Final Abstract

Final Report Approved on June 26, 2025

M.L. 2020 Project Abstract

For the Period Ending June 30, 2025

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

Project Manager: Christine Salomon

Affiliation: U of MN - College of Pharmacy

Mailing Address: 312 Church St SE 4-130 NHH

City/State/Zip: Minneapolis, MN 55455

Phone: (612) 626-3698

E-mail: csalomon@umn.edu

Website: https://www.pharmacy.umn.edu/

Funding Source:

Fiscal Year:

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

Appropriation Amount: $440,000

Amount Spent: $404,555

Amount Remaining: $35,445

Sound bite of Project Outcomes and Results

Our project outcomes include the refinement of methods to differentiate between live and dead Pseudogymnoascus destructans spores, the development of a spatial map of environmental persistence of the pathogen throughout three Minnesota hibernacula, and characterization of our best biocontrol fungal candidate on natural cave substrates.

Overall Project Outcome and Results

White-nose syndrome (WNS) is a devastating disease of hibernating bats caused by the fungal pathogen Pseudogymnoascus destructans (Pd) that has significantly impacted populations in Minnesota and throughout the US. Understanding Pd's persistence and viability in hibernation sites is critical to managing its spread and impact. This research focused on refining methods to assess viability of Pd spores, monitoring environmental persistence, and testing potential biocontrol fungi for efficacy on cave substrates.

We applied a new method of spore testing to assess viability of Pd samples. After several modifications to the standard protocol, we were able to detect significant differences in viability of control Pd samples. With some continued optimization, this method will be a useful tool to assess the effectiveness of treatment and management of infectious Pd

in environmental reservoirs.
We monitored Pd in several hibernacula to better understand where the pathogen is most abundant and likely to re-infect surviving/returning bats. We found low levels of pathogen on many substrate areas, in both hibernacula and non-hibernacula locations. Additionally, we sampled air for Pd spores in several areas and found low levels in two locations in Mystery Cave and the Soudan Iron Mine. We also found that after disinfecting entry surfaces that little Pd was further deposited onto them from the surviving population, and are likely not a source of reinfection for returning bats. However, the low but regular occurrence of P. destructans on both cave surfaces and in the air throughout both hibernacula suggests that mitigating pathogen levels broadly may be challenging.

Application of our biocontrol strain and extracts onto cave substrates with Pd showed promising inhibition at the direct application areas. These results suggest that the development of native, inhibitory microbes collected in hibernacula may be an effective approach towards developing nontoxic treatments and mitigation strategies for Pd.

Project Results Use and Dissemination

Our results have been shared with park managers and DNR researchers to keep them informed of the levels of Pd to add to bat monitoring data. We also participated in several outreach opportunities and scientific conferences to share our research with the public and scientific communities, including the Minnesota bat festival, Women in Natural Resources events with the DNR, President's Emerging Scholars training program (UMN), the Minnesota Wildlife Society Annual Meeting, American Society of Pharmacognosy, and several invited seminars at other institutions including the University of Florida and University of North Carolina. We also developed educational signage for sensitive caves.

Environment and Natural Resources Trust Fund

M.L. 2020 Approved Final Report

General Information

Date: November 12, 2025

ID Number: 2020-004

Staff Lead: Michael Varien

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

Project Budget: $440,000

Project Manager Information

Name: Christine Salomon

Organization: U of MN - College of Pharmacy

Office Telephone: (612) 626-3698

Email: csalomon@umn.edu

Web Address: https://www.pharmacy.umn.edu/

Project Reporting

Final Report Approved: June 26, 2025

Reporting Status: Project Completed

Date of Last Action: June 26, 2025

Project Completion: November 30, 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? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & 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	Approximate 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	Approximate 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	Approximate 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 work 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	$ Amount Spent	$ Amount Remaining
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**	**$342,025**	**$27,740**
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	$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	$200	$2,800
	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	$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	$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	$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	$12,000	-
							Sub Total	**$61,235**	**$58,435**	**$2,800**
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.	For collection of samples from caves/mines throughout Minnesota, all activities x 3 yrs					$4,000	$2,659	$1,341

		5-6 trips/yr (1-3 days each trip) for 3 yrs								
							Sub Total	**$4,000**	**$2,659**	**$1,341**
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	$108	$1,892
							Sub Total	**$2,000**	**$108**	**$1,892**
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	$1,328	$1,672
							Sub Total	**$3,000**	**$1,328**	**$1,672**
							Grand Total	**$440,000**	**$404,555**	**$35,445**

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	$ Amount Spent	$ Amount Remaining
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...

