



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2015 Work Plan

Date of Report: October 15, 2014

Date of Next Status Update Report: January 1, 2016

Date of Work Plan Approval:

Project Completion Date: June 30, 2018

Does this submission include an amendment request? No

PROJECT TITLE: Preventing a New Disease of Pines in Minnesota

Project Manager: Robert A. Blanchette

Organization: University of Minnesota

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Location: All counties in Minnesota

Total ENRTF Project Budget:

ENRTF Appropriation: \$371,000

Amount Spent: \$0

Balance: \$371,000

Legal Citation: M.L. 2015, Chp. 76, Sec. 2, Subd. 06d

Appropriation Language:

\$371,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to establish early detection for heterobasidion, an invasive root rot fungus, and develop efforts to prevent its spread and reduce its impact. This appropriation is available until June 30, 2018, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Preventing a New Disease of Pines in Minnesota

II. PROJECT STATEMENT:

Heterobasidion root rot is a serious invasive disease of red and white pine trees that has been recently found in Winona County, Minnesota. The goal of this project is to survey for *Heterobasidion* root rot in forest and urban landscapes where native red and white pines are located so infection sites can be detected as early as possible. New molecular screening methods will be used to identify the pathogen, management plans to fight the disease will be prepared and native biological control agents studied so we can reduce the impact of this disease to conifers growing in Minnesota forests and urban landscapes.

A new invasive tree disease called *Heterobasidion* root rot is a serious threat to Minnesota's red and white pines as well as other conifers. It is considered the most economically important disease of pines throughout the Northern temperate regions. In the United States it causes over 1 billion dollars in losses annually. It also has tremendous ecological impacts on forest health and productivity. Minnesota has been free of this very destructive tree disease until 2014. Over the past decade it has become well established in Wisconsin and has spread quickly throughout the state. The Wisconsin Department of Natural Resources reports in 2014 that it has been confirmed in 24 counties and three of these counties are adjacent to Minnesota. This pathogen attacks the roots of trees and moves from tree to adjacent tree underground causing circles of dead trees. Once in an area, the fungus grows through the roots and expands causing greater and greater mortality. The fungus produces fruiting bodies with spores at the base of dead trees and these disseminate overland to start new infections. The disease is caused by a complex of *Heterobasidion* species but the species currently in Wisconsin and recently found in southeastern Minnesota is *H. irregulare*. This project will establish effective guidelines to manage this disease which can be implemented immediately as new disease infection centers are found. It will also investigate potential biological control methods that can be used for long term prevention.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of [January 1, 2016]:

Project Status as of [September 1, 2016]:

Project Status as of [April 1, 2017]:

Project Status as of [September 1, 2017]:

Project Status as of [January 1, 2018]:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1:

Description: Develop new diagnostic tools for rapid detection.

Molecular primers and probes (DNA sequences) that are specific to the pathogen, will be designed for use in a diagnostic kit that can be used to rapidly identify the pathogen directly from wood samples obtained from the field. DNA will be extracted from *Heterobasidion* cultures and PCR products obtained. These products will then be cloned into *E. coli* and sequenced. Specific primers for *Heterobasidion* will be designed for nested and real time PCR assays on the basis of the alignment of the nuclear ribosomal ITS region of *Heterobasidion* from Gen Bank accessions. Multiple pairs of primers will be screened *in silico* using Basic Local Alignment Search Tool (BLAST) to explore all available *Heterobasidion* sequences and other fungi that can potentially cross-react. Primers that only match *Heterobasidion irregulare* will be tested using genomic DNA from different

Heterobasidion isolates. Wood tissue samples collected from forest stands (known *Heterobasidion* infected trees and healthy trees for controls) will be homogenized in lysis buffer. DNA will be extracted, tested and nested PCR methods completed to confirm the primers are effective at detecting the pathogen and not other wood inhabiting fungi. The primers will also be optimized for the real time PCR protocol and specific probes will be designed and optimized in a similar fashion as previously published. Standard fungal isolation will be attempted for each tree sample to compare detection rates with those for the PCR methodologies. This will provide a highly sensitive and accurate detection method to target this pathogen. The developed methodology will be used to assay field samples from surveys (see Activity 2) carried out in forested and urban landscapes over the duration of this grant. A service for identifying this disease will be established in the Plant Disease Clinic, which will become a center for diagnosing the disease for the North Central region of the US.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 119,230
Amount Spent: \$ 0
Balance: \$ 119,230

