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

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

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

**II. PROJECT ACTIVITIES AND OUTCOMES**

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| **Activity 1:** Testing of most active microbial biocontrol agents with relevant substrates and field studies  **Description:**We have identified >100 microbial strains that inhibit the growth of *P. destructans*. Among these strains, approximately 10 have demonstrated consistent production of antifungal extracts, and we have purified, characterized and identified most of the active compounds. We have also tested these compounds against cultured fibroblast (skin) cells from two species of bats (Northern long eared and Gray bats) to assess their toxicity. The strains that produce the most antifungal but least toxic compounds were prioritized, and these will be inoculated onto natural substrates from the three major hibernacula locations together with *P. destructans.* Once these experiments are analyzed, successful trials will be translated into small-scale field experiments in collaboration with the DNR. These experiments will be focused first in human-made hibernacula (mines) and smaller sandstone caves*. P. destructans* and antagonist growth will be assessed using microscopy and a sensitive qPCR DNA quantification method.  **ENRTF BUDGET: $149,522** |  |

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

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| **Activity 2:** Quantification of *P. destructans* in hibernacula: Seasonal and spatial dynamics  **Description:** Bats afflicted with WNS change their hibernation patterns within caves, and will often move towards the entrance of caves. However, little is known about the extent of the environmental reservoirs of *P. destructans* on substrates in these different locations over time. We will map the occurrence and quantity of *P. destructans* along transects of hibernacula at twice per year from substrate locations (walls, sediments, ceiling) to better understand the spatial and seasonal dynamics of *P. destructans* growth and potential spread. This information will be especially helpful to cave managers for focusing treatments or interventions. For example, the top of one door at the entrance of Mystery Cave in Forestville was found to have 3000x more *P. destructans* DNA than areas much deeper in the cave. These “pinch points” for bat entry/exit might be an obvious place for reinfection when bats return in the fall, and could be specifically disinfected. Our regular sampling and quantification before and after any treatments will also provide empirical data to assess management strategies.  **ENRTF BUDGET: $128,590** |  |

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

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| **Activity 3:** Assessment of WNS-free cave  **Description:** We identified a cave (not named here to minimize potential disturbance) with a healthy population of tricolor bats (*Perimyotis subflavus*) and no signs of WNS (as of 02/2019) with animal numbers consistent with pre-WNS census data. A preliminary analysis of a small number of substrates were all negative, suggesting that *P. destructans* is not present or not abundant in sampled areas. This is surprising and unexpected due to the widespread occurrence of WNS in all other Minnesota hibernacula. We propose to conduct a more thorough mapping of *P. destructans* throughout this cave (see activity 2) and from bats over the next three years, and methodically test a number of different possible factors that might be responsible for the absence of WNS. Various substrates (rocks, water, sediment, etc.) will be tested for their ability to host (or inhibit) *P. destructans* growth, and volatile air samples may be collected and tested. Concurrently, bacterial and fungal samples will be collected and tested for *P. destructans* inhibition. If the substrate or volatile materials show significant activity, the microbial experiments will be minimized. The results of these experiments should provide information about a naturally disease suppressive environment which will inform management strategies.  **ENRTF BUDGET: $166,524** |  |

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

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

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