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
ENTRF Background Check form	dcd15cf1-ef9.pdf
Figures 1-3 for Research Report October 2022	8beeb9e8-f34.pdf
Educational signage for sensitive cave	813b35b3-0a1.jpe
education sign at cave entrance	d8e93a40-0d5.jpe
MN bat festival 2024	db3872b3-346.jpe
Mystery Cave outreach event 2024	79f93a08-efc.jpe
ENTRF acknowledgement slide in all presentations	5038eaa6-7b4.png
Wandering Naturalist Podcast WNS and MN bats-link in document	39a17036-942.docx
Scientific manuscript on anti-Pd compounds from cave fungi	ad15f3f7-994.pdf

Media Links

Title	Link
Episode-217-intriguing-bats-of-minnesota-white-nose-syndrome	https://thewanderingnaturalist.libsyn.com/episode-217-intriguing-bats-of-minnesota-white-nose-syndrome

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 understand that travel expenses are only approved if they 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 understand the UMN Policy on travel applies.

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

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 (1) the source and estimated amounts of any revenue and (2) how you propose to use those revenues:

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

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Explanation & justification for Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Completion Date	Previous Completion Date: 06/30/2024 New Completion Date: 11/30/2024	This extension is requested because of an unexpected shaft repair in the Soudan Mine and road repair in Mystery Cave this past spring that prevented us from accessing both of these primary field sites for our last set of samples that we needed for genomic sequencing. We also need to conduct some additional sequence analysis from these samples which will not be available until after the project end date.	June 28, 2024	Yes	July 2, 2024

Status Update Reporting

Final Status Update January 14, 2025

Date Submitted: April 30, 2025

Date Approved: May 5, 2025

Overall Update

White-nose syndrome, caused by the fungal pathogen Pseudogymnoascus destructans (Pd), has significantly impacted bat populations in North America. Understanding Pd's presence and viability in hibernation sites is critical to managing its spread and impact. This research focused on refining methods to differentiate between live and dead Pd spores, improving detection sensitivity, monitoring environmental persistence, and testing biocontrol strains and extracts for efficacy on cave substrates.

To achieve these goals, we tested and optimized a method to distinguish between live and dead Pd spores using propidium monoazide (PMA) in combination with qPCR. Additionally, we assessed Pd presence in bat hibernacula through surface swabs and air sampling, while also evaluating alternative techniques for differentiating live and dead spores. Various concentrations of PMA (6-75 µM) were tested to determine the most effective treatment for differentiating live and dead Pd spores. Additives such as bovine serum albumin (BSA) and Tween 80 were introduced to stabilize live cells and improve assay accuracy. Alternative viability assays using ethidium monoazide bromide (EMA) and DNase treatment were also tested. Our biocontrol experiments on natural substrates showed efficacy using both a live fungal biocontrol as well as with fungal extracts applied to surfaces.

Activity 1

We cultured Pd on natural substrates from each of the hibernacula to test for the ability of the fungal biocontrol strain and fungal extracts to inhibit Pd. Both the live biocontrol and the extracts clearly inhibit Pd growth, but the effects were limited to the exact locations where the treatments had been applied, and did not spread beyond these areas. Although this result is disappointing, we can envision testing other methods of treatment dispersal to apply a more homogeneous inoculant or biocide, such as using aerosols or nebulizers.

