Project Title: Plant and Fungal Dynamics in American Chestnut Restoration

Summary: This grant application seeks funding for the following objectives: 1) fifth year survival and growth data of American and backcrossed chestnuts on a field site in Dresden, Ohio, 2) document natural cankers and report on the field resistance of the backcrossed chestnut lines, and 3) conduct an ectomycorrhizal (ECM) survey on chestnuts and incorporate beneficial fungi into inoculum for future Ohio plantings. Undergraduate students will work on research programs applicable to the TACF, while collaborating with professionals from Ohio institutions: Miami University, Ohio University, The Ohio Chapter of TACF, *The Wilds* Conservation Science Training Center, and the US Forest Service.

Principle Investigators:

Dr. Jenise M. Bauman
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Delaware, Ohio
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Duration of the Project: April 2012 - April 2013

Total Amount requested: $6,500.00

Short and Long Term goals of this project:

There are **three short-term goals** of this current grant application. **1)** This field study is designed to investigate ectomycorrhizal (ECM) fungal colonization, ECM community composition, and chestnut seedling host response, five years after initial field establishment. The natural colonization of ECM fungi will be documented and DNA sequencing will be used to identify species colonizing on chestnut roots. ECM communities will be compared between pure American and backcrossed chestnuts. ECM species colonization will be related to chestnut growth (height and basal diameter) to determine ECM species that are most beneficial to chestnut. **2)** Working collaboratively with scientists with the US Forest Service, the second short-term goal is to produce chestnut specific ECM inoculum for use in future plantings. Selecting fungi that readily colonize chestnut roots and can economically be cultured *in vitro* may aid in improving Ohio chestnut planting protocols. **3)** Lastly, this study will collect the fifth year's growth data and relate chestnut growth rates to the original soil treatments (described below). Working with Ohio University, data will be used to analyze survival through time. This field assessment will document, measure, and map the early cankers that have entered the restoration planting site. Results will relate the disease resistance potential of the two backcrossed chestnut genotypes established in this field site.

There are **three long-term objectives** of this current application. **1)** Our first long-term goal is to document ECM species composition overtime. This will be an ECM survey on chestnut after trees have been established for five growing seasons. The 2008 survey (sampled after two growing seasons) illustrated that certain ECM species were inadvertently transplanted on chestnut roots from the field nursery. Recording this fifth year data will provide greater insight to below-ground interactions of chestnut. Assessment will determine the competitive ability of introduced ECM species as they interact with native ECM source populations. **2)** The second long-term objective will focus on the facilitation of woody species recruitment in restoration plantings by an ECM host (chestnut). To determine this, a vegetation study will be conducted in chestnut stands. This will also allow for the comparison of the original planting methods to explore how the sub-surface treatments influence herbaceous community assembly. **3)** Lastly, the third long-term objective is to document the changes in ECM colonization on a diseased plant host. The hypothesis being tested is that the mutualism is contingent on the availability of carbon produced by the plant host. This current study will record ECM root colonization on cankered chestnuts and use this as a preliminary data that will be used to describe the nature of the plant-fungal mutualism under a disease model.

Project Narrative:

Introduction:

American chestnut (*Castanea dentata*) was eliminated as a canopy tree from the eastern U.S. forests with the introduction of chestnut blight (*Cryphonectria parasitica*) in the early 1900s. This hardwood species, valued for its economic and ecological qualities, was highly susceptible to the canker producing *C. parasitica*. Early control measures such as eradication of infected tissue and trees, treatment with Bordeaux mixture, and quarantine of infected hosts were unsuccessful in slowing the spread of the pathogen (Kuhlman 1978). By the 1950s, 200 million acres of American chestnut had succumbed to the disease and this once prominent species was relegated to a minor place in the eastern forests. Backcross breeding programs initiated by Burnham (1988) have been successful in incorporating blight-resistant genes from Chinese chestnuts (*C. mollissima*). Resulting in progeny contain ~ 94% *C. dentata* genes and display blight resistance with the desired morphological characteristics of the American chestnut (Hebard 2005).

American chestnut's fast growth rate, early nut production, and quality of timber make this a desired species for use in reforestation projects (Jacobs et al. 2009; Rhoades et al. 2009). This species tolerates a wide range of ecological conditions, including tolerance to drought and low pH, typical of coal mined sites (Jacobs 2007). Coupled with proper planting methods, chestnuts have the ability to quickly establish on reclaimed coal mine sites (McCarthy et al. 2008). Using backcrossed varieties in mine reclamation projects provides a reliable restoration tree species. This also provides field trials for chestnut lines in the native range of American chestnut. Further assessment of existing sites will provide valuable insight to field growth, long-term survival, and blight-resistance potential of the backcrossed genotypes.

