

### **Environment and Natural Resources Trust Fund**

M.L. 2024 Approved Work Plan

#### **General Information**

**ID Number: 2024-198** 

Staff Lead: Michael Varien

Date this document submitted to LCCMR: June 5, 2024

**Project Title:** Early Detection of Invasive Viruses in Native Pollinators

Project Budget: \$200,000

#### **Project Manager Information**

Name: Declan Schroeder

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#### **Project Reporting**

Date Work Plan Approved by LCCMR: June 20, 2024

**Reporting Schedule:** June 1 / December 1 of each year.

Project Completion: June 30, 2026

Final Report Due Date: August 14, 2026

# **Legal Information**

Legal Citation: M.L. 2024, Chp. 83, Sec. 2, Subd. 06d

**Appropriation Language:** \$200,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to create a baseline inventory of the quantity and diversity of viruses in, and determine the threat of these

viruses to, Minnesota native bees.

Appropriation End Date: June 30, 2027

#### **Narrative**

**Project Summary:** Forewarned is Forearmed: Our goal is to protect the newly described MN DNR native bees from invasive virus-derived diseases and population declines.

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

There are thousands of insect pollinator species in Minnesota, including over 500 species of native bees. Bees are the most efficient pollinators because their bodies are designed to collect and store pollen to feed to their young. Pollinators are integral parts of functioning environments. The plants they pollinate provide food and habitat for animals, buffer waterways, and store carbon. Without pollinators, we would not have many nutritious fruits and vegetables. Pollinator conservation provides economic benefits through improved crop pollination, and intrinsic value in beautiful, flowering landscapes. Numerous pollinators are in decline with disease being a potential leading cause. However, we have little to no information on the viruses present in our native bees. Through an ongoing ENRTF 2021-309 grant entitled Bee Minnesota – Protect our Native Bumblebees, we were the first to uncover at least two new invasive viruses not previously known to infect bumblebees, mainly Bombus impatiens. How many other invasive bee viruses are we missing? Without knowing the viral landscape, we cannot be prepared to mitigate their impact on our bees and thus pollination services.

# 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 collect baseline data of virus in Minnesota bee populations, conduct a threat analysis, and develop a pathogen list to determine whether neutralizing protocols should be in acted against invasive virus bee pathogens. Our first objective is to target five main native bee pollinator families, namely Andrenidae, Apidae, Colletidae, Halictidae and Megachilidae. These were selected because 1) these bees are on the MN DNR native species list, 2) they are relatively common and we are confident that we can collect the 20-25 species within these families from the funded study locations. Our second objective is to use the NGS Oxford Nanopore Technologies metagenome sequencing platform to describe the RNA virus diversity in native populations in Minnesota. We will create virus distribution lists as it relates to species & family, ecological province, abundance of pollinator and their life history traits Finally, we will carry out a threat analysis (hazard vs risk) on the potential and therefore invasive threat these native bee viruses pose. This will provide new data for a hazard vs risk threat analysis on whether viruses pose a real and present danger to the health and longevity of bee pollinators in Minnesota

# 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?

This project complements the ongoing research currently funded by ENRTF. The importance of identifying and profiling the true viral landscape in our bee pollinators cannot be overstated. A project outcome is a pathogen list. The data generated in this project will create a new body of work that lists the potential viral pathogens damaging native bee pollinator communities in Minnesota. This will provide natural resource managers the information needed to develop and implement solutions to better protect Minnesota's native bee populations from invasive virus-derived diseases and population declines. Project outcomes and updates will be posted on the Bee Minnesota website.

## **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

#### **Activities and Milestones**

#### Activity 1: Sampling, cataloging and extraction of viruses from sentinel native MN bees

**Activity Budget: \$71,700** 

#### **Activity Description:**

Dr Cariveau has secured funding from USDA and US Fish and Wildlife Service, to collect native bees across Minnesota from new study sites. We will target five main families, namely Andrenidae, Apidae, Colletidae, Halictidae and Megachilidae. These were selected because 1) these bees are on the MN DNR native species list, 2) they are relatively common and we are confident that we can collect the 20-25 species within these families from the funded study locations, 3) spans five of the six bee families in Minnesota (the sixth family is too rare, there are only a total of seven bee families globally and one is relegated to Australia), 4) includes a mix of important life-history traits such as social vs. solitary and ground nesting vs. stem nesting and 5) we can reliably identify them quickly. Funding from ENRTF will be used to transfer samples into the correct containers and to transfer them to the Schroeder Virology Lab, where we will use a new virus extraction workflow, first developed in the ENRTF 2021-309 Bee Minnesota grant, to extract any resident virus from the bees.

#### **Activity Milestones:**

Description	Approximate	
	Completion Date	
Collection of MN native bees for only this project	April 30, 2025	
Taxonomic identification of native bees collected	May 31, 2025	
Synthesize an agency report of samples collected	June 30, 2025	
Extraction of viruses from the native bee samples	September 30, 2025	

#### Activity 2: Screening for viruses in native bees by applying genomic sequencing technologies

Activity Budget: \$128,300

#### **Activity Description:**

High through-put DNA/RNA extraction on bee pools per location will be achieved using a commercially available Viral DNA/RNA Isolation Kit. Quantity and quality of nucleic acids extracted will be assessed using a fluorometer. We will apply a custom designed sequencing pipeline to describe the viral metagenomic diversity. Briefly, gDNA/cDNA will be sequenced on the GridION device (Oxford Nanopore Technologies), which allows multiple library preparations to be run simultaneously. The sequence reads will be assembled into contigs using a Minimap2 de novo assembler, which was designed to assemble highly heterogeneous virus populations and is well suited to the computational challenge that the virus quasispecies present. The virus genomes will be visualized in Anvi'o. Outputs include read count tables by sample, virus load and diversity per sample, whole-recombinant and partial viral genomes, and identification of quasispecies. A threat analysis will be undertaken to assess which invasive viruses to target for mitigation or eradication.