Throughout our monitoring, we have focused on developing methods to test for viable Pd, rather than just the presence or absence of Pd DNA. Our results demonstrated that PMA treatment successfully reduced DNA amplification from dead Pd spores, however, we've faced challenges because PMA sometimes affects live cells, leading to unexpectedly high Cq values in those live cells. This complicates the accurate differentiation between live and dead cells, making the optimization of the PMA protocol essential. Adjustments, including additives and staining time, improved differentiation between live/dead spores. Alternative methods (EMA and DNase) did not provide sufficient differentiation. Next steps should involve continued optimization of the PMA assay to improve its accuracy.
(This activity marked as complete as of this status update)

Activity 2

We continued monitoring P. destructans in several hibernacula to better understand where the pathogen is most abundant and likely to re-infect surviving/returning bats. We found that there is a low level of P. destructans fungus on many substrate areas of Mystery Cave and the Soudan Iron Mine, in both areas with hibernating populations before the numbers dropped dramatically, as well as areas not known to harbor bats. Additionally, we sampled air for P. destructans spores in several areas and found low levels in two locations in Mystery Cave, and on two levels of the Soudan Iron Mine. Our previous studies identified high levels of pathogen near the entrance of Mystery Cave II, with especially high levels at “pinchpoints” of doors where bats fly through as they enter and exit the cave. We found that after disinfecting the door surfaces that little P. destructans was further deposited onto them from the surviving

population, and are likely not a source of reinfection for returning bats. However, the low but regular occurrence of P. destructans on both cave surfaces and in the air throughout both hibernacula suggests that mitigating pathogen levels broadly may be challenging.
(This activity marked as complete as of this status update)

Activity 3
Because the population of tricolor bats in the local Twin cities sandstone cave has not declined as precipitously as the little brown bats in other major hibernacula (~80% versus >95%), we have continued to monitor the cave walls, ceiling and floors for Pd. The frequency and distribution of Pd throughout this cave has remained at a consistent and relatively low, but detectable level. During the last bat survey (winter 2024/25), none of the remaining tricolor bats showed any obvious symptoms of WNS (although the bats were not swabbed for direct Pd detection). It is not yet clear if the surviving bats are more resistant to infection, are more able to survive infection, or if some other characteristic of this cave provides some protection. We hypothesized that one protective feature could be the frequent unsanctioned visitation of the cave by local explorers that might result in an increased temperature (from body heat and candles), as well as disruption of torpor. Measurement of temperatures in five locations over six months indicated that there was no appreciable change in temperature due to human presence. It is not yet known if more frequent torpor disruptions might result in lower levels of infection.
(This activity marked as complete as of this status update)

Dissemination
Christine Salomon gave a presentation on our biocontrol research at the Minnesota Wildlife Society Annual Meeting in St. Cloud, MN on Feb 19, 2025. She also presented this research in a lecture for a Grand Challenges class on Antibiotic Resistance (Feb 26, 2025)

Status Update Reporting

Status Update October 1, 2024

Date Submitted: April 30, 2025

Date Approved: May 5, 2025

Overall Update

We have continued to monitor the P. destructans pathogen on hibernacula surfaces and have added additional monitoring of P. destructans conidia in cave air using long term air samplers. We can detect P. destructans throughout WNS positive caves and mines and we have also confirmed that they are present in the air in both bat hibernacula areas as well as areas not known to harbor bats, suggesting a widespread, low level distribution P. destructans in hibernacula. We have also developed a useful assay to test both biocontrol strains and biocontrol extracts on natural rock substrates from the three major hibernacula cave types found in Minnesota. Visual inspection of the initial assays suggest good inhibition of the pathogen using the live biocontrols, and additional molecular quantification is in progress. Additionally, we have observed some promising results using the biocontrol extracts, instead of live fungi, which may be a more controllable and desirable method for pathogen suppression on substrates.