Like other members of Fagaceae, American chestnut forms ectomycorrhizas with certain fungal species (Hiremath and Lehtoma 2007; Palmer et al. 2008; Bauman et al. 2011). Over 6,500 species are ectomycorrhizal (ECM). They are generally characterized by the formation of a fungal sheath, Hartig net, and radiating hyphae (Smith and Read 2008). Previous studies have

documented the benefits that this symbiosis has on many conifers and angiosperms in reforestation projects (Marx 1991). These benefits include greater access to water, nutrients, alleviation of metal toxicity, and protection from root pathogens (Cordell et al.1999). In turn, these fungi receive carbon in the form of photosynthates from their plant host forming a mutualistic relationship between plant and fungi. Because chestnut was eliminated as a canopy tree from the Eastern deciduous forests by the 1950s, very little is known about these ECM interactions.

Chestnut in Ohio Coal Mine Restoration:

Using the Forestry Reclamation Approach (FRA) proposed by The Appalachian Regional Reforestation Initiative (ARRI), McCarthy et al. (2008) established a mixed genotype chestnut stand on reclaimed coal mine in the Tri-Valley Wildlife Area in central Ohio, USA. FRA treatments such as deep ripping coupled with traditional plow and disking alleviated soil compaction and disturbed the herbaceous canopy formed by invasive species. Importantly, these soil treatments greatly aided in the establishment of the $15/16^{th}$ backcrossed chestnut (i.e., $1/16^{th}$ *C. mollissima*) which had an 80% survival rate after three growing seasons (McCarthy et al. 2010). This backcrossed genotype expressed the upright, fast growth rates similar to pure American chestnut. After four field seasons in this field site, chestnut canopies surpassed invasive species (such as tall fescue and lespedeza) and formed chestnut burrs (Figure 1).

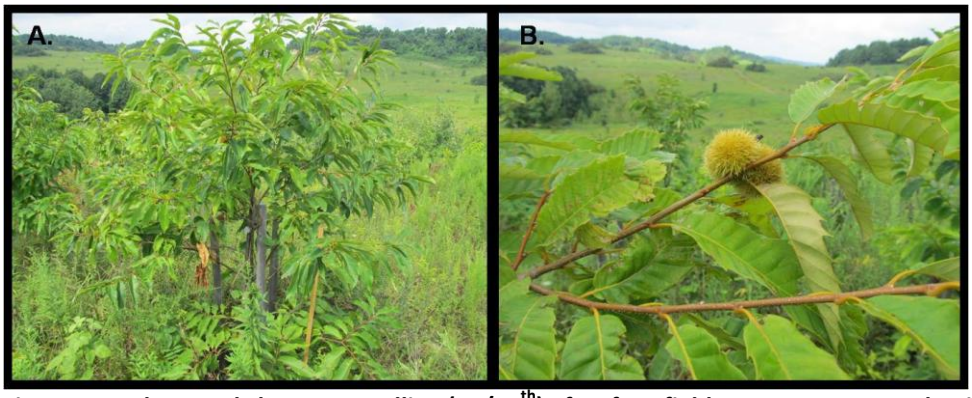


Figure 1: Backcrossed chestnut seedling ($15/16^{th}$) after four field seasons. Burr production was recorded from certain individuals.

In addition, these mechanical treatments increased the activity of ECM fungi resulting in greater root colonization on chestnut (Bauman et al. 2011). Chestnut seedlings found naturally colonized by ECM in the mechanically treated plots had the greatest growth rates. DNA laboratory analysis has identified 16 different species of ectomycorrhizal (ECM) fungi on chestnut roots in the field (Bauman et al. in review). Of these, a few abundant species appeared to have a high affinity for chestnut and invoked a positive host response. Employing methods that encourage the formation of chestnut ectomycorrhizas maximizes shoot growth in the early years of establishment. Learning how to culture these fungi from chestnut root tips and use these cultures as inoculum for future plantings would be the next step in developing protocols used to establish advanced backcrossed genotypes for long-term survival.

