Environment and Natural Resources Trust Fund
2016 Request for Proposals (RFP)

Project Title: **ENRTF ID: 124-D**

White Nose Bat Syndrome Biological Control - Phase 2

Category: D. Aquatic and Terrestrial Invasive Species

Total Project Budget: $ 452,532

Proposed Project Time Period for the Funding Requested: 3 years, July 2016 to June 2019

Summary:

Phase 2 continuation of research towards discovery and optimization of biocontrol for White Nose Bat Syndrome. Work scope is expanded to additional statewide hibernacula, and characterization of total bat microbiomes.

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Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

map of hibernacula, examples of microbial isolates and assay set up

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

I. PROJECT STATEMENT

Our primary goal is to identify, develop and optimize biological control agents for prevention and/or treatment of White Nose Bat Syndrome (WNS) in Minnesota and eventually other locations. WNS is a devastating fungal disease that has decimated bat populations throughout the Northeast and Canada, killing more than 7 million bats to date. Although diseased bats have not been found at any of the major hibernation locations in Minnesota (Soudan Iron Mine and Mystery Cave as of February 2015), WNS *is likely to develop within the next 1-3 years*. The consequences of these massive bat declines are devastating losses of biodiversity, local species extinctions, and the loss of pest control for forests and agriculture. In the state of Minnesota, the economic value of bats has been estimated to be at least $1.4 billion per year, which does not include the additional downstream "costs" of water and environmental degradation due to increased pesticide use.

This work is an extension of our current ENTRF project (Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol) during which we have amassed a large collection of microbes (>200) collected from both bats and roost areas in the Soudan Mine hibernation areas to test as biocontrol agents. Additional bacterial and fungal test isolates will be obtained from bats and roosts from Mystery Cave and other hibernation areas throughout the state and assessed. We previously used non-pathogenic, faster growing fungi as "proxy" species of the real pathogen to test the biocontrol agents, but have since acquired an authentic culture of the *Pseudogymnoascus destructans* fungal pathogen for all future studies. We are especially interested in further studying and developing fast growing fungi as potential competitors or biocontrol agents and have identified a number of non-pathogenic species as candidates. An additional goal is to characterize the total microbiome of bats from each of the hibernation areas using culture-independent methods (DNA sequencing of all microbes from bat swabs). Since the disease has not yet developed among bat populations, this provides a critical window for obtaining samples from healthy bats throughout the state (which we will start to obtain now since we can't predict the arrival date of disease). This foundational data will allow us to compare how the microbial community changes over time due to either application of biocontrol agents or to WNS.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Characterization of culturable fungi and bacteria (~300 isolates) from bats and roosts in hibernacula throughout Minnesota **Budget: $218,446**

Bacteria and fungi will be collected from bats, roosts, and surfaces from Mystery Cave State Park and other minor hibernation areas in Minnesota. We will compare the diversity and biocontrol characteristics of these microbes to those already obtained from the Soudan Iron Mine. We will especially focus on the non-pathogenic species of fungi found associated with bats as promising and abundant competitors of the WNS fungal pathogen *Pseudogymnoascus destructans*.

Outcome	Completion Date
1. Isolation and culture of bacteria and fungi (~300 isolates) from bats/roosts in Mystery Cave State Park and minor hibernation caves near the Twin Cities	*07/01/17*
2. Characterization of growth and inhibition capacity of bacteria and fungi	*07/01/18*
3. Determination of mechanism of growth inhibition of best biocontrol agents (top 3)	*07/01/19*

Activity 2: Characterization of total microbiome associated with bats and roosts (from at least 10 bats and roosts per location per year) **Budget: $131,530**

In addition to the culture-dependent methods to study bat microbes, we will also use a culture-independent approach to obtain a more complete picture of the bacterial and fungal species associated with bats sampled from different locations, both before and presumably after development of WNS. The timing of the experiments may change due to the unknown nature of when WNS will fully arrive in Minnesota.

Outcome	Completion Date

1. Collection of microbial samples from bats in Soudan, Mystery Cave and other locations for DNA analysis	*07/01/17*
2. DNA isolation and sequencing from samples	*07/01/18*
3. DNA analysis of total microbial communities and comparison with cultured populations	*07/01/19*

Activity 3: Development of dissemination methods for application of biocontrol agents **Budget: $102,556**
Once the best biological microbes and materials are identified, they will be tested for efficacy and specificity under different application environments. This component will require optimization of formulation (how the materials will be physically used and applied to roosts and/or bats). Future studies will incorporate these findings for direct testing with live bats.