Activity 1

The combination of results from the latest set of experiments have demonstrated that the candidate biocontrol fungus Oidiodendron truncatum fungus is a specific inhibitor of the bat pathogen P. destructans, is non-toxic to bat skin cells, and does not have a significant effect on the four most common non-pathogenic fungi isolated from the Soudan Iron Mine. We therefore focused on developing tests on natural rock substrates from three Minnesota hibernacula (Soudan Irone Mine, Mystery Cave, and a sandstone cave in the Twin Cities). We developed conditions to grow P. destructans on UV-disinfected rock slabs, and then applied spores of the biocontrol strain to the growing pathogen. After allowing both strains to grow for two additional weeks, all microbial growth was removed from the rocks using enzymes and detergents, and the viability dye PMA was added to the cells. This was also repeated using extracts from the biocontrol strain. Visual inspection of the infected rocks treated with the biocontrol show strong growth of Oidiodendron, and we are in the process of quantifying the amount and level of viability of P. destructans that remained after treatment.

Activity 2

We are continuing to quantify the Pd pathogen in hibernacula and non-hibernacula areas within the same caves. Additionally, we are using air samplers (spore traps) to assess the quantity of airborne Pd conidia. Substrate swabs were also collected in hibernacula and non-hibernacula areas in February in addition to the placement of two air samplers in hibernacula during a four week period in Feb-March. The air samplers were placed in levels 10 and 12 where bats are known to hibernate and collected air samples for 6 minutes every hour. Very few bats were present at the time of placement. The attached graph shows the results of the qPCR assay which shows Pd present in the air on the 10 and 12 levels. In addition, Pd was detected from substrates from level 10. The presence of Pd in hibernacula show that these areas may remain infectious to bats that hibernate in these areas. However, the quantity detected is very small, approximately a spore or less (detection can be less than a spore because there are many copies of the gene region in the contents of a Pd spore). These areas should continually be monitored for changes of the spore load of Pd.

Activity 3

Because one of the P. destructans-positive sandstone caves in the Twin Cities had a small, healthy population (25) of tricolor bats for several years longer than the largest hibernacula (Soudan Mine and Mystery Cave), we hypothesized that there could be some unique microbial or geochemical features that were protecting the bats there. However, we observed that this population did eventually start to decline, and this year was reduced to just 5 individuals. Although these remaining bats have no visible signs of WNS, we do continue to detect P. destructans throughout the cave on walls

and ceiling surfaces.Given the limited size of this cave, and small bat population, this may be an ideal location for the first field testing of our biocontrols and biopesticides.

Dissemination

Christine Salomon presented the WNS project during a Chemistry departmental seminar at the University of North Carolina in Wilmington in August. She also helped co-organize the Minnesota Bat Festival held at the Minnesota Valley Wildlife Refuge (10/26/24), gave a public lecture on WNS research, and hosted a research table with her lab during the event.

Status Update Reporting

Status Update April 1, 2024

Date Submitted: April 30, 2025

Date Approved: May 5, 2025

Overall Update

Our goals for this project are to develop tools and data about the White Nose Syndrome fungal pathogen in caves and mines in Minnesota and to test our best biocontrols in ways that mimic natural cave surfaces. We have completed the testing needed to set up the final experiments of biocontrols and extracts on actual cave and mine rock samples. This will allow us to most closely mimic the application of the biocontrols (or biopesticides) on cave walls in a controlled setting. Another accomplishment is that we have developed a reproducible new method to quantify live versus dead pathogen in laboratory samples, which is an important step in quantifying the effectiveness of our treatments. So far, we have completed these viability studies on straightforward laboratory cultures, and will try to apply this method to natural rock substrates and on environmental samples. We are also preparing to conduct a final sample transect survey of P. destructans to map Pd in Mystery Cave and the Soudan Iron Mine. However, due to road construction (Mystery Cave) and shaft maintenance (Soudan), we will not be able to access these sites until late May/June, and we requested an extension for the final report.

Activity 1

One of our priorities is to identify biocontrol strains that are effective at killing or stopping the growth of the bat pathogen (P. destructans) but that are not toxic towards bats (or other animals) and that do not indiscriminately kill native microbial communities. We have previously focused on testing the top biocontrol strains against non-pathogenic common fungi found in the Soudan Mine, and identified five that specifically inhibit the pathogen. We have also isolated common bacteria from the three hibernacula (Soudan Mine, Mystery Cave and Heinrich Brewery cave) to test for potential anti-bacterial activities as an additional measure of specificity. Additionally, we have started biocontrol inoculation experiments on non-sterile rocks to also assess potential effects on the natural microbial communities.

