**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**

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| **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**

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| **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**

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| **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**