Outcome	Completion Date
1. Develop and test molecular primers and probes for diagnostics	December 31, 2015
2. Molecular diagnostic kit developed for evaluation of field samples	August 30, 2016
3. Molecular diagnostic methods used for pathogen detection on samples from field surveys	June 30, 2018

Activity Status as of [January 1, 2016]:

Activity Status as of [September 1, 2016]:

Activity Status as of [April 1, 2017]:

Activity Status as of [September 1, 2017]:

Activity Status as of [January 1, 2018]:

Final Report Summary:

ACTIVITY 2:

Description: Survey and identify infected trees.

A statewide survey will be initiated utilizing Department of Natural Resources, county and federal data bases and information from state, county, industry, and federal foresters to identify plantations and other sites in the state that may be infected. These will include locations near the identified disease centers that are in adjacent counties to Wisconsin, sites identified using information such as stand age and thinning history and locations of unexplained pockets of mortality. Additional surveys will be done on ornamental pines located in the areas of greatest concern in Minnesota (cities in counties next to existing Wisconsin infection centers). Wood samples obtained from the surveys will be placed in sterile collection bags and brought to the laboratory for processing. The new diagnostic procedures described above will be used to detect the fungus in wood samples in its earliest stage of colonization. Additional microbiological testing will be done to confirm the pathogen and extent of its colonization.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 137,935
Amount Spent: \$ 0
Balance: \$ 137,935

Outcome	Completion Date
1. Complete first surveys for the disease in all Minnesota counties adjacent to Wisconsin	December 31, 2016
2. Complete surveys of pine in other forest and urban areas of Minnesota	June 30, 2018

Activity Status as of [January 1, 2016]:

Activity Status as of [September 1, 2016]:

Activity Status as of [April 1, 2017]:

Activity Status as of [September 1, 2017]:

Activity Status as of [January 1, 2018]:

Final Report Summary:

ACTIVITY 3:

Description: Establish a monitoring network for diagnostics and study methods for control including the evaluation of biological control agents.

There are several integrated control procedures that have been used to limit the continued spread of this disease in other parts of the country. We will evaluate the various cultural methods used to limit disease spread such as preventing movement of infected material from an area, cut and burn infected trees that could sporulate, clear cut stands that have extensive infection, thin stands during periods of the year when spores are not present, treat stumps with fungicides (boron stump treatment) to prevent new infections, clean logging equipment used in infected sites etc. In addition investigations evaluating our native pioneer colonizing fungi for potential use for biocontrol will be carried out. Fungi will be isolated from field locations, identified by DNA sequencing and tested for biological control activity in laboratory assays. Successful candidate organisms will also be tested under field conditions. This work will follow previous protocols that have proven to be successful to identify fungi that out compete forest pathogens.

Guidelines for controlling the disease in woodlands as well as in urban landscapes in Minnesota will be developed. An integrated approach that considers each of these control measures will be developed with our state cooperators and formalized into a decision-support framework based on forest age, proximity to known infections, and long-term economic and ecological objectives. Short term and long range management plans will be developed with stakeholders from Minnesota and Wisconsin and a decision-support framework for guiding disease management plans obtained. This framework will take into consideration long-term economic and ecological objectives, forest conditions (e.g., age, density, and site quality) and proximity to known infections to help aid the development of management prescriptions for minimizing impacts and spread of this disease.

Summary Budget Information for Activity 3:

ENRTF Budget: \$ \$113,835
Amount Spent: \$ 0
Balance: \$ 113,835

Outcome	Completion Date
1. Control guidelines developed for woodlands and urban landscapes	June 30, 2016
2. Biological control agents found and tested	June 30, 2018
3. Informational and training programs for detection and control completed	June 30, 2018

Activity Status as of [January 1, 2016]:

Activity Status as of [September 1, 2016]:

Activity Status as of [April 1, 2017]:

Activity Status as of [September 1, 2017]:

Activity Status as of [January 1, 2018]:

Final Report Summary:

V. DISSEMINATION:

Description:

A comprehensive outreach plan utilizing web based and other materials will be initiated. Online resources will include e-newsletter articles, extension factsheets, a website devoted to this project, webinars, and social media updates. Printed materials will consist of scientific journal articles and extension bulletins as well as more general outreach methods using columns in newspapers and various on line informational sites. In addition, the University of Minnesota Plant Disease Clinic will be directly involved in analyzing samples for this work and will be another informational source to the public through their bulletins, outreach activities and first responder plant disease program.

