores fall within one meter of the cap-a field and modeling based study. Mycologia 103:1175-1183.

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Lilleskov EA, Hobbie EA, Horton TR (2011) Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecology 4: 174-183.

O'Brien MJ, Gomola CE, Horton TR (2011) The effect of forest soil and community composition on ectomycorrhizal colonization and seedling growth. Plant Soil 341: 321-331.

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Professional Experience

Director of the Council on Biotechnology in Forestry — 2001-present
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Associate Professor — 1995-2003
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Assistant Professor — 1989-1995
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Postdoctoral Associate — 1986-1989
Area: Molecular Plant Pathology
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Teaching within the past 5 years

EFB307 Principles of Genetics
EFB308 Principles of Genetics Lab
BTC497 Research Design & Professional Development
BTC425/EFB625 Plant Biotechnology

Publications within the past 5 years

Book chapters:

H. Liang, P.A. Kumar, V. Nain, W.A. Powell, J.E. Carlson (2010) Selection and Screening Strategies. In: C. Kole, C.H. Michler, A. G. Abbott and T.C. Hall (eds) Development and Deployment of Transgenic Plants. Springer-Verlag – Berlin, Heidelberg, New York, Tokyo, Vol.1, pp. 85-143
pp169-192

Peer reviewed journals:

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Professional Experience

Professor of Forest Policy and Law — 2011-present
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Syracuse University College of Law

Adjunct Professor and Visiting Instructor — 1996-1999
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Attorney-at-Law — 1989-1995
Bagley, Lynett & Saia, Buffalo, NY

Teaching within the past 5 years

- FOR 465: Natural Resources Policy
- FOR 485/685: Business Law
- FOR 487/687: Environmental Law and Policy
- FOR 489/689 & LAW 865: Natural Resources Law

Publications within the past 5 years

Malmsheimer, R.W., J.L. Bowyer, J.S. Fried, E. Gee, R. Izlar, R.A. Miner, I.A. Munn, E. Oneil, W.C. Stewart. 2011. Managing Forests Because Carbon Matters: Integrating Energy, Products, and Land Management Policy. Forthcoming: Journal of Forestry 109(7s):S7-S51.

Mortimer, M.J., and R.W. Malmsheimer. 2011. The Equal Access to Justice Act and Federal Forest Service Land Management: Incentives to Litigate? Journal of Forestry 109(6):352-358.

Kuehn, D., P. D'Luhosch, V.A. Luzadis, R.W. Malmsheimer, and R. Schuster. Attitudes and intentions of off highway vehicle riders towards trail use: Implications for forest managers. Journal of Forestry 109(5):281-287.

Mortimer, M.J., M.J. Stern, R.W. Malmsheimer, D.J. Blahna, L. Cerveny, and D. Seesholtz. 2011. Environmental and social risks: Defensive NEPA in the US Forest Service. Journal of Forestry 109(1):27-33.

Keele, D.M., R.W. Malmsheimer, D.W. Floyd, and L. Zhang. 2009. An analysis of ideological effects in published versus unpublished judicial opinions. Journal of Empirical Legal Studies 6(1):213-239.

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Christopher Newport University, Newport News, VA

Professional experience

SSAI consultant, NASA DEVELOP September 2012-November 2012

- Worked in ArcGIS with Landsat and MODIS imagery to analyze the hydrology of the Great Dismal Swamp
- Provided information on how water should be allocated during the growing season

SSAI consultant, NASA DEVELOP February 2013-April 2013

- Worked in ArcGIS with VIIRS and Hysplit to track particulate matter during a large wildfire in Oregon
- Together, with a team, put together a video on the project that came in first place in a national contest

PGRP intern, Boyce Thompson Institute for Plant Research June 2012-August 2012

- Worked in the Giovannoni lab, investigating the role of carotenoids in tomato fruit development
- Measured carotenoid concentrations in ABA-deficient mutants and ZDS knockouts to look for a build-up resulting from a block in the pathway
- Results supporting the hypothesis were presented at BTI's annual student symposium

Research Assistant, SUNY Research Foundation January 2014-present

- Investigating the nutrient content of decomposing leaf litter belonging to two transgenic American chestnut types, a hybrid type, and a wild-type chestnut

- Investigating the species of mycorrhizal fungi that colonize leaf litter of two transgenic American chestnut types and wild-type chestnut
- Field work involving planting and collecting litterbags, and lab work involving nutrient analysis and mycorrhizal fungi DNA analysis

Teaching Assistant, SUNY ESF August-December 2013, 2014

- Teaching a lab section of Introduction to Soils, FOR 34, and grading assignments
- Field portion of the lab involves traveling to sites and digging soil pits to identify layers of the soil profile, lecturing on Best Management Practices in forests, lecturing on created wetlands as described in section 404 of the Clean Water Act
- Lab portion involves CEC analysis, soil acidity, and soil fertility