Environment and Natural Resources Trust Fund
2019 Request for Proposals (RFP)

Project Title: **ENRTF ID: 217-F**

Protecting Minnesota Birch by Sustainable Chaga Cultivation

Category: F. Methods to Protect, Restore, and Enhance Land, Water, and Habitat

Sub-Category:

Total Project Budget: $ 385,232

Proposed Project Time Period for the Funding Requested: June 30, 2022 (3 yrs)

Summary:

Development of methods to cultivate medicinal chaga fungus to protect birch trees from unsustainable harvesting and damage and to provide access to a valuable Minnesota commodity

Name: Christine Salomon

Sponsoring Organization: U of MN

Title: Associate Professor

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Images of chaga conks on birch trees, harvested chaga and commercial chaga products, and several representative bioactive compounds isolated from chaga with anti-oxidant, anti-viral, and anti-cancer activities

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%
____ If under $200,000, waive presentation?			

PROJECT TITLE: Protecting Minnesota birch by sustainable chaga cultivation

I. PROJECT STATEMENT

This proposal is focused on developing new methods of cultivating the medicinal chaga fungus to protect Minnesota birch tree and forest resources from unsustainable harvesting and damage. The chaga fungus (*Inonotus obliquus*) infects birch trees and develops large black/brown fungal masses called conks. Chaga only grows in cold, northern climates on a few different species of birch (white, yellow and black), and takes 10-15 years to fully mature. The conks, used in traditional medicines globally, are a rich source of many potent compounds with anti-oxidant, anti-cancer and wound healing activities. A recent surge in interest in natural medicines has resulted in over harvesting to obtain chaga for use in medicinal teas, tinctures and extracts. However, slow fungal growth and extensive harvesting has led to its virtual disappearance from all Minnesota state parks. Although collectors have turned to state forests and private lands for chaga, these sources are not sustainable for long term production, and soon this resource will be depleted and result in significant damage to birch trees.

We propose to develop new cultivation methods and field inoculations that would provide sustainable production of chaga and a new forest industry for Minnesota. We have established a preliminary study to test this in a field inoculation setting. As part of this proposal, we would expand our field inoculation trials and develop lab-based cultivation of chaga. We will systematically characterize all of the bioactive chemical compounds and their biological activities, including anti-oxidant potential, anti-microbial and anti-cancer activity and wound healing properties. We also anticipate the discovery of new compounds with potentially different activities from the proposed cultivation conditions. Our goal is to optimize an efficient culture system that promotes fungal growth with chemical/medicinal properties as similar to wild chaga as possible. This new source of chaga would potentially make wild-harvesting of the fungus from Minnesota forests unnecessary, protecting our forest resources and promoting access to a new commodity for Minnesota.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Development of chaga culture conditions

The fungus that produces chaga can be grown in culture but the conditions for optimum growth and factors that induce chaga formation are poorly understood. The fungus produces different types and quantities of bioactive compounds depending on growing conditions, substrate and strain. We have already obtained several Minnesota isolates from chaga and will collect many additional strains to select those with superior growth rates and high levels of medicinal compounds. These cultures will be grown in laboratory decay chambers using different substrates, growth promoting substances and environmental conditions. The cultivated mycelium, chaga biomass and wood/fungus combinations will be harvested and used in Activity 2. In addition, field cultivated chaga using sustainable forestry practices will also be investigated. A preliminary study initiated this past year at the University of Minnesota Cloquet Forestry Station has inoculated birch with isolates of the fungus. Although field production could take time (5 to 10 years), there are methods that can be used to accelerate chaga formation in the tree. Previous work in the Blanchette lab has been able to produce a valuable resin in a tropical tree using fungal inoculation and cutting the time down to only 2 years for production from what normally takes decades. This previously developed technology could be applied to birch trees to produce chaga in just a few years. However, to fully evaluate these methods, field trials are needed. Methods include making small wounds in trees and inoculating with the various growth substances that accelerate decay followed by inoculation with the chaga producing fungus. Once the methodology has been optimized, birch trees, which do not have a high value for wood products, could be grown specifically for their use to produce high valued chaga. Chemical analyses confirming the compounds produced and their concentrations will be done under Activity 2.

ENRTF BUDGET: $117,904

Outcome	Completion Date
1. Establish new culture conditions for chaga using birch wood/bark substrates	06/30/2021
2. Establish field trials of accelerated birch inoculations	06/30/2022
3. Optimize culture conditions and harvest timing to optimize activity	06/30/2022

Activity 2: Purify, characterize and quantify the biologically active components of chaga

More than 50 unique compounds have been previously isolated and characterized from wild harvested chaga that are believed to contribute to its medicinal properties. These compounds include members of different structural classes (terpenes, polyphenols, polysaccharides, melanins) with diverse biological activities such as anti-cancer, antioxidant, antimicrobial, antiviral and immuno-stimulant. We will extract each of the materials obtained in activity 1 (wild chaga, tree inoculated material and various cultures) and characterize the chemistry using chromatographic and spectroscopic methods. Extracts and pure compounds will also be tested in biological assays to characterize their activities. This information will be used to optimize culture conditions and field inoculations. Additionally, we anticipate that new, previously undiscovered compounds will be identified during these cultivations. These compounds will be structurally characterized and tested for novel bioactivities, potentially enhancing the value of any commercial chaga products that are developed.