At the end of the fourth growing season, chestnut blight (*C. parasitica*) cankers have been detected on pure American chestnuts (Figure 2). With these natural cankers, come an interesting assessment of the resistance potential of the 7/8 and 15/16 chestnut hybrids. In addition, this field site will provide interesting insight to the dynamics of the plant and fungal mutualism. It has been documented following herbivory that diminished hyphae development correlated to photosynthetic tissue lost, suggesting carbon limitation as a mechanism driving the decrease in ECM colonization (Saikkonen et al. 1999). It can be hypothesized that ECM colonization will show a similar pattern when host tissue is lost due to blight. However, certain species whose ectomycorrhizas persist under changing carbon allocation may become important for coppice sprout production.

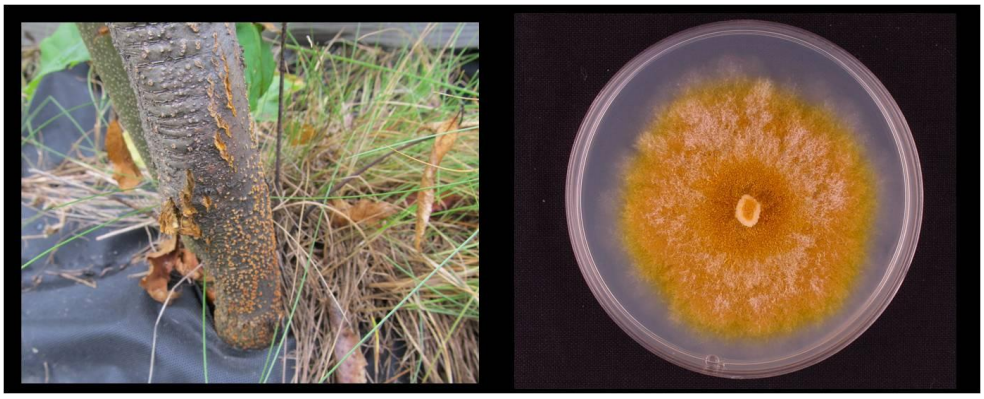


Figure 2. Chestnut-blight canker recorded on a pure American chestnut seedling after four field seasons.

2012 TACF Funding Project Objective:

This current grant application seeks funding to continue this field research with regard to plant and fungal dynamics of backcrossed chestnuts. Funds awarded by The American Chestnut Foundation will be allocated to the following objectives: 1) record and analyze the fifth year survival and growth data as it relates to original soil treatments, 2) map cankers and report on the mortality and disease resistance of the backcrossed chestnut lines, and 3) conduct an ECM survey on chestnuts and incorporate the most beneficial fungi into inoculum for future plantings. Research proposed in this grant application will take place on an established field site in Dresden, Ohio. The workload will network five Ohio institutions: Miami University, Ohio University, The Ohio Chapter of The American Chestnut Foundation, The Conservation Science Training Center of The Wilds, and USDA Forest Service, Northern Research Station.

To accomplish the workload proposed, this project will form the basis of a summer training program for undergraduate interns. *The Wilds* Scholar Internship Program is a 10-week intensive training program designed to mentor the professional growth of undergraduate students. This program couples hands-on field training with intern project ownership for the summer of 2012 (June 11, 2012 – August 17, 2012). Three specific research objectives will be assigned to three interns that will allow for summer study of TACF history and breeding programs, concepts of disease ecology, field applications of chestnut seedlings, and DNA extraction and sequencing of ECM fungi.

Research Methods:

Study Site:

The study site was initiated in the spring of 2007. Three experimental blocks, each containing the control and three soil treatments, were set-up prior to planting. Each block was 73 × 36 m with four 18 × 36 m treatment plots contained within (Figure 3). Each block was replicated three times. The following treatment plots were established: 1) a control left undisturbed (C), 2) a plot cross-ripped at a depth of approximately 1 meter created by a D-6 dozer with a 1.0 m steel ripper bar attachment (R), 3) a plowed and disked plot installed by a conventional tractor (PD), and 4) a ripped + plowed and disked plot (RPD). A total of 1200 one-year-old chestnut seedlings were planted in the treatment plots (12 plots, 100 seedlings per plot) as bare rootstock in April of 2007 at a spacing of 2.15 × 2.15 m. Chestnut seeds were comprised of the following: 400 pure American chestnuts (*C.dentata)*, 400 7/8th backcrossed chestnuts, and 400 15/16th backcrossed chestnuts.