Outcome	Completion Date
1. Characterize and compare the best methods for applying/disseminating biocontrol microbes/materials (whole cells or cultures, volatiles or direct inhibition)	*07/01/18*
2. Optimize treatment formulation (live or dead cells, quantity, application timing, etc.)	*07/01/18*
2. Measure effects of each treatment method on viability of WNS fungus and spores	*07/01/19*

III. PROJECT STRATEGY

Project Team/Partners funded by ENRTF

Dr. Christine Salomon (UMN) Assistant Professor, BioTechnology Institute and Center for Drug Design is an expert in microbial culturing, testing and characterization and will oversee the project and contribute to Activities 1 and 2.

Dr. Robert Blanchette (UMN) is a Professor in Plant Pathology and an expert in fungal biology. He will lead the fungal collections and characterizations in both Activities.

Additional partners (not funded by ENRTF) include **Jim Essig** (DNR Park Manager of Soudan Mine State Park) and **Dr. Gerda Nordquist** (DNR, State Mammologist) who will help coordinate research activities and provide logistical support for sampling and experiments. We are also in communication with key managers with the US Fish and Wildlife Service: **Richard Geboy**, Midwest Regional WNS Coordinator and **Jonathan Reichard**, National WNS Assistant Coordinator and we will participate in their hosted monthly national conference calls.

B. Project Impact and Long-Term Strategy

At the very minimum, our work will provide foundational information about the diversity, abundance and geographical characteristics of microbial communities associated with both healthy and sick bats (anticipated in the near future) throughout the state of Minnesota. If we are successful at identifying biocontrol agents that inhibit the pathogen, these could be developed into therapeutic tools for disease management in Minnesota and other affected states. We are also applying for additional grants from the US Fish and Wildlife Federation to expand this work. Due to the rapid spread of the disease and dynamic nature of how diseases change the microbial landscape of their hosts, we anticipate needing to change our focus in the future to characterizing the microbes of surviving bats. We may also need to apply for "Phase 3" round of funding to support the testing of treatments or preventative measures in live bats during hibernation periods, in collaboration with bat disease experts.

C. Timeline Requirements

We are requesting 3 years of funding so that we can include at least 2 full hibernation periods for microbial sampling and in depth characterization of the potential biocontrol agents. We also anticipate that the WNS disease will arrive within this time frame and want to continue to collect, sample and compare the microbial populations associated with the bats as they change over time due to infection.

2016 Detailed Project Budget

Project Title: White Nose Bat Syndrome Biological Control: Phase 2

IV. TOTAL ENRTF REQUEST BUDGET: 3 years

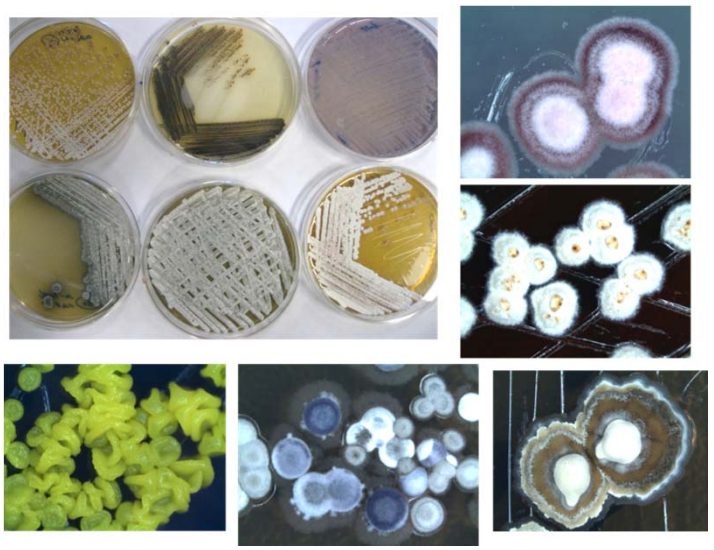
BUDGET ITEM	AMOUNT
Personnel:	$ -
Christine Salomon, Project Manager and Principle Investigator (75% salary, 25% benefits): 5% FTE for each of 3 years	$ 19,836
Postdoctoral researcher (82% salary, 18% benefits): 100% FTE for each of 3 years, sample collections, testing, assay development, biocontrol formulation and optimization, data/statistical analysis	$ 165,388
Technician (79% salary, 21% benefits): 50% FTE for each of 3 years, sample collections, DNA extractions and analysis, biological assays, media and reagent preparations, data organization	$ 70,881
Research Scientist (75% salary, 25% benefits): 25% FTE for each of 3 years, sample collections with focus on fungi, fungal taxonomy and characterization, data analysis and management	$ 64,427
Undergraduate student technicians (100% salary): 50% FTE for each of 3 years, media and sample prep, sample management, fungal cultivations, general lab support	$ 21,000
Equipment/Tools/Supplies:	$ -
Activity 1:	
Supplies for microbial isolations (growth media, reagents, antibiotics, petri dishes, tubes), DNA isolation supplies (extraction kits $350 per kit x 4 per year) general lab supplies (gloves, tips, tubes, etc.), chemicals, solvents, glassware. For 2 FTE scientists for 3 years.	$ 50,000
Sequencing for phylogenetic analysis of bacterial and fungal isolates: (AGAC sequencing facilities, $3.50 per reaction x ~1000 reactions per year, over 3 years)	$ 10,500
Repair of equipment and instrumentation (e.g. vacuum pumps, water baths, incubators, shakers, etc.) and replacement of glassware/parts due to inevitable breakage: estimated at $1000 per year for 3 years	$ 3,000
Microscopy (Scanning Electron, Light, Confocal microscopes-hourly instrument fees at CBS Biological Imaging Facility $25-$37 per hour plus specimen preparation fees, ~ 20hrs/year)	$ 2,500
Activity 2:	
Microbiology reagents for DNA amplification and cleanup kits (3 years)	$ 3,000
DNA sequencing fees for total genomic microbiome analysis (100 samples per run at $2000 each x 2 per year for 3 years)	$ 12,000
Activity 3:	
supplies for biological testing (microbiology supplies, consumables, petri dishes, antibiotics), general lab supplies, glassware. For .5 FTE scientist over 2 years	$ 10,000
Instrumentation/core facility fees for chemical analysis (NMR, Gas chromatography, mass spectrometry): hourly charges of $10-40 per hour or per sample, estimated at $1000 per year x 2 years.	$ 2,000
Travel: In-state round trip travel between St. Paul and Soudan Mine Park: room/board for 2-4 researchers, mileage, est. 5-6 trips/yr (1-3 days each trip) for 3 yrs	$ 16,500
Publication fees (~3 total, $500 per publication-page/color fee charges for publishing scientific manuscripts)	$ 1,500
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 452,532