ENRTF BUDGET: $ 267,328

Outcome	Completion Date
1. Establish quantification methods for active metabolites from wild grown chaga	06/30/2020
2. Compare the chemical compounds and bioactivity of compounds from cultivated, field-inoculated and wild chaga	06/30/2022
3. Characterize new compounds isolated from cultivated chaga	06/30/2022

III. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role
Christine Salomon	Associate Professor	UMN	Project manager and lead for activity 2
Robert Blanchette	Full Professor	UMN	Lead for activity 1

IV. LONG-TERM- IMPLEMENTATION AND FUNDING: We anticipate developing several promising chaga cultivation systems that will vary in chemical composition and biological activities. Our goal is to demonstrate that we can produce chaga products in a controlled and efficient setting that are equal to or more potent than wild chaga. Once this phase of the project is complete, a second phase might require an educational component to demonstrate to the public that wild chaga on birch trees should not be harvested due to this more accessible and sustainable source. Additional field trials might also be needed for promising birch inoculations that are not fully completed in this initial phase.

V. TIME LINE REQUIREMENTS: We are requesting 3 years of funding due to the time required to grow the fungus under various conditions. Natural chaga on birch trees takes 10-15 years to mature, but we are hoping to significantly reduce this growth time using optimized culture conditions of fungal mycelia.

VI. SEE ADDITIONAL PROPOSAL COMPONENTS:

A. Proposal Budget Spreadsheet

B. Visual Component or Map

F. Project Manager Qualifications and Organization Description

2019 Proposal Budget Spreadsheet

Project Title: Protecting Minnesota birch by sustainable chaga cultivation

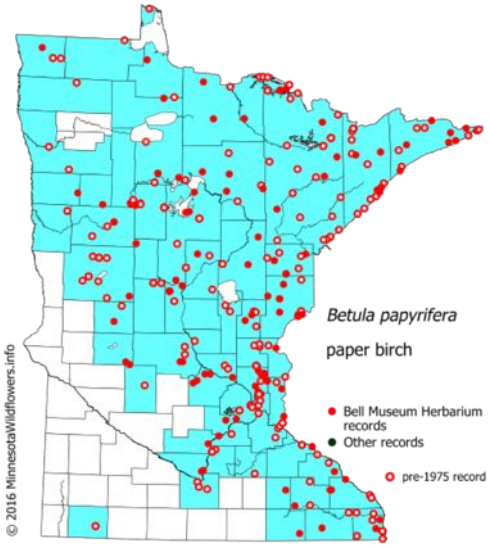
IV. TOTAL ENRTF REQUEST BUDGET: 3 years

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
Christine Salomon, Project Manager and Principle Investigator (75% salary, 25% benefits): 5% FTE for each of 3 years ($26,250)	$ 326,141
Postdoctoral researcher (82% salary, 18% benefits): 100% FTE for each of 3 years, chemical analysis, compound purificaiton and characterization, compound quantification and data analysis. Biological activity testing ($188,993)	
technician (79% salary, 21% benefits): 20% FTE for each of 3 years, biological assay testing, database management for chemistry and activity ($13,540)	
Research Scientist (75% salary, 25% benefits): 25% FTE for each of 3 years, chaga collection, field inoculations, lab cultivations, culture optimization ($79,358)	
Undergraduate student technicians (100% salary): 50% FTE for each of 3 years, media and sample prep, sample management, fungal cultivations, general lab support ($18,000)	
Equipment/Tools/Supplies:	$ -
Activity 1	
Field collection/inoculation and lab cultivation supplies (gloves, collection bags, petri dishes, culture tubes, pipettes and tips, media, sterilization supplies, chemical reagents for field inoculation trials, growth chambers, humidifiers)	$ 12,000
Activity 2	
Supplies for compound purification, structure elucidation and quantification (organic solvents, chromatography supplies, NMR solvents, filters, vials, pipettes)	$ 18,000
Supplies for biological assays (pipettes, pipette tips, epi tubes, culture tubes, petri dishes, media, 96 well plates, antibiotic standards, gloves)	$ 18,000
Travel: In-state round trip travel between St. Paul and various forest locations and field stations (ie. Cloquet Forestry Station) in northern Minnesota: room/board for 2-3 researchers for overnight trips, mileage, est. 5-6 trips/yr (1-3 days each trip) for 3 yrs	$ 6,000
Publication costs: ~2 total, $1000 per publication-page/color fee charges and/or open access charges for publishing scientific manuscripts	$ 2,001
Euipment repair and calibration: Repair of equipment and instrumentation (e.g. vacuum pumps, water baths, incubators, shakers, etc.) and calibration of instruments (pipettes, balances) estimated at $1000 per year for 3 years	$ 3,090
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 385,232