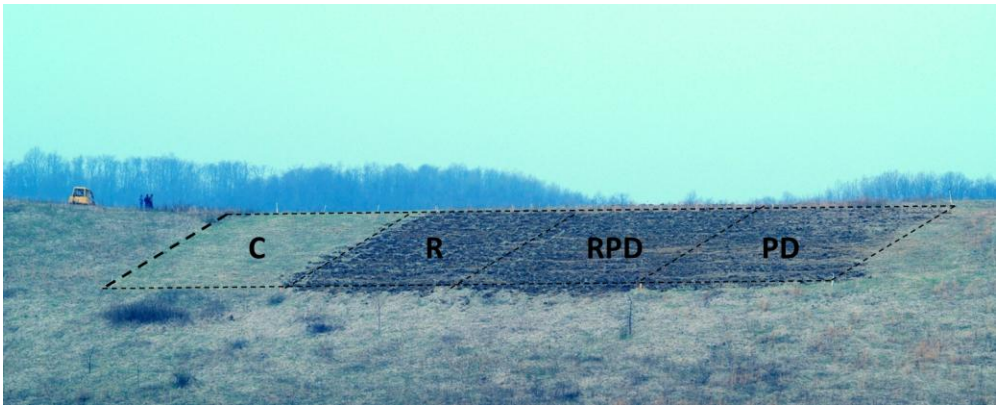


Figure 3. Field block design: Each consisted of four treatments: 1) a control (C), 2) a plot cross-ripped (R), 3) a ripped + plowed and disked plot (RPD), and 4) a plowed and disked plot (PD). Each block consisted of 400 chestnut seedlings and was replicated three times for a total of 1200 chestnuts.

2012 ECM Field Sampling and DNA Sequencing

A total of 100 American chestnut seedlings (50 pure American and 50 of the 7/8th backcrossed chestnuts) will be selected for non-destructive root sampling. To control for seasonal variation with regard to ECM composition, two root collection dates are being proposed: 50 seedlings in April of 2012 (right before bud break) and another 50 in October 2012 (when trees enter dormancy). The 15/16th backcrossed chestnuts will not be sampled. All trees with notable cankers will also be selected for root sampling during both sampling dates. To ensure roots are being sampled from chestnut and not a part of the surrounding vegetation, soil will be carefully removed with a spade to expose the chestnut root system at a depth of 25 cm and a width of 45 cm. Roots will be carefully sifted away from the soil and then stored on ice. Once in the laboratory, roots will be washed and transferred into a Petri dish containing sterile

water. Roots will be washed, examined, and morphotyped under the stereoscope for ectomycorrhizal sheaths.

A 3 mm root tip segment will be used for isolating the fungal DNA using the internal transcribed spacer (ITS) region. Root tips will be grouped by similar morphology and one root tip belonging to each morphotype will be sampled for ECM fungi. The DNA will be extracted using the QIAgen DNeasy® Plant Mini Kit. PCR primers (ITSF1 and ITS4) will be used to amplify the ITS region of fungal ribosomal DNA. Products will be analyzed by electrophoresis on 0.5% agarose gel and the PCR product bands will be purified using the Wizard® SV Gel and PCR Clean-Up system by Promega (described in Bauman et al. 2011). DNA will then be sequenced using the ITSF1 primer and capillary Sanger sequencing at the Plant-Microbe Geomonics Facility of The Ohio State University.

ECM Culturing in vitro:

A 10 mm segment of root, per morphotype, will be sampled from chestnuts and stored on moist filter paper at 4° C. Root segments will be surface sterilized with a 10% bleach solution and plated onto Modified Melain-Norkrans Agar (MMN). Cultures will be grown at room temperature and inspected for fungal mycelium two times daily. Mycelium from targeted ECM species will be transferred to maintain a pure culture of ECM. Approximately 10 mg of hyphae per species will be selected for DNA sequencing to verify that the intended fungal culture is being maintained in culture. Once the ECM species is confirmed, culture will be transferred to sterilized peat and vermiculite medium and grown in the dark for 3-6 weeks. Medium will then be mixed with greenhouse soil used as planting medium for chestnuts grown by nut. Inoculation potential will be confirmed using DNA sequencing of root fungi. Culturing and DNA sequencing of inoculum will be completed at the US Forest Service in Delaware, OH.