Activity 2

Quantification of viable P. destructans

This primary objective of this activity is to develop and optimize a chemical tool that we can use to differentiate between live versus dead P. destructans pathogen in the environment. This will be essential for analyzing the results of any treatments we employ in the field, because both dead and live pathogen will yield very similar DNA and it is impossible to determine if a biocontrol or biopesticide was successful at reducing the pathogen reservoir.

Experiments continued focusing on optimization of the live/dead assay using propidium monazide (PMA) to differentiate between live and dead Pd spores in samples. Due to some results in which PMA was having an effect on the amount of DNA measured in llive cells, further titration of PMA concentration (6, 12, 25, 50, and 75 µM) was trialed. Control samples of live cells treated with PMA had higher cq values than untreated control live samples. This would have confounding effects on an analysis between live and dead cells. Upon consultation with the manufacturer, alternative methods involving additives to the buffer will be tested in order to obtain consistent results from this assay.

Activity 3

One of our goals is to replicate the antagonism of biocontrols against Pd that we observe on agar plates with similar systems using natural cave substrates. This is especially challenging because natural substrates can either be sterilized (reducing microbial complexity but removes all associated nutrients required for growth) or left colonized with native microbes (which are very complex to analyze, quantify and study). We have been conducting additional testing of the ability of both P. destructans and the biocontrol Oidiodendron truncatum to grow on sterile and non-sterile natural

substrates from the three hibernacula environments, with and without additional nutrients. These substrate/antagonism experiments will be analyzed using quantitative PCR and with and without additional viability dye treatments. Some early promising results show that the established P. destructans colonies are completely covered by the biocontrol strain using visual (microscopy) observation. An important question to address is whether the original P. destructans mycelia and conidia are killed or simply inhibited but still alive and viable.

Dissemination

Christine Salomon was a co-organizer for the Minnesota Bat Festival (October 28, 2023) held at the Minnesota Valley Wildlife Refuge which drew more than 1500 attendees. Dr. Salomon also gave a presentation on white nose syndrome in bats to the general public and hosted a research table during the festival event. Dr. Salomon presented research talks on WNS in bats at an international Biotechnology Symposium at the University of Tokyo (October 2023), The Whitney Institute and University of Florida (January, 2024), St. Catherine University in St. Paul (February 2024), and Hamline University in St. Paul (February, 2024).

Status Update Reporting

Status Update October 1, 2023

Date Submitted: December 8, 2023

Date Approved: January 19, 2024

Overall Update

Our primary goals for this project are to develop tools and data about the White Nose Syndrome fungal pathogen in caves and mines in Minnesota and to test our best biocontrols in ways that mimic natural cave surfaces. We are on track to complete most of our objectives focused on sampling and testing of quantification methods, as well as developing systems to culture the pathogen on natural cave substrates (ie. rocks) for environmental testing. We have also successfully determined which biocontrol candidates are the least harmful to native fungi found in caves, which allowed us to re-prioritize the top strains to continue testing. We now have 5 active biocontrols that meet all of our requirements so far (not toxic to mammalian cells or native fungi, stops the fungal pathogen from growing), and need to test them against native bacterial species. We are not yet ready to start any field experiments with either biocontrol strains or extracts (biopesticides) because we have not yet fully optimized our tools for measuring these experiments. The primary challenge we have faced is difficulty in optimizing a chemical tool (PMA) to quantify DNA from live versus dead pathogen.

