Environment and Natural Resources Trust Fund
2020 Request for Proposals (RFP)

Project Title: **ENRTF ID: 037-A**

Foundational Research on Fungi and Protecting Minnesota Trees

Category: A. Foundational Natural Resource Data and Information

Sub-Category:

Total Project Budget: $ 362,196

Proposed Project Time Period for the Funding Requested: June 30, 2023 (3 yrs)

Summary:

Collecting the medicinal fungus "Chaga" in Minnesota results in damage to trees and is being over harvested. This project develops new methods for sustainable production/harvest and characterizes its medicinal properties.

Name: Robert Blanchette

Sponsoring Organization: U of MN

Job Title: Professor

Department: Department of Plant Pathology

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Location:

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

The medicinal fungus grows on birch trees found throughout Minnesota. Trees with Chaga and several bioactive compounds are shown. Goal: develop sustainable cultivated Chaga with high medicinal properties.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Foundational Research on Fungi and Protecting Minnesota Trees

I. PROJECT STATEMENT

Fungi produce many different compounds used in medicine and there has been a surge in interest of natural products used as medicine. In Minnesota, one medicinal mushroom called chaga has had large scale collecting leading to over harvesting, damage to trees in state and public areas, and depletion of the resource. Chaga is a fungal outgrowth on birch trees that is collected and used in medicinal teas, tinctures and extracts. The chaga fungus (*Inonotus obliquus*) infects birch trees and develops large black/brown fungal masses. Chaga only grows in cold, northern climates and takes 10-15 years or more to fully mature. Chaga, used in traditional medicines globally, is a rich source of many potent compounds with anti-oxidant, anti-cancer and wound healing activities. The extensive harvesting has led to its virtual disappearance from all Minnesota state parks and many state forests. Tree damage also occurs because the chaga is firmly attached to the tree and harvesting requires an axe to remove the medicinal mushroom. The high value for this fungus has many collectors indiscriminately collecting the fungus from wherever it can be found. This proposal is focused on developing new methods of cultivating the medicinal chaga fungus to protect Minnesota trees and forest resources from unsustainable harvesting and damage.

We propose to develop new cultivation methods and field inoculations that would provide sustainable production of chaga and a new forest industry for Minnesota. It appears possible to speed up the growing process of chaga to produce sufficient amounts of the medicinal mushroom to meet the growing demand. As part of this proposal, we would carry out 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 and accelerates fungal growth with chemical/medicinal properties that are similar to wild chaga. This new source of chaga would potentially make wild-harvesting of the fungus from Minnesota forests unnecessary, protecting our forest resources from damage ad indiscriminate cutting. In addition, the project would promote cultivated chaga which would be 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 the laboratory 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. Birch trees at the University of Minnesota Cloquet Forestry Station and also in State Forests will be used for field trials. Although field production of the fungus can take a long period of time, new methods to accelerate chaga formation in the trees will be tested. Previous work in the Blanchette lab on a different project has been able to produce a valuable medicinal resin in a tropical tree using fungal inoculation and reduced 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 as well. 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: $125,463

Outcome	Completion Date
1. Establish new culture conditions for chaga using birch wood/bark substrates	06/30/2022
2. Establish field trials of accelerated birch inoculations	06/30/2023
3. Optimize culture conditions and harvest timing to optimize activity	06/30/2023

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: $ 236,733

Outcome	Completion Date
1. Establish quantification methods for active metabolites from wild grown chaga	06/30/2021
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	06/30/2023

III. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

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

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 outreach activities will demonstrate to the public that wild chaga on birch trees should not be harvested due to this more accessible and sustainable source. This new information should move easily into widespread use.

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 years to mature, but we are planning to significantly reduce this growth time using optimized culture conditions of fungal mycelia and significant results will be obtained within 3 years.