Summer Internship Training Projects

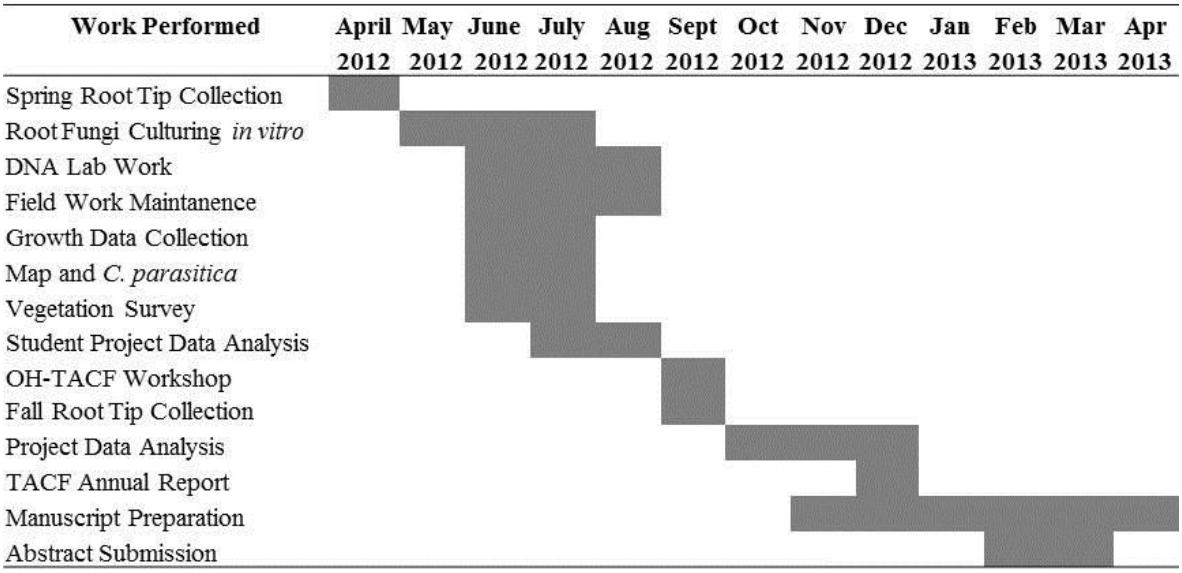
In June of 2012, field projects for student interns will begin. Three interns will be assigned three separate student projects in the Dresden, OH field site: 1) growth and survival of chestnuts on mine sites, 2) ECM community analysis by fungal DNA extraction and sequencing, and 3) a vegetation survey of the herbaceous plant community. Students will be mentored by The Wilds' Director of Science Training, Dr. Jenise M. Bauman.

1) **Chestnut Growth and Survival**: Each surviving chestnut will be measured, height and basal diameter. Height (cm) was measured using a meter stick from soil level to the tip of the main stem. Basal diameter (mm) will be measured 3 cm above the root collar recorded by using a digital caliper. A volume index (height × basal diameter^2) will be used to estimate the volume of each chestnut seedling. Growth will be derived from the difference between the original measurements (recorded April 2007). (*Growth data will be submitted to Dr. Brian McCarthy, Ohio University, for additional reporting). In addition, natural chestnut cankers will be measured (length and width) and GIS coordinates of blighted trees will be recorded.
2) **ECM Community Analysis:** Root tips sampled in April of 2012 (and stored -80) will be used for this student project. ECM species will be determined by DNA sequencing of the ITS region (as described above). Sequences generated by the Plant-Microbe Geomonics Facility (The Ohio State University) will be used to

identify the ECM fungi sampled from roots. Samples will be edited using Sequence Scanner v 1.0 and compared to vouchered sequences in GenBank using the BLAST search (Altschul et al., 1997). The genera of the fungi reported in this study will be based on the best matches of those in the GenBank. Fungal identity will then be compared to growth rates to determine feedback potential. ECM community composition will be compared between pure American and backcrossed chestnuts using a non-metric multidimensional scaling (NMDS) ordination.

3) **Vegetation Survey of the Herbaceous Plant Community:** A vegetation survey will be conducted in July of 2012. Plant community composition will be compared among the treatments described in Figure 3. To accomplish this, 12, 1 × 1 m quadrats will be placed in the center of each subplot where all vegetation will be recorded. Description of herbaceous plant diversity and species richness will be calculated. A NMDS ordination followed by a permutational MANOVA will be used to determine if these soil treatments influenced plant vegetation, five years after implementation.

