Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report: 11/30/15

Date of Next Status Update Report: January 2017

Date of Work Plan Approval:

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

PROJECT TITLE: Biological Control of White Nose Bat Syndrome - Phase 2

Project Manager: Christine Salomon

Organization: University of Minnesota, Center for Drug Design

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Location: *Ramsey County, St. Paul / Hennepin County, Minneapolis / St. Louis County, Soudan (Breitung Township)/ Fillmore County, Forestville*

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$452,000**
	Amount Spent:	**$0**
	Balance:	**$452,000**

Legal Citation: M.L. 2016, Chp. 124-D, Sec. 116p, Subd. 06

Appropriation Language:

I. PROJECT TITLE: White Nose Bat Syndrome Biological Control - Phase 2

II. 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 (>500) 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 ~50 non-pathogenic bacteria and fungi as candidates. An additional goal is to characterize the total microbiome of bats from each of the hibernation areas using culture-dependent and 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.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January, 2017**:**

Project Status as of July, 2017**:**

Project Status as of January, 2018**:**

Project Status as of July, 2018**:**

Project Status as of January, 2019**:**

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Microbial Antagonist Library
Description: Our goal is to identify the best microbial antagonists that can be applied to roost areas and or directly to bats to provide dynamic and long lasting protection against fungal infection. Although we have successfully identified over 50 bacterial and fungal isolates from the Soudan Mine that inhibit the growth of *P. destructans* under laboratory conditions, we do not yet know which species can grow efficiently on either roost substrates or on bats themselves. We also plan to determine which microbial antagonists can successfully grow

on various substrates related to bat habitats throughout Minnesota including limestone, sandstone and greenstone/banded iron (hematite, jasper, chert): See activity 3.

Bacteria and fungi will be collected from bats, roosts, and surfaces from Mystery Cave State Park and other minor hibernation areas in Minnesota that provide representatives from the various different types of substrates. 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*. Live colonies of each strain will be tested on solid media with an agar overlay spread with spores from *P. destructans* (for bacterial antagonists) or with side by side mycelial plugs (for fungal antagonists). Additional assays will include spore germination inhibition and non-contact dependent antagonism.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 222,209
Amount Spent: $ 0
Balance: $ 222,209

Outcome	Completion Date
1. Isolation and culture of bacteria and fungi (~500 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 Pd 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

Project Status as of January, 2017**:**

Project Status as of July, 2017**:**

Project Status as of January, 2018**:**

Project Status as of July, 2018**:**

Project Status as of January, 2019**:**

Final Report Summary:

ACTIVITY 2: Characterization of total microbiome associated with bats and roosts throughout Minnesota (culturable and culture-independent).

Description: We will compare the diversity and biocontrol characteristics of the new microbial library (~500 strains) obtained in activity 1 to those already obtained from the Soudan Iron Mine. Each isolate will be characterized using DNA sequencing (16s rRNA gene for bacteria and the ITS region for fungi). These data, together with the morphological and growth inhibition characteristics, will be compared and analyzed to identify any potential patterns of exceptional bioactivity. In addition to the comparing the culturable microbial communities associated with bats and roosts, we will use culture-independent methods to obtain a more complete picture of bacterial and fungal species associated with bats from different locations. DNA will be isolated from bat swabs taken from each location and purified and submitted to the UMN Genomics center for sequencing. We will begin obtaining samples immediately so that we can ideally have a pre-WNS sample set. We will also use these methods to eventually compare the outcomes of treatments on animals and roost materials. The timing of subsequent sampling for full microbiome sequencing will depend on the arrival of WNS disease to the hibernacula. These data will also allow us to compare the microbial communities between bat species and among the different habitats and geographic regions of the state.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 130,922

Amount Spent: $ 0
Balance: $ 130,922

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 and pre-disease samples	07/01/19

Project Status as of January, 2017**:**

Project Status as of July, 2017**:**

Project Status as of January, 2018**:**

Project Status as of July, 2018**:**

Project Status as of January, 2019**:**

Final Report Summary:

ACTIVITY 3: : Development of dissemination methods for application of biocontrol agents
Description: Understanding the life cycle of *P. destructans* (Pd) is key to developing the best treatments for WNS. Pd is infamous for producing tough spores (conidia) that can lie dormant in caves for many years until they find their way onto a hibernating bat. It is unknown if Pd can successfully reproduce on natural substrates outside of its bat hosts, which could mean that this pathogen may persist indefinitely in a cave. We need to develop treatments for both bats and their cave environments to successfully combat WNS, as a treatment strategy that only attacks Pd in one place is likely to fail. We propose to screen our Soudan mine microbes that were active against Pd in our initial tests for their ability to combat Pd on bat skin punches and in Minnesota cave roosts and sediments.