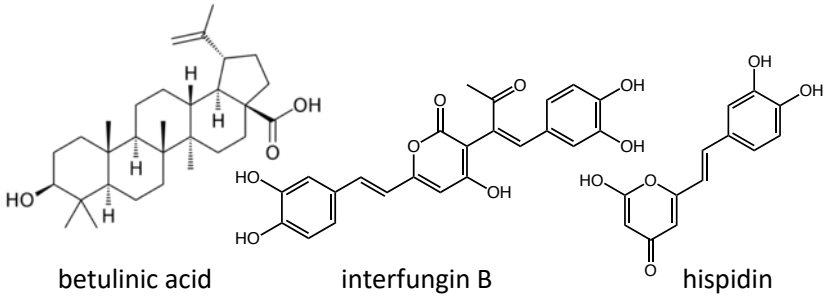
V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period: none	N/A	*none*
Other State $ To Be Applied To Project During Project Period: none	N/A	*none*
In-kind Services To Be Applied To Project During Project Period: Salary support for PI (Salomon) and co-PI (Blanchette) 5% FTE for 3 years	$ 57,489	*secured*
Past and Current ENRTF Appropriation: ENTRF 2013-2016 to conduct research on White Nose Syndrome as a sub-aim of a larger Soudan Mine Microbe project ("Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol", ML 2013-03F)	$ 838,000	*secured*
ENTRF 2016-2019 to expand research on biocontrol agents for treating white nose syndrome in bats (""White Nose Bat Syndrome Biological Control: Phase 2", M.L. 2016-124-D)	$ 452,000	*secured*
Other Funding History: none		

Protecting Minnesota birch by sustainable chaga cultivation



- Chaga “conks” are produced after infection of birch trees with the fungus *Inonotus obliquus*.
- The chaga material is highly valued as a medicinal product due to its anti-oxidant, anti-cancer and anti-viral activities.
- Chaga grows slowly (10-15 years) and has been over-harvested from many areas in Minnesota
- Harvesting practices frequently damage birch trees



Structures of some of the bioactive compounds isolated from chaga: betulinic acid (antiviral), interfungin B (anti-oxidant), hispidin (antitumor and anti-oxidant)

Goals and outcomes:

- Develop an alternative and efficient source of chaga to minimize wild collection, over-harvesting and damage to birch trees
- Provide data comparing the chemistry and bioactivity of cultured or field inoculated samples to wild chaga
- Develop cultivated chaga as a new commodity for Minnesota

VI. PROJECT MANAGER QUALIFICATIONS AND ORGANIZATION DESCRIPTION

Project Manager Qualifications

Dr. Christine E. Salomon, Project Manager and Principle Investigator

Dr. Salomon is an Associate Professor at the Center for Drug Design and a faculty member in the Biotechnology Institute at the University of Minnesota. Dr. Salomon earned her Ph.D. at the Scripps Institution of Oceanography, UCSD, in the area of natural products chemistry from invertebrates and microbes. She continued her training as a postdoctoral fellow in the Department of Microbiology at the University of Minnesota where she worked on understanding how microbes biosynthesize chemical compounds. Dr. Salomon's current research program is focused on the discovery and utilization of novel microbes that can be used for biological control of animal and plant pathogens and production of unique compounds for biomedical and biotechnological applications. She has successfully secured both internal (Academic Health Center, Masonic Cancer Center, Healthy Foods Healthy Lives Institute) and external (United States Department of Agriculture and US Fish and Wildlife) support for her research program.

Dr. Salomon is the manager/PI on a currently funded ENRTF project " White Nose Bat Syndrome Biological Control: Phase 2", M.L. 2016, Chp. 124-D, Sec. 116p, Subd. 06. Her ongoing work on the isolation, characterization and testing of novel bacteria and fungi in this unique environment utilizes many of the same chemical analytical tools that will be used in this proposal to characterize chaga chemistry. She has collaborated and published with Dr. Robert Blanchette, the lead for activity 1. Dr. Blanchette brings extensive experience in fungal and tree biology, and has conducted parallel work in a different tree/fungus system to produce a valuable resin-rich wood called agarwood. This work was developed into a company (Cultivated Agarwood LTD) to commercially produce the product in Vietnam. https://tinyurl.com/agarwoodLTD

As a member of the Biotechnology Institute, Dr. Salomon has access to industrial companies interested in partnering with academic researchers for future development efforts. She is experienced in patent protection of intellectual property and has worked closely with the Office of Technology Commercialization at the University of Minnesota. These connections will be essential for the commercial development of any biotechnologies discovered through the proposed research.

Organizational Description

The University of Minnesota Biotechnology Institute was initially established to catalyze the development of a biotechnology industry in Minnesota. It plays a central role in providing training and coordinating research in biological, chemical and engineering sciences at the University of Minnesota. It also serves as an important resource for industry by providing connections with academic research partners.

The primary mission of the Center for Drug Design at the University of Minnesota is to promote scientific research to advance health. As an independently funded research center, the focus of the institute is applied research. Members of the CDD have an excellent track record of successfully developing technologies that have then been licensed by a variety of industrial partners for commercialization.