Activity 1

One of our priorities is to identify biocontrol strains that are effective at killing or stopping the growth of the bat pathogen (P. destructans) but that are not toxic towards bats (or other animals) and that do not indiscriminately kill native microbial communities. We have prioritized our top five biocontrol fungal strains based on their activity against the bat pathogen (P. destructans) as well as their lack of toxicity towards mammalian cells and against several diverse representatives of native fungi. Four of our strains are good at killing P. destructans, but also killed most or all of the other native fungi, so were de-prioritized. We also tested the top five biocontrol strains against P. destructans on several different medias to compare growth, and found that they differed in growth rate and effectiveness under some conditions. These results will have implications for how we consider testing them in caves on natural rock substrates.

Activity 2

Quantification of viable P. destructans

This primary objective of this activity is to develop and optimize a chemical tool that we can use to differentiate between live versus dead P. destructans pathogen in the environment. This will be essential for analyzing the results of any treatments we employ in the field, because both dead and live pathogen will yield very similar DNA and it is impossible to determine if a biocontrol or biopesticide was successful at reducing the pathogen reservoir. We are starting to see some success with the chemical reagent (a PCR dye called PMA), which shows some differences in quantification experiments of DNA from live versus dead spores. We are still working to optimize conditions to expand the difference so that we can use this as a tool in field and lab experiments. Consultation with the manufacturer of the reagent has provided us with some additional conditions to test.

Activity 3

One of our goals is to replicate the antagonism of biocontrols against Pd that we observe on agar plates with similar systems using natural cave substrates. This is especially challenging because natural substrates can either be sterilized (reducing microbial complexity but removes all associated nutrients required for growth) or left colonized with native microbes (which are very complex to analyze, quantify and study). We have been testing the ability of Pd to grow on sterilized, natural substrates from the Heinrich Brewery Cave to develop a simple antagonism assay with candidate biocontrols. We inoculated Pd with different broth agar medias and found that SD broth but not SD agar allows growth

on Heinrich rock slabs. Once we have optimized this growth we will introduce the biocontrol strains to quantify antagonism.

Dissemination

Christine Salomon presented research updates including the WNS project at the UMN Biotechnology Institute seminar series (March 2023) and the UMN Microbiology Club (March 2023).

Status Update Reporting

Status Update April 1, 2023

Date Submitted: April 21, 2023

Date Approved: May 15, 2023

Overall Update

As part of our overall goal of developing a microbial biocontrol strategy, we are focusing on quantifying the amounts of infectious (live) Pseudogymnoascus destructans fungus on surfaces in hibernacula. In addition to identifying the surfaces with the highest levels of pathogens and greatest need for disinfection/treatment, we would like to correlate the seasonal deposition rate of fungal conidia with the number of bats that fly over surfaces. In this reporting period, we installed electronic bat counters and trail cameras to quantify the numbers of bats entering and exiting 3 caves in Mystery Cave State Park, but were stymied by rodents chewing through the power cables for the instruments. We plan to repair and armor the cables before redeployment. Additionally, we have completed the chemical and activity characterization of one of our nine top fungal biocontrol strains and are writing this up as a manuscript for publication.

Activity 1

We are also still working on characterizing and testing the top biocontrol strains that we have previously identified and are focused on writing the manuscript on the chemistry and activity from an Illyonectria sp. fungus collected from the Soudan Iron mine that showed good inhibition of P. destructans on solid media. We have shared in previous progress reports the structures and identities of the anti-fungal and non-active compounds identified as well as the levels of cytotoxicity towards two species of bat fibroblast (skin) cells. This strain showed intermediate levels of specificity towards the most common, culturable non-pathogenic fungi found in the Soudan Iron Mine and could still be a viable biocontrol or source of biopesticides.

Activity 2

We applied for a small equipment grant from USFW for three battery powered bat detectors which use infrared beam-break technology to count animals that pass through the frame. The counters were installed at three locations at Mystery Cave State park in September: the main entrances to Mystery I and II, and a ground level entrance for Mystery II (Figure 1). Before installing, we obtained swab samples from each location to quantify the amount of P. destructans on surfaces, and then disinfected the door sill surfaces so that we can quantify new depositions of P. destructans on our next sampling trip.