Once the most potent biological control microbes are identified (Activity 1), they will be tested for efficacy and specificity under different application environments. The first assay will involve growing each candidate on medias made from each of the three major roost materials: Soudan Mine rock material, limestone (Mystery Cave) and sandstone (Metro area caves). We will test the ability of strains to grow in both liquid and solid medias made from extracts of each substrate type. Once we have identified growth-positive antagonists, they will be tested against Pd in natural substrate materials. Pd-inoculated soils/substrates will be challenged with inoculations of Soudan mine microbes and changes in Pd growth will be compared with the previously established baseline. Similar studies will be conducted on bat wing punch explants to identify microbes that could be used directly on bats. Microbes that inhibit Pd and grow at an acceptable rate will be considered for scaled-up testing in natural environments once their environmental safety is evaluated. 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.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 98,869
Amount Spent: $ 0
Balance: $ 98,869

Outcome	Completion Date

1. Test the growth of *P. destructans* on each representative substrate material (Soudan Iron Mine material, Mystery Cave limestone and Metro area sandstone)	07/01/2017
2. Test the growth of best antagonist strains on representative substrate materials and bat wing punch explants	07/30/2018
3. Measure inhibition of Pd growth by antagonist strains on substrates and bat wing punch explants	01/30/2019
4. Optimize treatment formulation for best inhibitors that can grow on each kind of substrate.	07/01/2019

Project Status as of January, 2017**:**

Project Status as of July, 2017**:**

Project Status as of January, 2018**:**

Project Status as of July, 2018**:**

Project Status as of January, 2019**:**

Final Report Summary:

V. DISSEMINATION:

- Publications to primary scientific journals will be submitted covering all aspects of this proposal. Seminars and lectures will be given at scientific conferences and to local stakeholders. Strains of interest will be made available through the American Type Culture Collection (ATCC, with appropriate usage restrictions agreed to by the University of Minnesota, LCCMR and the DNR).

- Intellectual Property will be kept confidential so that patent protection can be coordinated by the University of Minnesota Office of Technology Commercialization, LCCMR and the DNR. This will be done in accordance with Statute 116p.10, "royalties, copyrights, patents, and sale of products and assets".

- Relevant results will also be communicated to the general public through an interactive display at the Soudan Mine Visitor Center that was developed as part of our previous ENRTF project. We are also pursuing the development of a small exhibit at the Science Museum of Minnesota to educate the public about the threat of White Nose Bat Syndrome as well as related current research efforts.

Project Status as of January, 2017**:**

Project Status as of July, 2017**:**

Project Status as of January, 2018**:**

Project Status as of July, 2018**:**

Project Status as of January, 2019**:**

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel		
Christine Salomon,	$ 19,836	Project Manager and Principle Investigator (75% salary, 25% benefits): 5% FTE for each of 3 years
1 postdoctoral Research Associate	$ 165,388	(82% salary, 18% benefits): 100% FTE for each of 3 years, sample collections, testing, assay development, biocontrol formulation and optimization, data/statistical analysis
1 Technician	$ 70,881	(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
1 Research Scientist	$ 64,427	(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
1 undergraduate student technician	$ 21,000	50% FTE for each of 3 years, media and sample prep, sample management, fungal cultivations, general lab support
Equipment/Tools/Supplies:	$	
Activity 1		
Supplies for microbial isolations and characterization	$ 50,000	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.
Microscopy	$ 2,500	Scanning electron, light, confocal microscopes-hourly instrument fees at CBS Biological Imaging Facility $25-37 per hour plus specimen preparation fees, ~20 hours per year
Activity 2:		
DNA and sequencing supplies	$ 3,000	DNA amplification reagents and consumables, DNA cleanup kits (for ~ 500 strains)
DNA sequencing (Sanger sequencing)	$ 10,968	Sequencing for phylogenetic analysis of bacterial and fungal isolates (AGAC sequencing facilities, $3.60 per reaction x ~1000 reactions per year x 3 years)
DNA sequencing (MiSeq)	$ 11,000	DNA library preparation and amplification services (10.95 x ~ 150 samples per run), MiSeq sequencing paired-end single lane, 300 cycles ($1,968 per lane) x 3 runs over 3 years.
Activity 3:		
Bioassays (antifungal testing)	$ 10,000	Reagents, compounds and consumables (microbiology supplies, antibiotics, plasticware) for biological testing, general lab supplies, glassware. For 0.5 FTE scientists over 2 years
Instrumentation/core facility fees for chemical analysis	$ 2,000	Fees for core facilities for chemical analysis of active strains (NMR spectroscopy, gas chromatography, mass spectrometry). Hourly charages of $10-40 per hour or per sample,