Activity Status as of [January 1, 2016]:

Activity Status as of [September 1, 2016]:

Activity Status as of [April 1, 2017]:

Activity Status as of [September 1, 2017]:

Activity Status as of [January 1, 2018]:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$ 312,540	Funding for a graduate student, research scientist and 3 undergraduate students to carry out the proposed activities. Undergraduate students: \$18,000 (100% salary, 0% benefits). 30% FTE each year for 3 years. Graduate Student: \$132,000 (56% salary, 44% benefits) 50% FTE for 3 years. Research Laboratory Scientist. \$162,540 (71% salary, 29% benefits) 75% FTE each year for 3 years.
Equipment/Tools/Supplies:	\$14,460	Supplies for the lab and field work
Capital Expenditures over \$5,000:	\$32,000	Real Time PCR for the rapid and efficient diagnostics to be conducted on field samples obtained from throughout the state.
Travel Expenses in MN:	\$12,000	Travel for disease surveys and sample collection as well as to conduct the biological control investigations

TOTAL ENRTF BUDGET: \$371,000

Explanation of Capital Expenditures Greater Than \$5,000:

Real time PCR diagnostic system (\$32,000.)- This is a molecular diagnostic system that allows precise detection of pathogens (using specific DNA primers) and is essential for developing a rapid and efficient method of disease identification from field samples. This state of the art equipment will be used for assays to detect the fungus in wood and will be used for all sample assays during the 3 year period. It will then be used in the Plant Pathology Diagnostic lab to continue a service of disease detection on samples sent by the public for analysis.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

4.65 over 3 years

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
	\$0	\$0	
State			
In Kind Support for Project Leader	\$225,897	\$0	Salary and fringe for 1 month per year for 3 years (41,900). University indirect costs at 52% (\$183,997)
TOTAL OTHER FUNDS:	\$297,897	\$0	

VII. PROJECT STRATEGY:

A. Project Partners:

Professor Robert Blanchette will be the project leader and coordinate activities. He will take part in the development of the diagnostic kits oversee the surveys and direct outreach activities. Dr. Brett Arenz from the Department of Plant Pathology will take part in outreach and training programs and lead the laboratory diagnostic activities in the Plant Disease Clinic. Dr. Benjamin Held is a research scientist in the Department of Plant Pathology and will be involved with the development of rapid molecular procedures for identifying the pathogen, take part in the field surveys and training programs. A graduate student in the Department of Plant Pathology will investigate the use of new biological (aggressive pioneer fungal colonists) control agents. Faculty in the Department of Forest Resources at the University of Minnesota will be involved with the development of management plans for Minnesota’s woodlands, will help coordinate surveys and contribute to training programs. Non funded partners that will help with surveys and sample collection include the MN Dept. of Agriculture, Minnesota DNR, Wisconsin DNR and U.S. Forest Service.

B. Project Impact and Long-term Strategy:

The main goal of this project is to identify this new invasive tree disease as early as possible as it moves into Minnesota, prepare an effective defense to fight the disease and reduce its impact to our native conifers growing in forests and urban landscapes. Once the diagnostic procedures are developed, the Plant Disease Clinic can continue to evaluate samples for the people of Minnesota after the end of this proposal . Other State agencies will use the control management guidelines developed from this project long into the future.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
University of Minnesota Rapid Response Funds. To begin preliminary surveys and develop sample processing protocols for the project	2013 to 2014	\$72,000

IX. VISUAL COMPONENT or MAP(S):

Proposal # O84-D

Title: Preventing a New Disease of Pines in Minnesota

Project Manager: Blanchette



Circles of dead trees in Wisconsin killed by the root rot fungus



Fruiting bodies of the fungus on the base of dying pine trees

The Problem:

Heterobasidion is an invasive root rot fungus that attacks all conifers. Once it gets into a tree it moves through the roots from tree to tree causing expanding circles of death. The Disease is in Wisconsin causing serious losses with infection centers at the border to Minnesota

What is being proposed:

Develop rapid screening methods of detection

Survey and sample trees to identify the disease

Initiate a campaign to educate the public about the disease.

