



Environment and Natural Resources Trust Fund

2022 Request for Proposal

General Information

Proposal ID: 2022-207

Proposal Title: White Nose Bat Syndrome Biological Control: Phase 3

Project Manager Information

Name: Christine Salomon

Organization: U of MN - College of Pharmacy

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Project Basic Information

Project 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

Funds Requested: \$449,000

Proposed Project Completion: June 30 2025

LCCMR Funding Category: Aquatic and Terrestrial Invasive Species (D)

Project Location

What is the best scale for describing where your work will take place?

Region(s): NE, SE, Metro,

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Narrative

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

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). This quantification work will be combined with a method to assess viable (live) versus dead cells and spores, which should allow for a more accurate assessment of treatments.

What is your proposed solution to the problem or opportunity discussed above? i.e. What are you seeking funding to do? You will be asked to expand on this in Activities and Milestones.

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 recently positive for the presence of the pathogen *P. destructans*. We propose to determine how bats in this cave have remained free of WNS by studying the physical environment, substrates, and microbial populations. This information may help to develop a treatment strategy for other hibernacula, and to support conservation efforts for surviving bats.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

Our primary goal is to develop a safe and effective product and treatment strategy to reduce the reservoir of WNS pathogen in hibernacula and ultimately reduce infection rates for our remaining bats (and new pups). Our regular sampling and quantification before and after any test treatments will also provide empirical data to assess management strategies. We will also be studying a hibernacula with healthy bats to provide information about a naturally disease suppressive environment which could inform management strategies.

Activities and Milestones

Activity 1: Testing of most active microbial biocontrol agents with relevant substrates and field studies

Activity Budget: \$172,168

Activity Description:

We identified >100 microbial strains that inhibit the growth of *P. destructans*. Among these strains, 10 have demonstrated consistent production of antifungal extracts, and we have purified 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 applied to natural substrates from the three major hibernacula locations together with *P. destructans*. We will also test extracts and the active compounds on substrates with established *P. destructans*. Experimental substrate challenges will be treated with a viability reagent propidium monoazide (PMA) before DNA extraction to differentiate between DNA from live versus dead *P. destructans*. qPCR with a specific Taqman probe will be used to quantify DNA from live cells. 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 on human-made hibernacula including mines and smaller historic sandstone brewery caves that serve as hibernacula. *P. destructans* and antagonist growth in these field studies will be assessed by qPCR.

Activity Milestones:

Description	Completion Date
Optimization and testing of top (3-5) biocontrol agents/extracts on natural substrates	December 31 2023
Field testing of best biocontrol agents, extracts and pure compounds	June 30 2024
Quantification of <i>P. destructans</i> on substrates in lab and field experiments	June 30 2025

Activity 2: Quantification of viable *P. destructans* in hibernacula: Seasonal and spatial dynamics

Activity Budget: \$132,415

Activity 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 viable *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. Samples will be analyzed by treating with PMA (as in activity 1) before isolation of DNA and subsequent qPCR quantification of *P. destructans*. 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.

Activity Milestones:

Description	Completion Date
qPCR quantification and viability of samples collected from hibernacula transects, 2x per year, 3 years	June 30 2025

Activity 3: Assessment of WNS-free cave

Activity Budget: \$144,417

Activity 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 02/2020) with animal numbers consistent with pre-WNS census data. A preliminary analysis of a small number of substrates were recently positive for *P. destructans*, suggesting that WNS may develop in these bats soon. 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.

Activity Milestones:

Description	Completion Date
Substrate testing against <i>P. destructans</i>	June 30 2023
Microbial sampling, isolation and testing (depending on results of substrate testing)	June 30 2024
qPCR quantification of <i>P. destructans</i> on bat and substrate samples throughout cave	June 30 2025

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Robert Blanchette	University of Minnesota, Department of Plant Pathology	Dr. Blanchette will be responsible for the qPCR analysis of pathogen populations and isolations/characterization of fungi from caves and mines	Yes

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this be funded?

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 (which funded this work through a grant from 2019-2020), and will seek additional funds from the National Science Foundation to expand studies of the natural history and environmental reservoir of *P. destructans*.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Biological Control of White Nose Syndrome in Bats - Phase II	M.L. 2016, Chp. 186, Sec. 2, Subd. 06d	\$452,000

Project Manager and Organization Qualifications

Project Manager Name: Christine Salomon

Job Title: Associate Professor

Provide description of the project manager's qualifications to manage the proposed project.

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 was the Principal Manager on a previously 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. 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.