		estimated at $1000 per year x 2 years
Other expenses (all activities)		
Repair of equipment and instrumentation	$ 3,001	Repair for instruments such as vacuum pumps, water baths, incubators, shakers, etc. and replacement of glassware/components due to inevitable breakage. Estimated at $1000 per year x 3 years.
Publication fees	$ 1,500	~3 total publications, $500 per publication charge for open access journals
Travel Expenses in MN:	$16,500	In-state round trip travel between St. Paul and Soudan Mine Park, Mystery Cave and metro area caves: room/board for 2-4 researchers, mileage, est. 5-6 trips/yr (0.5-2 days each trip) for 3 yrs
TOTAL ENRTF BUDGET:	**$ 452,000**	

Explanation of Use of Classified Staff:

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 2.3 FTE per year x 3 years

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
US Fish and Wildlife	$ 240,000		Related project on White Nose Bat syndrome—sharing of sampling data and strains between projects.
State			
University of Minnesota (Dept. Center for Drug Design)	$ 39,672	$	In kind PI salary support, 3 years at 10% FTE
University of Minnesota (Dept. Center for Drug Design)	$ 239,560		In kind overhead/indirect costs: U of M rate of 53% of direct costs for 3 years
TOTAL OTHER FUNDS:	**$519,232**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

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 all activities.

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 all 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 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 andgeographical 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 Minnesotaand 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. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
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. 100% obligated	July 2013-July 2016	$838,000
		$
		$

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached

X. RESEARCH ADDENDUM: Continuation from previous ENTRF 2013 Research Addendum

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 2017, July 2017, January 2018, July 2018 and January 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

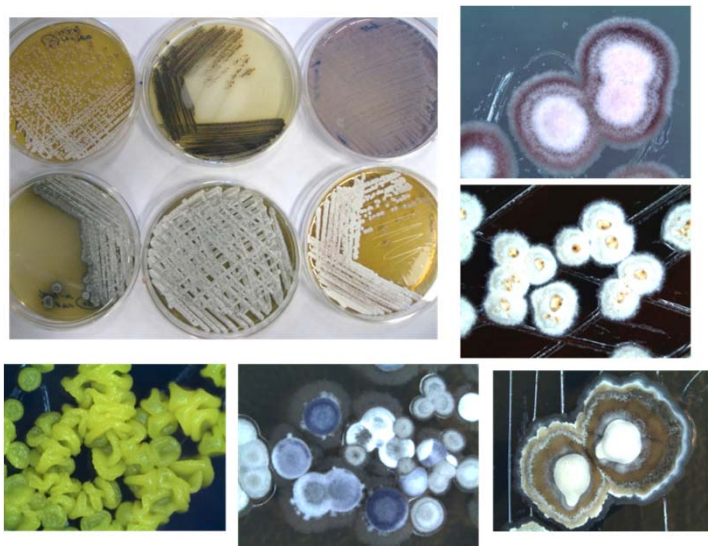
Project Title: *Biological Control of White Nose Bat Syndrome-Phase 2*
Legal Citation:
Project Manager: *Christine Salomon*
Organization: *University of Minnesota, Center for Drug Design*
M.L. 2016 ENRTF Appropriation: $ *452,000*
Project Length and Completion Date: *3 Years, July 1, 2016-June 30, 2019*
Date of Report: *December 2015*

