Environment and Natural Resources Trust Fund

M.L. 2020 Approved Work Plan

General Information

ID Number: 2020-004

Staff Lead: Michael Varien

Date this document submitted to LCCMR: August 19, 2021

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

Project Budget: $440,000

Project Manager Information

Name: Christine Salomon

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Project Reporting

Date Work Plan Approved by LCCMR: August 20, 2021

Reporting Schedule: April 1 / October 1 of each year.

Project Completion: June 30, 2024

Final Report Due Date: August 14, 2024

Legal Information

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 06c

Appropriation Language: $440,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to continue assessing and developing a biocontrol agent for white-nose syndrome in bats.

Appropriation End Date: June 30, 2024

Narrative

Project Summary: Testing of best biocontrol microbes for controlling white nose syndrome (WNS) in bats: Mapping of fungal pathogen in environmental reservoirs and field testing with biological control candidates.

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 identified one hibernaculum that surprisingly had a healthy population of tricolor bats through 2020 (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, but this cave was recently determined to be WNS positive. We propose to determine how this cave remained free of P. destructans and WNS-positive bats for longer than any other cave in Minnesota 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 hibernaculum that remained free of WNS through 2020 to provide information about the development of an environmental reservoir of pathogen and potential interventions to minimize reinfection of healthy bats.

Project Location

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

Statewide

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

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, 2022
Quantification of P. destructans on substrates in lab and field experiments	June 30, 2024
Field testing of best biocontrol agents, extracts and pure compounds	June 30, 2024

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, 2024

Activity 3: Assessment of WNS positive cave

Activity Budget: $135,417

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 through 2020 with animal numbers consistent with pre-WNS census data. A preliminary analysis of a small number of substrates were all negative in 2019, but P. destructans was detected in 2020. In early spring of 2021, we observed the first signs of WNS on bats. 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 delayed occurrence 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. The small size of this cave and detailed population numbers will allow us to carefully monitor the extent of the environmental reservoir of P. destructans and progression of disease. This cave may also be a candidate for trial treatments of the most promising biocontrols.

Activity Milestones:

Description	Completion Date
Substrate testing against P. destructans	June 30, 2022
Microbial sampling, isolation and testing (depending on results of substrate testing)	June 30, 2023
qPCR quantification of P. destructans on bat and substrate samples throughout cave	June 30, 2024

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 the WNS-free cave	Yes

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
The results of this research will be shared with both the public and scientific community. Examples of communications include giving seminars at public forums, participating in DNR sponsored events (i.e. Bat Week), sharing of data and results with DNR staff and state park managers, and updates on our lab website. Scientific communications will be provided through the publication of scientific manuscripts and posters and talks at scientific conferences. ENRTF will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENTRF Acknowledgment Guidelines.

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 2019-2021), 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

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Principle Investigator		Project mananager and chemistry and bioactivity testing lead.			36.5%	0.15		$28,128
Postdoctoral researcher		Collecting and testing substrates and volatile samples, fractionation and identification of active components. Microbial isolations and characterizations. Field experiments with test biocontrol strains			25.4%	3		$199,126
Research Associate		Sample collection and qPCR analysis of samples for P. destructans quantification in field and laboratory experiments. Fungal isolations and characterizations.			36.5%	0.6		$81,127
Technician		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	**$369,765**
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 and assays, activity 1, 3 yrs					$8,235
	Tools and Supplies	Microscopy supplies: microscope use, fixatives, sample prep instrumentation, sample supplies	microscopic characterization of biological control experiments, Activity 1, 3 yrs					$3,000
	Tools and Supplies	Microbiology supplies (media, reagents, petri dishes, tubes, gloves, field sampling materials)	Collection and analysis supplies for quantifying viable P. destructans					$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					$12,000
							Sub Total	**$61,235**
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					$4,000
							Sub Total	**$4,000**
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	**$440,000**

Classified Staff or Generally Ineligible Expenses

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

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 and typical surfaces from 3 major bat hibernacula areas, including an iron mine from northern Minnesota, a sandstone cave from the metro area, and a karst cave from the southern part of the state. The major objectives of the project are listed as "Testing of best biocontrol strains on substrates from 3 diverse hibernacula, small scale field trials, mapping of P. destructans in hibernacula, assessment of microbial community and physical characteristics...

Optional Attachments

Support Letter or Other

Title	File
ENTRF Background Check form	dcd15cf1-ef9.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

Since this proposal was first submitted in 2019, one of the WNS-free caves was recently noted as WNS positive (spring 2021). This required us to change our Activity 3 slightly by focusing more on mapping and quantifying the pathogen in the environmental reservoir while we continue to test the microbial and physical characteristics of that location, and continue to monitor the progression of WNS in the small bat population that hibernates there. We also reduced several budget lines slightly to match the reduction in budget that was recommended (by 9k).

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

N/A

Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I agree to the Commissioner's Plan.

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 any revenues are realized during this project through the development of an effective biocontrol, we would like to reinvest this into additional development, assessment, optimization and expansion of treatment for hibernacula throughout the state.

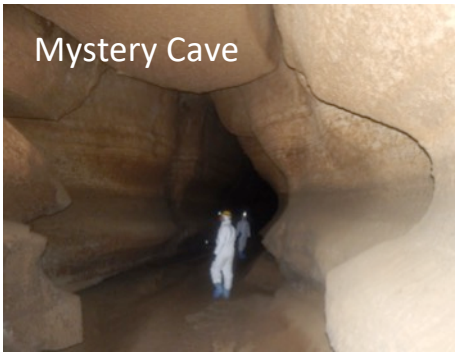
Does your project include original, hypothesis-driven research?

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