Establish a monitoring network for the state at the University of Minnesota's Plant Disease Clinic.

Development management plans for Minnesota to reduce the impact of the disease

Goals:

To identify the disease as early as possible as it moves into Minnesota and prepare an effective defense to fight this disease and reduce its impact to our native conifers growing in forests and urban landscapes. The program will help keep our pine resources healthy.



X. RESEARCH ADDENDUM:

See attached

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 30, 2016, September 30, 2016, April 30, 2017, September 30, 2017 and January 30, 2018. A final report and associated products will be submitted between June 30 and August 15, 2018.



Project Title: Preventing a New Disease of Pines in Minnesota
 Legal Citation:
 Project Manager: Robert A. Blanchette
 Organization: University of Minnesota
 M.L. 2015 ENRTF Appropriation: \$371,000
 Project Length and Completion Date: 3 Years, June 30, 2018
 Date of Report: October 15, 2014

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Fill in your activity title here.</i>			<i>Fill in your activity title here.</i>			<i>Fill in your activity title here.</i>				
Personnel (Wages and Benefits)	\$81,270	\$0	\$81,270	\$124,635	\$0	\$124,635	\$106,635	\$0	\$106,635	\$312,540	\$312,540
3 Undergraduate Students. \$18,000 (100% salary, 0% benefits). 30% FTE each year for 3 years											
1 Graduate Student. \$132,00 (56% salary, 44% benefits) 50% FTE each for 3 years											
Benjamin Held, Laboratory Scientist. \$162,540 (71% salary, 29% benefits)75% FTE each year for 3 years											
Equipment/Tools/Supplies	\$5,960	\$0	\$5,960	\$3,800	\$0	\$3,800	\$4,700	\$0	\$4,700	\$14,460	\$14,460
Field Supplies:collection bags, GPS unit, hatchets, saws, sampling materials, storage containiers.											
Lab supplies: Culture media for growing microorganisms, agar, antibiotics for culturing, ethyl alcohol and other chemicals, costs for molecular sequencing, molecular primers, probes and reagents, petri dishes, culture containers, microbiology diagnostic reagents, sterile bags, pipettes and pipette tips,DNA extraction kits, cloning kits, autoclave bags, culture tubes, isolation tools, laboratory gloves, incubation chamber, biocontrol microcosm plates and jars.											
Capital Expenditures Over \$5,000	\$32,000	\$0	\$32,000		\$0	\$0	\$0	\$0	\$0	\$32,000	\$32,000
Real time PCR diagnostic system (\$32000.)- a molecular diagnostic system that is able to detect specific DNA primers. Real-time PCR is being proposed for use in this project because it has become the standard for rapid and accurate pathogen detection and analysis. This equipment is different than traditional PCR that is used for DNA amplification because it involves using florescent probes attached to primers that measure amplification as it occurs, in one reaction. It offers a more sensitive and precise analysis, a more rapid turnaround time and has reduced sample handling thereby reducing the possibility for contamination. Target DNA can also be quantified which aids in determining the extent of colonization by the pathogen in field samples.This specialized new equipment is currently not available. We do not have this equipment in our research lab, the Plant Disease Diagnostic Lab or other research laboratories in the facility. For the work outlined in our proposal, a real time pcr is essential since large numbers of samples will be processed and it would be used continuously for the project.											
Travel expenses in Minnesota	\$0	\$0	\$0	\$9,500	\$0	\$9,500	\$2,500	\$0	\$2,500	\$12,000	\$12,000
Travel to survey sites throughout Minnesota. Mileage \$9,000; lodging \$2,000; meals \$1,000 over 3 years											
COLUMN TOTAL	\$119,230	\$0	\$119,230	\$137,935	\$0	\$137,935	\$113,835	\$0	\$113,835	\$371,000	\$371,000