We returned to the sites in January 2023 to retrieve the data from the counters. Although there is some bat count data for this time period, we found that 2 of the 3 counters had wires chewed through and found some moisture infiltration into two of the units. We are currently trying to repair the devices and hope to reinstall the counters soon. We also installed infrared trail cameras with motion detectors in front of each cave entrance, and will analyze this data once we return to collect the data cards in the spring.

Activity 3

We have tested several samples of rock and sediment materials collected from the Heinrich Brewery Cave against P. destructans to test the hypothesis that chemical or mineral components of hibernacula might inhibit or slow the growth of P. destructans. From previous field expeditions and consultations with geologists, we observed a strong sulfur smell in some sections of the cave, and there are areas with prominent elemental sulfur deposits. However, using a simple growth assay with P. destructans plated next to various substrates indicated that none of the tested materials inhibited growth (Figure 2). One of the substrate assays showed increased growth of P. destructans and lack of melanin production (a stress response) in the presence of autoclaved (sterilized) sand from the cave. Non-sterilized sand did

result in some inhibition due to microbial growth, but it is not clear if this is due to chemical inhibition or nutrient competition.

Dissemination

Salomon presented research seminars on this WNS biocontrol project for the UMN Plant Microbiology Colloquium (Dec 2022), the Biotechnology faculty seminar series (March 2023), the Mycological Society of Minnesota meeting (March 2023), and the UMN student Microbiology Club (March 2023).

Status Update Reporting

Status Update October 1, 2022

Date Submitted: November 3, 2022

Date Approved: November 7, 2022

Overall Update

We have focused our efforts on developing and optimizing new methods to quantify live (viable) versus dead (non-viable) P. destructans from environmental samples. Experiments focused on the optimization of the live/dead assay using propidium monazide (PMA) continued. This assay has been used in other bacterial and fungal systems has the benefit of discriminating between live and dead cells as screen using qPCR. However, optimization is necessary for each specific use of PMA on a given organism. We have made some improvements and will continue to optimize and test the system moving forward. We also completed the anti-fungal specificity experiments for the top biocontrol agents. Additionally, we completed a long term study of viability of P. destructans conidia (spores) on sterile cave substrates as a necessary foundation for later testing of top biocontrol strains on natural substrates. Figures 1-3 are shown in the attachment.

Activity 1

Biocontrol anti-fungal specificity

We have completed the replicates that were previously missing in our anti-fungal challenge experiments using the top four most common fungi found in the Soudan Iron Mine. The complete data table is shown below (Figure 3) and shows that four of the biocontrol strains have high specificity towards P. destructans and are not active towards the four tested strains.

This specificity data together with the cytotoxicity information of pure compounds from these strains against bat fibroblast cells allows us to prioritize the most promising and least toxic strains. (indicated by green columns in figure 3). Although these strains may serve as living biocontrol agents for substrates, they may also provide a source of highly specific biopesticides. We will be exploring this application in the next phase of research.

Please see attachment for figure 1.

Activity 2

We focused on comparing concentrations of PMA when treating cells in addition to using different methods to kill cells (heat and UV light). A 50/50 mixture of live/dead cells was also tried to test the sensitivity of the assay (Fig 2). Several different extraction methods were also tested in preparation for sample processing.

Results show 50-75 µM PMA treatments are similarly effective (Fig. 3). There were also confounding results with the control live samples treated with PMA sometimes showing an increase in Cq value compared to the non PMA treated live cells. This could indicate that PMA may be affecting live cells in addition to dead cells in some instances. We are currently in the process of finding a solution by further titration of PMA concentration and/or adding stabilizing compounds to the buffer solution. The comparison of extraction methods showed that the Prepman Ultra extraction product, a streamlined and more economical method, was superior to the Qiagen column kit. This will save time and reduce costs for extractions of samples in preparation for qPCR. The mixture of 50/50 live and dead cells showed a slight reduction in Cq value.