V. OTHER FUNDS

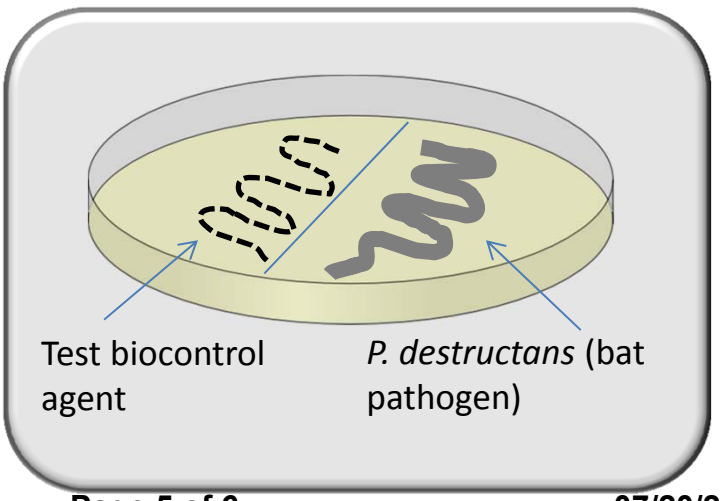
SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period: none	0.00	N/A
Other State $ To Be Applied To Project During Project Period: none	0.00	N/A
In-kind Services To Be Applied To Project During Project Period: Salary support for PI (Salomon) and co-PI (Blanchette) 5% FTE for 3 years	$ 43,639	secured
Funding History: 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) This investment has led directly to the applied work in the proposed application.	$ 838,000	secured
Remaining $ From Current ENRTF Appropriation: $376,609 remaining from ML 2013 03F (as of 04/30/2015)	$ 376,609	100% obligated

White Nose Bat Syndrome Biological Control: Phase 2

Expanded microbial sampling of bat hibernacula in MN: Soudan Iron Mine, Mystery Cave State Park and Metro area caves.



Bacteria and fungi isolated from bats/roosts in the Soudan Iron Mine (ENRTF 2012)



Which microbes isolated from bats best inhibit the growth of the WNS pathogen?

PROJECT MANAGER QUALIFICATIONS AND ORGANIZATION DESCRIPTION

Project Manager Qualifications

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

Dr. Salomon is an Assistant Professor and Assistant Director 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 soil 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 and wildlife pathogens and production of unique compounds for biomedical and biotechnological applications. She has successfully secured both internal (Academic Health Center, Healthy Foods Healthy Lives Institute) and external (United States Department of Agriculture) support for her research program.

Dr. Salomon is the Principal Investigator on a currently funded ENRTF project "Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol", ML 2013, Chp.52, Sec.2, Subd. 03f. Her ongoing work on the isolation, characterization and testing of novel microbes in this unique environment led directly to key components in the proposed continued research on biological control of the WNS pathogen. She has worked and collaborated with all of the investigators and partners listed and will serve as the project manager for the proposed interdisciplinary work.

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.