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Fill in your activity title here.***			***Fill in your activity title here.***			***Fill in your activity title here.***				
Personnel (Wages and Benefits)	$151,709	$0	$151,709	$104,454	$0	$104,454	$85,368	$0	$85,368	$341,531	$341,531
Christine Salomon, Project Manager and Principle Investigator (75% salary, 25% benefits): 5% FTE for each of 3 years, $19,836											
1 Postdoctoral researcher (82% salary, 18% benefits): 100% FTE for each of 3 years, $165,388											
1 technician (79% salary, 21% benefits): 50% FTE for each of 3 years, $70,881											
1 Research Scientist (75% salary, 25% benefits): 25% FTE for each of 3 years, $64,427											
1 Undergraduate student technicians (100% salary): 50% FTE for each of 3 years, $21,000											
Equipment/Tools/Supplies											
Supplies for microbiology collections, isolations and purifications: 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 (approximately $8000 per scientist per year)	$50,000	$0	$50,000							$50,000	$50,000
microscopy fees/core facilities: Scanning electron, light, confocal microscopes-hourly instrument fees at CBS Biological Imaging Facility $25-37 per hour plus specimen preparation fees, ~20 hours per year	$2,500	$0	$2,500							$2,500	$2,500
DNA sequencing and supplie: DNA amplification reagents and consumables, DNA cleanup kits (for ~ 500 strains)				$3,000	$0	$3,000				$3,000	$3,000
DNA sequencing (Sanger sequencing): Sequencing for phylogenetic analysis of bacterial and fungal isolates (AGAC sequencing facilities, $3.60 per reaction x ~1000 reactions per year x 3 years)				$10,968	$0	$10,968				$10,968	$10,968
DNA sequencing (MiSeq Illumina sequencing) DNA library preparation and amplification services (10.95 x ~ 150 samples per run), MiSeq sequencing paired-end single lane, 300 cycles ($1,968 per lane) x 3 runs over 3 years.				$11,000	$0	$11,000				$11,000	$11,000

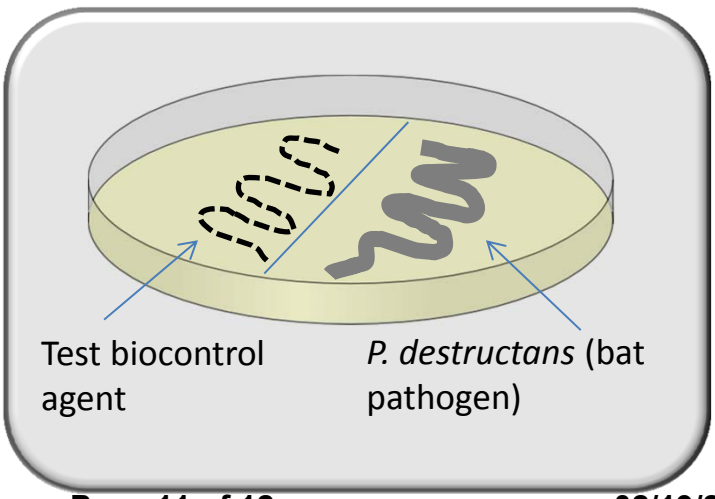
Bioassay supplies: Reagents, compounds and consumables (microbiology supplies, antibiotics, plasticware) for biological testing, general lab supplies, glassware. For 0.5 FTE scientists over 2 years							$10,000	$0	$10,000	$10,000	$10,000
Instrument and core facilty fees for chemical analysis: Fees for core facilities for chemical analysis of active strains (NMR spectroscopy, gas chromatography, mass spectrometry). Hourly charages of $10-40 per hour or per sample, estimated at $1000 per year x 2 years							$2,000	$0	$2,000	$2,000	$2,000
Travel expenses in Minnesota											
Travel between St.Paul and: Soudan Mine (444 miles round trip), Mystery Cave (240 miles) at $.52 per mile. Metro area cave trips (~10-20 miles round trip). Lodging for 2-4 researchers for 1-2 days per trip, plus meals. Estimated 2 trips to Soudan, 2 trips to Mystery Cave and 3-4 trips to Metro area caves per year x 3 years.	$16,500	$0	$16,500							$16,500	$16,500
Other											
publication fees ($500 per manuscript for open access publication x 3)	$500	$0	$500	$500	$0	$500	$500	$0	$500	$1,500	$1,500
Repair for instruments such as vacuum pumps, water baths, incubators, shakers, etc. and replacement of glassware/components due to inevitable breakage. Estimated at $1000 per year x 3 years.	$1,000	$0	$1,000	$1,000	$0	$1,000	$1,001	$0	$1,001	$3,001	$3,001
COLUMN TOTAL	**$222,209**	**$0**	**$222,209**	**$130,922**	**$0**	**$130,922**	**$98,869**	**$0**	**$98,869**	**$452,000**	**$452,000**

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?