Timeline:



Research outline and funding required:

April - May 2012 ($2,665.00 for DNA supplies, $575.00 for culturing supplies)
Order Supplies and Prepare Research Materials
Collect samples from 50 seedlings
Photograph and voucher root tips collected on chestnut in the spring of 2012
Store 3mm root tips at -80° C for summer intern DNA project
Begin culturing ECM *in vitro*
Interns will be selected for the Wilds Scholar internship program

June – August 2012 ($750.00 for sequencing, $1,000.00 for tech salary, $375.00 for research poster printing.
Wilds Scholar Internship (June 11th – August 17th, 2012)
Train interns on laboratory and field techniques: DNA extraction and purification, vegetation identification, GIS/GPS mapping, and sampling techniques.
Measure all chestnuts and provide maintenance to field plot
Documents and GIS chestnut blight cankers
DNA Extraction and Sequencing of ECM fungi from chestnut root tips
Analyze results of student projects
Students present research to Wilds staff

September-October 2012 ($375.00 for travel, $750.00 for sequencing)
Workshop for the Ohio Chapter of American Chestnut Foundation scheduled for mid-September.
Students will present final summer research project during workshop.
50 root samples collected Fall of 2012
DNA Extraction and Sequencing of ECM fungi from chestnut root tips

November – December 2012
Complete DNA Extraction and Sequencing of ECM fungi from chestnut root tips
Final reports organized for TACF
Begin manuscript preparation

January – March 2013
Continue manuscript preparation
Submit abstracts for student conference presentations

How Results will be measured and reported:

Results will be presented during the 2012 annual meeting of the Ohio Chapter of The American Chestnut Foundation. Students will be required to present their findings during this meeting. In addition, funding for poster printing will allow students an opportunity to present at regional conferences that may include research venues at their universities, the Ohio Academy of Science, and the Midwest chapter of the Society of Ecological Restoration. Student results will be synthesized into larger chestnut projects and presented during the 2013 The Ecological Society of America. Written research dissemination will include publishing results in journals including *Mycorrhiza, United States Department of Agriculture Forest Service Research* publication, and *The Journal of American Chestnut.*

Budget and Justification:

Item	Quantity	Unit Price	Price
Qiagen DNeasy 250 Reactions	1	925	$925.00
Microcentifuge Tubes 1.5	2	90	$225.00
PCR .5 Microcentifuge Tubes	2	90	$225.00
Dntps	1	120	$120.00
Taq Polyermerase	1	270	$270.00
Wizard Clean-up	1	400	$400.00
Pipette Tips	5	100	$500.00
Sequencing	250	6	$1,500.00
Sterile Petri Plates	1 case	295	$295.00
Glucose/yeast/malts			$110.00
Agar			$170.00
Research Poster Printing	125	3	$375.00
Wilds Lab Technician	1	0.5	$500.00
Wilds GIS/GPS Field Tech	1	0.5	$500.00
Field Travel			$385.00
		TOTAL	**$6,500.00**

Research Supplies: $5,115.00
Culturing fungi will require Sterile Petri plates ($295.00), Glucose/yeast/malts extracts ($110.00), Glucose ($125.00), and Agar ($170.00). Poster printing for undergraduate student projects @ $125.00 ($375.00), Quiagen DNeasy 250 Reaction Extraction Kit ($925.00), Microcentifuge and PCR tubes ($450.00), Dntps ($120.00), Taq polyermerase ($270.00), Wizard Clean-Up Kit ($400.00), Pipette tips ($500.00), Sequencing At $6.00 at samples for 250 samples ($1,500.00).

Field and Lab Assistance: $1,000.00
The amount of $500.00 is being requested to pay for 50% of the field technician's time. Field tech will assist with GIS/GPS mapping of cankers and field identification of plants sampled in the chestnut plots. An additional $500.00 is being requested for 50% of the Wilds lab technician time. The lab tech will assist with the daily operations of the Wilds DNA laboratory and will be available for assisting students, ordering supplies, and managing the lab space.

Travel: $385
This funding will be allocated to gasoline costs of travel between the Wilds in Cumberland, OH and the US Forest Service in Delaware OH.

Matching Funds:

The Wilds will match funds via three student lodging research stipends ($4,500.00) and 50% of field and lab technician's salary for the project ($1,000.00). Please note, in-kind match also includes Wilds director's (Dr. Jenise M. Bauman) time and effort on this project. In addition, the US Forest Service pledges in-kind match for salaries covering research scientist (Dr. Shiv Hiremath) and laboratory technician (Kirsten Lehtoma).

Literature Reviewed:

Altschul, S. F., Madden, T.L., Schaffer, A. A., Zhang J., Zhang Z., Miller W. & Lipman D.J. (1997) Gapped BLAST and PSIBLAST:A new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389–3402.

Bauman, J. M., McCarthy, B. C., Hiremath S., and Keiffer, C. H. 2012. Ectomycorrhizal fungal interactions and their influence on establishing American chestnut (*Castanea dentata*) in restoring grasslands. *Journal of Applied Ecology*. In review.