Activity 3

We have completed a study of conidia viability on sterile natural cave substrates from a local Twin Cities cave. Slabs of slate from the cave were boiled to disinfect, then cut into small 1 x 0.5 x 0.3 cm coupons and disinfected again. P. destructans conidia (1000) were pipetted onto each coupon which was then incubated in a sterile petri dish at 15

degrees celsius with 90-100% humidity for 6 months. Slabs were dried, preserved and treated for electron microscopy, and observed using a scanning electron microscope. No germination of any spores was observed, and the original conidia remained intact. Although we do not know if these conidia retained their viability after the experimental period, these results suggest that this system using sterile substrate will not be appropriate for testing biocontrol candidates with P. destructans.

Dissemination

Christine Salomon presented this research to the University of Minnesota President Emerging Scholars Program (August, 2022) and at the University of Illinois, Chicago for a seminar series. Manuscripts are in progress for publishing the structures and activities of compounds from two of the biocontrol strains.

Status Update Reporting

Status Update April 1, 2022

Date Submitted: April 27, 2022

Date Approved: May 24, 2022

Overall Update

The current protocol for detecting Pseudogymnoascus destructans (Pd) in environmental samples is very sensitive, enabling detection down to DNA in less than a single spore. However, this detection method cannot differentiate between DNA that originates from live or dead fungal material, which can have implications towards interpretations of qPCR results on the epidemiology of the pathogen and how it survives in the environment as well as the effectiveness of biological control treatments. To address this limitation, we have begun testing and optimizing a method using a viability dye call PMA (propidium monoazide). Our preliminary results show some differentiation between live and heat killed P. destructans conidia, but additional optimization is ongoing.

We are interested in understanding the specificity of antifungal activity for the top biocontrol candidate strains. An ideal biocontrol treatment will specifically kill P. destructans, but not inhibit the growth of native microbial communities. We used data from our recent fungal inventory study to identify the four most common fungi found throughout the Soudan Iron Mine and tested each biocontrol candidate against each of these strains. Five of the nine biocontrol strains showed very little or no inhibition of the environmental fungal strains, demonstrating a high level of specificity.

Activity 1

The anti-fungal specificity of the most promising biocontrol strains (Oidiodendron truncatum, Ilyonectria radicicola, Trichoderma oblongispora, Helicostylum sp, Trichoderma atroviride, Codophora melanii, Hypocrea pachybasiodes, Mortierlla minotissima and Mucor luteus) was tested using a side by side competition plug assay. (Wilson et al. PLoSONE, 2017, 12(6): e0178968) Four strains of the most common fungi from the Soudan Iron Mine were chosen from previous studies (Held, et al. PLoSONE, 2020, 15(6): e0234208) (Sistotrema brinkmanii, Postia floriformis, Scytalidium album & Marinnaeae compotospora).

Plugs (~5mm2) of each environmental test fungus taken from the actively growing edge of a colony were placed 1.5 cm away from a plug of the biocontrol candidate strain. Plates were made in duplicate, and incubated at 15 C and examined daily. Initial results suggest that four of the nine strains show significant inhibition of two or more environmental isolates. Five strains show minimal inhibition of the environmental isolates, suggesting a higher level of specificity for inhibition of P. destructans.

Activity 2

Propidium monoazide (PMA), a membrane impermeant dye which selectively penetrates dead cell membranes and cross-links to DNA, which thereby strongly inhibits PCR amplification. Effectively this would mean differentiating between live and dead Pd conidia from swabs or samples suspected of having Pd. While this method has been proven effective on a variety of microbes, validation experiments are needed to optimize using specific organisms, like Pd. We have done initial experiments using PMA on conidia collected from active cultures of Pd and using several live/dead controls in which we intentionally kill spores using UV light and heat. There were promising results in several of the PMA treated samples of killed conidia, where some suppression of amplification of target DNA using qPCR, was observed. However, we are carrying out further experiments hoping to optimize and obtain consistent results across all treated samples and clear amplification suppression of dead Pd spores.

Activity 3

No updates for this activity

Dissemination

Salomon presented lectures on White Nose Syndrome in bats in several classes at the University of Minnesota (GCC 3016: Science and Society: Working Together to Avoid the Antibiotic Resistance Apocalypse and Nature of Life: Biology Saves the World, Feb and March, 2022)