Organization: U of MN - College of Pharmacy

Organization Description:

Dr. Salomon is in the Center for Drug Design in the College of Pharmacy 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.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
Personnel								
Principle Investigator		Project manager and chemistry and bioactivity testing lead.			36.5%	0.15		\$28,128
Research Associate		Collecting and testing substrates and volatile samples, fractionation and identification of active components. Microbial isolations and characterizations. Field experiments with test biocontrol strains			36.5%	3		\$199,126
Research Associate		Sample collection and qPCR analysis of samples for <i>P. destructans</i> quantification in field and laboratory experiments. Fungal isolations and characterizations.			36.5%	0.75		\$84,627
Junior scientist		biological assay testing, database management for bioactivity, chemistry and microbiology samples, general lab support			0%	1.5		\$40,923
undergraduate research assistant		media and sample prep, sample management, fungal cultivations, general lab support			0%	0.75		\$20,461
Co-Principal Investigator		Manager for qPCR analysis of pathogen and fungal isolations			0%	0.15		-
							Sub Total	\$373,265
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	Microbiology supplies: media, reagents, petri dishes, tubes, gloves, field sampling materials	microbiology field work, microbiology isolations and assays, activity 1, 3 yrs					\$8,500
	Tools and Supplies	Microscopy supplies: microscope use (scanning electron microscope), fixatives, sample prep instrumentation, sample supplies	microscopic characterization of biological control experiments, Activity 1, 3 yrs					\$4,500

	Tools and Supplies	Microbiology supplies (media, reagents, petri dishes, tubes, gloves, field sampling materials)	Collection and analysis supplies for quantifying viable <i>P. destructans</i> -activity 2, 3 years						\$8,000
	Tools and Supplies	Molecular biology/sequencing costs: (DNA isolation kits, PCR supplies, enzymes, reagents, sequencing costs) x 300 samples/year	materials for isolating and sequencing DNA for activity 2, 3 years						\$12,000
	Tools and Supplies	Chemical supplies (solvents, chromatography materials, reagents, tubes, glassware, pipettes)	supplies for conducting chemical extractions, fractionation and analysis of substrates and microbial samples for activity 3, 2 years						\$18,000
	Tools and Supplies	Supplies for biological assays (pipettes, pipette tips, epi tubes, culture tubes, petri dishes, media, 96 well plates, reagents, gloves), estimated 300 samples per year	Supplies for conducting biological antifungal assays with extracts/compounds/substrates obtained for activity 3, 3 years						\$14,000
								Sub Total	\$65,000
Capital Expenditures									
								Sub Total	-
Acquisitions and Stewardship									
								Sub Total	-
Travel In Minnesota									
	Miles/ Meals/ Lodging	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	For collection of samples from caves/mines throughout Minnesota, all activities x 3 yrs						\$5,735
								Sub Total	\$5,735
Travel Outside Minnesota									
								Sub Total	-
Printing and Publication									
	Publication	~2 total, \$1000 per publication-page/color fee charges and/or open access charges for publishing scientific manuscripts	Publication of scientific data and results obtained during this project						\$2,000

							Sub Total	\$2,000
Other Expenses								
		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	Funds for inevitable breakage, repair of glassware and instrumentation and calibration of instrumentation					\$3,000
							Sub Total	\$3,000
							Grand Total	\$449,000

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
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Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	-
Non-State				
			Non State Sub Total	-
			Funds Total	-

Attachments

Required Attachments

Visual Component

File: [89f6590e-63e.pdf](#)

Alternate Text for Visual Component

A map of Minnesota showing the locations of the 3 major bat hibernacula areas, with images showing typical structures from these different types of caves/mines (iron mine, sandstone caves and calcium karst caves). There is a brief summary of the aims of the proposal: 1. Testing of best biocontrol strains on substrates from 3 diverse hibernacula. 2. Small scale field trials 3. Mapping of *P. destructans* in hibernacula. 4. Assessment of physical and chemical factors in WNS-free cave...

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Does your project have potential for royalties, copyrights, patents, or sale of products and assets?

Yes

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

Yes

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF? If so, describe here:

Yes, If we develop a biocontrol product that garners revenue, we would very much like to reinvest into continued optimization, assessment and dissemination to park managers.

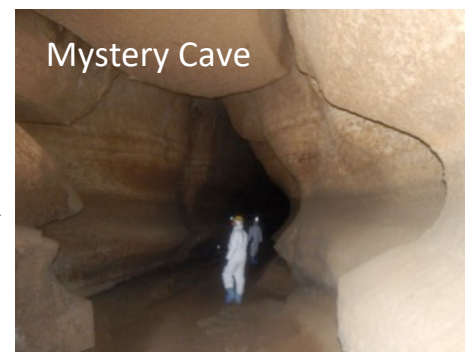
Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

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