Bauman, J. M., Keiffer, C. H , McCarthy, B. C., and Hiremath S. 2011. Methods promoting ectomycorrhizal interactions on establishing American chestnut seedlings during coal mine land reclamation. *The Journal of the American Chestnut.* **2:** 9-10.

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Jacobs, D.F., Selig, M.F. & Severeid, L.R. 2009. Aboveground carbon biomass of plantation grown American chestnut (*Castanea dentata*) in absence of blight. *Forest Ecology and Management*. 258: 288-294.

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McCarthy, B. C., Bauman, J. M., and Keiffer, C. H. 2008. Mine reclamation strategies for the Restoration of American chestnut (*Castanea dentata*). *Ecological Restoration.* **26:** 292 - 294.

Palmer, J. M., Lindner, D. L., and Volk, T. J. 2008. Ectomycorrhizal characterization of an American chestnut (*Castanea dentata*)-dominated community in Western Wisconsin. Mycorrhiza 19: 27-36.

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SHORTENED CURRICULUM VITAE

JENISE M. BAUMAN, PH.D.
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Zanesville Ohio, 43701
Phone: (513) 593-1243
Email: baumanjm@muohio.edu

CURRENT EMPLOYMENT:

Postdoctoral Researcher
The Wilds, Cumberland, OH **August 2010 – present**
This research position worked collaboratively with academic partners to facilitate long-term research projects in the conservation and environmental sciences. I have created and currently manage The Wilds Scholar Internship program, an intensive training program that mentors undergraduate students. I manage a laboratory and an active research program investigating plant and fungal interactions in ecological restoration.

EDUCATION:

Ph.D. in Botany, Miami University, Oxford OH **December 2010**
Advisor: Dr. Carolyn H. Keiffer
Dissertation Title: Ectomycorrhizal communities associated with restoration planting of American chestnut (Castanea dentata) seedlings on Ohio mine lands

M.S. in Plant Pathology, West Virginia University **May 2005**
Advisor: Dr. William L. MacDonald
Thesis Title: A comparison of the growth and asexual reproduction by Cryphonectria parasitica isolates infected with hypoviruses via anastomosis and transfection

B. S. in Horticulture, Eastern Kentucky University **July 1998**
School of Agriculture, emphasis in ornamental horticulture

AWARDS AND HONORS:

2010: E. Lucy Braun Award for Excellence in Ecology, Ecological Society of America
2010: The Heimsch Award in Botany, Miami University
2009: Women Breaking Barriers, Graduate Student Award recipient, Miami University
2008: Graduate Students' Research Achievement Award, Miami University
2007: William Niering Award, Society of Ecological Restoration

GRANTS AND FUNDING:

National Fish and Wildlife Five Star Restoration Grant	*$20,000.00*	*July 2011*
US Dept. of Interior, Office of Surface Mining	*$24,100.00*	*January 2007*
Joint Research Venture Grant USDA Forest Service	*$28,000.00*	*July 2006*

SELECTED CHESTNUT PUBLICATIONS:

Bauman, J. M., Keiffer, C. H., McCarthy, B. C., and Hiremath S. Methods promoting ectomycorrhizal interactions on establishing American chestnut (*Castanea dentata*) seedlings in coal mine restoration. *Journal of Applied Ecology*. In Review.

Bauman, J. M., Keiffer, C. H. and Hiremath, S. 2012. Facilitation of American chestnut (*Castanea dentata*) seedling establishment by *Pinus virginiana* in mine reclamation. *International Journal of Ecology.* In Press.

Bauman, J. M., Keiffer, C. H. and Hiremath, S. 2011. The Influence of Inoculated and Native Ectomycorrhizal Fungi on Morphology, Physiology and Survival of American Chestnut. Pages 16-37 in: Barnhisel, R.I., (ed.). *The American Society of Mining and Reclamation Proceedings. Sciences Leading to Success.* Lexington, KY.

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McCarthy, B.C., K.E. Gilland, K.E., Bauman, J.M., and Keiffer. C.K. 2010. Factors affecting performance of artificially regenerated American chestnut on reclaimed mine sites. Pages 582-597 in R.I. Barnhisel (Ed.), *Bridging Reclamation, Science, and Community*. Proceedings of the National Meeting of the American Society of Mining Reclamation. Lexington, KY.

McCarthy, B.C., Bauman, J.M., and Keiffer, C.H. 2008. Mine reclamation strategies for the Restoration of American chestnut (*Castanea dentata*). *Ecological Restoration.* 26: 292 - 294.

2011 CONFERENCE PRESENTATIONS :

Bauman, J. M. 2011. Site Influence and Soil Variation on Ectomycorrhizal Composition and Colonization. Appalachian Regional Reforestation Initiative. August 2-4, Knoxville TN.

