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

Project Title: **ENRTF ID: 157-D**

White Nose Bat Syndrome Biological Control: Phase 3

Category: D. Aquatic and Terrestrial Invasive Species

Sub-Category:

Total Project Budget: $ 444,636

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

Summary:

Testing of best biocontrol microbes for controlling white nose syndrome (WNS) in bats: Mapping of fungal pathogen, field testing, and assessment of a WNS-free cave with healthy bats

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Sponsoring Organization: U of MN

Job Title:

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Location:

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Map of Minnesota showing locations of 3 major hibernacula (Soudan Mine, Metro area caves, Mystery Cave)

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

PROJECT TITLE: White nose bat syndrome biological control: Phase 3

I. PROJECT STATEMENT: White nose syndrome (WNS) is a devastating fungal disease of hibernating bats which has killed at least 90% of little brown bats (*Myotis lucifugus*) at many sites in Minnesota. Our proposal is focused on developing a biocontrol strategy to treat substrates and to monitor the fungal pathogen, *Pseudogymnoascus destructans*, in Minnesota hibernacula. Our previous research supported by LCCMR and USFW has allowed us to build a library of potential biocontrol microbes (>2000 strains) collected from major hibernacula (Soudan Iron Mine, Mystery Cave, and several sandstone caves). We have screened many of these strains, identified the most potent inhibitors, and are ready to test these strains/extracts on natural substrates and in limited field settings. We have also optimized a sensitive DNA based detection method (qPCR) and used this approach to measure the occurrence and abundance of *P. destructans* along transects of Mystery Cave and in the Soudan Mine (the two largest hibernacula in Minnesota).

We propose to continue monitoring *P. destructans* to better understand where the fungus is most abundant and likely to re-infect surviving/returning bats. This approach will be used to monitor treatment experiments, and this data will also be available to park managers to identify specific locations in Soudan Mine and Mystery Cave State Parks to focus treatments, decontamination of equipment, or to help regulate visitors/staff in those areas. Additionally, we recently identified one hibernaculum that surprisingly has a healthy population of tricolor bats (another Minnesota bat species that has been decimated in other nearby locations). An initial, small-scale test of some of the surfaces of this cave were negative for the presence of *P. destructans*. We propose to determine how this cave has remained free of *P. destructans* and WNS by studying the physical environment, substrates, volatile compounds present, and microbial populations. This information may help to develop a treatment strategy for other hibernacula, and to support conservation efforts for surviving bats.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Testing of most active microbial biocontrol agents with relevant substrates and field studies
Description: We have identified >100 microbial strains that inhibit the growth of *P. destructans*. Among these strains, approximately 10 have demonstrated consistent production of antifungal extracts, and we have purified, characterized and identified most of the active compounds. We have also tested these compounds against cultured fibroblast (skin) cells from two species of bats (Northern long eared and Gray bats) to assess their toxicity. The strains that produce the most antifungal but least toxic compounds were prioritized, and these will be inoculated onto natural substrates from the three major hibernacula locations together with *P. destructans.* Once these experiments are analyzed, successful trials will be translated into small-scale field experiments in collaboration with the DNR. These experiments will be focused first in human-made hibernacula (mines) and smaller sandstone caves. *P. destructans* and antagonist growth will be assessed using microscopy and a sensitive qPCR DNA quantification method.

ENRTF BUDGET: $149,522

Outcome	Completion Date
1. Optimization and testing of top (3-5) biocontrol agents/extracts on natural substrates	*12/31/2021*
2. Field testing of best biocontrol agents	*06/31/2023*
3. Quantification of P. destructans on substrates in lab and field experiments	*06/31/2023*

Activity 2: Quantification of *P. destructans* in hibernacula: Seasonal and spatial dynamics
Description: Bats afflicted with WNS change their hibernation patterns within caves, and will often move towards the entrance of caves. However, little is known about the extent of the environmental reservoirs of *P. destructans* on substrates in these different locations over time. We will map the occurrence and quantity of *P. destructans* along transects of hibernacula at twice per year from substrate locations (walls, sediments, ceiling) to better understand the spatial and seasonal dynamics of *P. destructans* growth and potential spread. This information will be especially helpful to cave managers for focusing treatments or interventions. For example, the top of one door at the entrance of Mystery Cave in Forestville was found to have 3000x more *P. destructans* DNA than areas much deeper in the cave. These "pinch points" for bat entry/exit might be an obvious place for reinfection when bats return in the fall, and could be specifically disinfected. Our regular sampling and quantification before and after any treatments will also provide empirical data to assess management strategies.

ENRTF BUDGET: $128,590

Outcome	Completion Date
1. qPCR quantification of samples collected from hibernacula transects, 2x per year, 3 years	*06/2023*

Activity 3: Assessment of WNS-free cave
Description: We identified a cave (not named here to minimize potential disturbance) with a healthy population of tricolor bats (*Perimyotis subflavus*) and no signs of WNS (as of 02/2019) with animal numbers consistent with pre-WNS census data. A preliminary analysis of a small number of substrates were all negative, suggesting that *P. destructans* is not present or not abundant in sampled areas. This is surprising and unexpected due to the widespread occurrence of WNS in all other Minnesota hibernacula. We propose to conduct a more thorough mapping of *P. destructans* throughout this cave (see activity 2) and from bats over the next three years, and methodically test a number of different possible factors that might be responsible for the absence of WNS. Various substrates (rocks, water, sediment, etc.) will be tested for their ability to host (or inhibit) *P. destructans* growth, and volatile air samples may be collected and tested. Concurrently, bacterial and fungal samples will be collected and tested for *P. destructans* inhibition. If the substrate or volatile materials show significant activity, the microbial experiments will be minimized. The results of these experiments should provide information about a naturally disease suppressive environment which will inform management strategies.