VI. SEE ADDITIONAL PROPOSAL COMPONENTS:

A. Proposal Budget Spreadsheet

B. Visual Component or Map

F. Project Manager Qualifications and Organization Description

2020 Proposal Budget Spreadsheet

Project Title: Protecting Minnesota birch by sustainable chaga cultivation

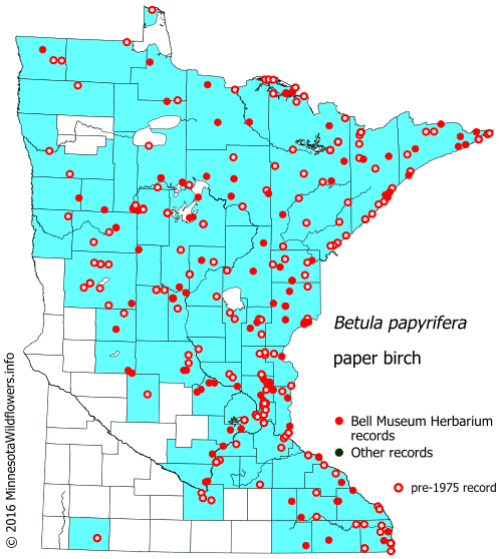
IV. TOTAL ENRTF REQUEST BUDGET: 3 years

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
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	$ 82,073
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, sequencing cultures)	$ 16,500
Activity 2	
Supplies for compound purification, structure elucidation and quantification (organic solvents, chromatography supplies, NMR solvents, filters, vials, pipettes)	$ 18,200
Supplies for biological assays (pipettes, pipette tips, epi tubes, culture tubes, petri dishes, media, 96 well plates, antibiotic standards, gloves)	$ 13,800
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	$ 2,000
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 =	$ 362,196

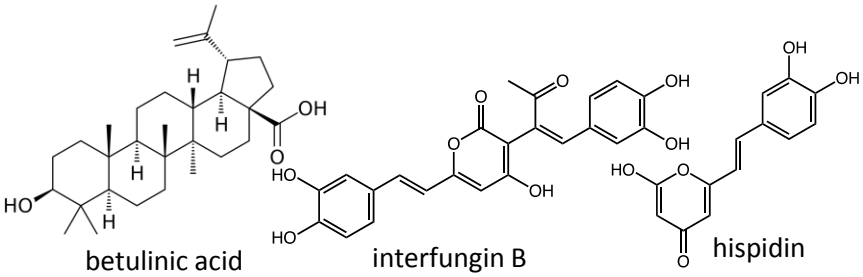
V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

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	$ 61,157	*secured*
Past and Current ENRTF Appropriation:		
ML2015, Chp. 76, Sec. 2, Subd. 06d - Preventing a New Disease of Pines in Minnesota	$ 371,000	*Ending 6/30/19*
ML2016, Chp. 186, Sec. 2, Subd. 06f - Dutch Elm Disease Resistance - Phase II	$ 200,000	*Ending 6/30/19*
Other Funding History: none		

Foundational Research on Fungi and Protecting Minnesota Trees



- The medicinal mushroom “Chaga” is highly valued due to its anti-oxidant, anti-cancer and anti-viral activities.
- Chaga is produced on birch trees by the fungus *Inonotus obliquus*.
- Chaga is being over harvested in natural forests and harvesting damages trees.
- The demand for chaga is very high and collectors are removing it from wherever it grows.
- New methods are needed for sustainable production and to stop the extensive damage caused by harvesting



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 produced sustainably and stop 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

Robert A. Blanchette, Project Manager and Principle Investigator

Dr. Robert Blanchette (Co-PI) is a professor in the Department of Plant Pathology. He has been involved with research and teaching of forest and landscape trees at the University for over 30 years. He currently teaches undergraduate and graduate classes at the University of Minnesota on forest and shade tree diseases. Research is in the area of forest pathology and wood microbiology with investigations underway on the biology and ecology of tree pathogens, tree defense mechanisms and managing tree diseases using integrated control procedures. He has received many honors for his research accomplishments including Fellow of the American Association for the Advancement of Science, Fellow of the American Phytopathological Society, Fellow of the International Academy of Wood Science, and Hans Merensky Fellow for Wood Science. He will serve as Project Manager and coordinate the overall project and take an active part in the proposed research. Dr. Blanchette has served as project leader on several past projects including 2015-084 Preventing a new disease of pines in Minnesota, 2013-19B Finding Disease Resistant Elm Trees in Minnesota and 2016-131-D Winning the Dutch elm disease battle Phase II.

Dr. Christine Salomon will be a co-investigator on this project. She 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 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 agricultural 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.

Organizational Description

The Department of Plant Pathology at the University of Minnesota is one of the oldest Plant Pathology departments in the country. Since 1907 the department has had a strong impact on plant health, agricultural development, and ecosystem vitality on a local, national, and international scale. It is involved with solving today's complex plant health problems through cutting-edge research. The department provides sound plant health advice to stakeholders throughout Minnesota and around the globe and is educating the next generation of plant health professionals and change-makers through a modern and broad plant pathology curriculum. Dr. Salomon is in the Center for Drug Design at the University of Minnesota and a member of the Biotechnology Institute. These centers play a central role in providing training and coordinating research in biological, chemical and engineering sciences at the University of Minnesota.