Bauman, J. M., Keiffer, C. H. and Hiremath, S. 2011. The Influence of Inoculated and Native Ectomycorrhizal Fungi on Morphology, Physiology and Survival of American Chestnut. *The American Society of Mining and Reclamation: Sciences Leading to Success.* June 11-16, Bismarck, ND.

Bauman, J. M., Keiffer, C.H., McCarthy, B.C., and Hiremath, S. 2011. Soil surface treatments to alleviate competition of invasive forbs and graminoids on Ohio coal mined lands. Joint Meeting of the 2nd Kentucky Invasive Species Conference and the 13th Annual Southeast Exotic Pest Plant Council Conference. May 3-5, Lexington, Kentucky.

SHORTENED CURRICULUM VITAE
Shivanand T. Hiremath

EDUCATION:

B.Sc.	1971	Chemistry	Karnatak University, Dharwar, India
M.S.	1973	Biochemistry	Karnatak University, Dharwar, India
Ph.D.	1978	Biochemistry	National Chemical Lab, Pune, India

EMPLOYMENT HISTORY:

1988-present	Research Mol Biologist, NRS-4, USDA Forest Service, Delaware, OH.
1996-present	Adjunct Assoc Prof., Dept of Entomology, Ohio State University, Columbus, OH.
1987-1988	Dept. of Entomology, University of California, Riverside, CA.
1983-1987	Dept. of Biochemistry, University of Kentucky, Lexington, KY.
1977-1982	Research Associate, Dept. of Cell and Molecular Biology, SUNY, Buffalo, NY.

CURRENT RESAERCH INTERESTS

- Development of improved ectomycorrhizal inoculum mixtures for use in reforestation efforts on normal soil as well as in regions that have unfavorable conditions such as sandy, drought-affected, and reclaimed areas.
- Isolation, identification, and utilization of novel mycorrhizal fungi that can survive in abnormal soil conditions. Development of markers for tracking and quantitation of mycorrhizal fungi.
- Genomics of mycorrhizal symbiosis to identify genetic markers of beneficial traits in the mycorrhizal fungi. These markers will help identify suitable fungus/tree combinations that will be successful in a given soil condition.
- Genetic improvement of mycorrhizal fungi for biological control of insect pests, and for improvement of tree health and vigor.
- Molecular mechanism of hypovirulence in the chestnut blight fungus; structure/function of a plant potyvirus (Post doctoral work); purification/characterization of a bacterial inducible citrate metabolizing enzyme (Ph. D.).

REPRESENTATIVE PUBLICATIONS:

Hiremath, Shiv; Lehtoma, Kirsten; Podila, Gopi K. 2008. Identification of a small heat-shock protein associated with ras-mediated signaling pathway in ectomycorrhizal symbiosis. USDA-NRS Research Paper NRS-7 (8 pages).

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Varley, D.A., Podila, G.K., **Hiremath, S.T**. (1992). Cutinase in *Cryphonectria parasitica*, the chestnut blight fungus: Suppression of cutinase gene expression in isogenic hypovirulent strains containing double-stranded RNAs. Mol. Cell. Biol. **12**: 4539-4544.

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Hiremath, S.T., Jones, D. (1992). Juvenile hormone regulation of vitellogenin in the gypsy moth *Lymantria dispar*. Suppression of mRNA in the fat body. J. Insect Physiol. **38**: 461-474.

Hiremath, L., **Hiremath, S.T**., Rychlik, W., Joshi, S., Domier, L., Rhoads, R.E. 1989. *In vitro* synthesis, phosphorylation, and localization on 48S initiation complexes of human protein synthesis initiation factor 4E. J. Biol. Chem. **264**: 1132-1138.

Jones, G., **Hiremath, S.T**., Hellman, G.M., Rhoads, R.E. 1987. Inhibitory and stimulatory control of developmentally regulated hemolymph proteins in *Trichoplusia ni*. In Molecular Entomology, ed. John Law, Vol. **49**, pp295-304, Alan R. Liss, Inc. New York.

Hellmann G.M., **S.T.Hiremath**, J.G.Shaw and R.E.Rhoads (1986). Cistron Mapping of Tobacco Vein Mottling Virus. Virology **151**, 159-171.

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Hiremath, S.T., R.A. Maciewicz and T.Y. Wang (1981). The loosely-bound non-histone chromosomal proteins of rat prostate in androgen action. BBA, **653**, 130-138.