ENRTF BUDGET: $166,524

Outcome	Completion Date
1. Substrate and volatile testing against P. destructans	*06/2021*
2. Microbial sampling, isolation and testing (depending on results of substrate testing)	*06/2022*
3. qPCR quantification of P. destructans on bat and substrate samples throughout cave	*06/2023*

III. PROJECT PARTNERS AND COLLABORATORS: Our primary partners will continue to be DNR park managers at State Parks (Soudan Mine and Mystery Cave) as well as Gerda Nordquist, MN DNR State mammalogist.

IV. LONG-TERM IMPLEMENTATION AND FUNDING: If we accomplish the goals in this proposal, we expect this to be the last request for the basic research components of this WNS project. Depending on the success of the field trials, we may request additional funds to expand treatments or interventions (such as targeted disinfection) to more hibernacula sites. We plan to continue to request funds from USFW to leverage effort towards WNS treatment, and will seek additional funds from the National Science Foundation to expand studies of the natural history and environmental reservoir of *P. destructans*.

2020 Proposal Budget Spreadsheet

Project Title: Biocontrol of white nose bat syndrome: Phase 3

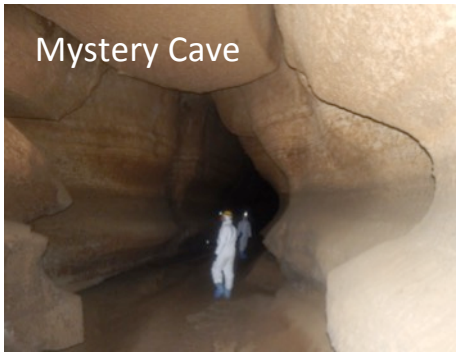
IV. TOTAL ENRTF REQUEST BUDGET: 3 years

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
PI Salomon 5% FTE for 3 years. Project mananager and chemistry and bioactivity testing lead.	$ 27,410
Co-PI Blanchette 5% (in kind) for 3 years. Lead for qPCR analysis and fungal work.	0
Postdoctoral researcher (82% salary, 18% benefits): 100% FTE for each of 3 years. Collecting and testing substrates and volatile samples, fractionation and identification of active components. Microbial isolations and characterizations. Field experiments with test biocontrol strains.	$ 197,380
Technician (79% salary, 21% benefits): 50% FTE for each of 3 years, biological assay testing, database management for bioactivity, chemistry and microbiology samples, general lab support	$ 40,923
Research Scientist (75% salary, 25% benefits): 25% FTE for each of 3 years. Sample collection and qPCR analysis of samples for *P. destructans* quantification in field and laboratory experiments. Fungal isolations and characterizations.	$ 82,462
Undergraduate student technicians (100% salary): 50% FTE for each of 3 years, media and sample prep, sample management, fungal cultivations, general lab support	$ 20,461
Equipment/Tools/Supplies:	$ -
Activity 1	
Microbiology supplies (media, reagents, petri dishes, tubes, gloves, field sampling materials)	$ 8,500
Microscopy supplies and costs (microscope use, fixatives, sample prep instrumentation, sample supplies)	$ 4,500
Activity 2	
Microbiology supplies (media, reagents, petri dishes, tubes, gloves, field sampling materials)	$ 8,000
Molecular biology/sequencing costs: (DNA isolation kits, PCR supplies, enzymes, reagents, sequencing costs)	$ 12,000
Activity 3	
Chemical supplies (solvents, chromatography materials, reagents, tubes, glassware, pipettes)	$ 18,000
Supplies for biological assays (pipettes, pipette tips, epi tubes, culture tubes, petri dishes, media, 96 well plates, reagents, gloves)	$ 14,000
Travel: In-state round trip travel : 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	$ 3,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 444,636

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 co-PI (Blanchette) 5% FTE for 3 years	$ 34,515	*secured*
Past and Current ENRTF Appropriation:		
Biological Control of White Nose Syndrome in Bats: Phase 2 (balance as of 12/2018)	$ 452,000	*bal: 171,293 Ending 06/19*
Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol, ML 2013-03F (WNS research was one of several sub-aims in this multi-PI project)	$ 838,000	*Ended 06/2016*

White nose bat syndrome biological control: Phase 3



- Testing of best biocontrol strains on substrates from 3 diverse hibernacula
- Small scale field trials
- Mapping of *P. destructans* in hibernacula
- Assessment of physical and chemical factors in WNS-free cave

F. Project Manager Qualifications

Dr. Salomon (PI) 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 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, Biotechnology Institute) and external (US Department of Agriculture and US Fish and Wildlife) support for her research program. Dr. Salomon is the Principal Investigator on a currently funded ENRTF project "Biological Control of White Nose Syndrome in Bats – Phase II", ML 2016 Chp 186, sec 2.

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. 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.

Organizational Description

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. The University of Minnesota Biotechnology Institute was established to catalyze the development of a biotechnology industry in Minnesota. It also serves as an important resource for industry by providing connections with academic research partners.

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.