#### **Activity Milestones:**

Description	Approximate Completion Date
Assembled virus genomes that includes a list of invasive native bee viruses	June 30, 2026
Quantification of virus load and diversity per sample and location.	June 30, 2026
Threat analysis of invasive viruses found	June 30, 2026
Submit draft manuscript to free and open access online preprint server such as bioRxiv	June 30, 2026

#### 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.

CVM and the Bee Lab manage several active social media channels as well as a website and an electronic newsletter. In addition to using these communication tools to disseminate project data, D. Schroeder and D. Cariveau frequently present to both the public and beekeepers throughout Minnesota and the US. We will also disseminate information using well developed communication channels within the Minnesota Extension system. Where appropriate, communications will acknowledge ENRTF as per 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?

The data generated in this project will create a new body of work that lists the potential viral pathogens damaging native bee pollinator communities in Minnesota. The College of Veterinary Medicine, University of Minnesota, has an active Extension and Outreach program that who will continue to disseminate results after project completion. Drs. Schroeder and Cariveau will publish research findings and present to scientific communities. Funds from this project will build on federal resources being used to pursue these goals, greatly expanding the scope of our efforts

#### Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Bee Minnesota – Protect Our Native Bumblebees	M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 03h	\$650,000

# **Budget Summary**

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineli gible	% Bene fits	# FTE	Class ified Staff?	\$ Amount
Personnel								
Project Manager		Dr Schroeder will project manage and to oversee and implement the molecular screening protocol previously developed in his lab. In addition, he will be directly responsible for all communication for the team			36.8%	0.32		\$71,000
Postdoc or Research Assistant		Responsible for running the sequencing assays for cataloging invasive viruses in native bees			25.7%	1.5		\$102,600
							Sub Total	\$173,600
Contracts and Services								
Contribution to the annual maintenance contract (for 2 years) on the ONT GridION sequencer	Professional or Technical Service Contract	Servicing and maintenance of GridION sequencing machine				-		\$8,000
·							Sub Total	\$8,000
Equipment, Tools, and Supplies								
	Tools and Supplies	Supplies to setup and collection of field samples	For the purchasing of nets, collection tubes and preservation material.					\$1,500
	Tools and Supplies	Virus screening molecular consumables ( Virus extraction, Nucleic acid extraction, molecular grade chemicals, NGS sequencing etc.) for bee samples	Surveying the viral pathogens in bee material collected					\$14,900
							Sub Total	\$16,400
Capital Expenditures								
							Sub Total	-

Acquisitions and						
Stewardship						
					Sub	-
					Total	
Travel In						
Minnesota						
					Sub	-
					Total	
Travel						
Outside						
Minnesota						
					Sub	-
					Total	
Printing and						
Publication						
	Publication	Scientific publication in open access journal	Outreach and publication of results			\$2,000
					Sub	\$2,000
					Total	
Other						
Expenses						
					Sub	-
					Total	
					Grand	\$200,000
					Total	,

# Classified Staff or Generally Ineligible Expenses

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

# Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount
State				
			State Sub	-
			Total	
Non-State				
In-Kind	United States Department of Agriculture (USDA)	Dr Cariveau (Co-PI, 0.1 FTE total) will advise on the sampling design and collecting of native bees (Activity 1) and will assist with data analysis and publication, and with all dissemination of results and outreach.	Secured	\$25,000
In-Kind	United States Department of Agriculture (USDA)	Postdoc time (0.1 FTE total) in Co-PI lab. Collect native bees and species identification	Secured	\$7,300
			Non State	\$32,300
			Sub Total	
			Funds	\$32,300
			Total	

#### **Attachments**

#### **Required Attachments**

#### Visual Component

File: bd5b401d-f1f.docx

#### Alternate Text for Visual Component

The five families of native bees targeted in our proposal is shown. We provide the results from our active project that found a new invasive virus in bumblebees. We the go on to outline the aim and outcome of the project....

#### Supplemental Attachments

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

Title	File
Audit	<u>52bf597b-dbb.pdf</u>
SPA Letter	<u>cd1de974-a51.pdf</u>
Research Addendum revised 2024-198	28360e57-d78.pdf
Updated Budget Research Addendum	ca4bed3f-311.docx
Research Addendum revised 2024-198 accepted	<u>9677b952-8bc.pdf</u>

#### Difference between Proposal and Work Plan

#### Describe changes from Proposal to Work Plan Stage

I made the following changes as per Comment ID:

- 1) I added the submission of an agency report (new milestone) to Activity one and similarly, added a 4th milestone to Activity two for the submission of a preprint to an open access journal server.
- 2) in the Narrative section (project outcomes) we rephrased the final sentence "It is essential that we collect baseline data." to read "A project outcome is a pathogen list."
- 4) in the Narrative section, we removed the reference in the text that mentions almonds.
- 5) the USDA grant will fund the person hours to collect the bees for this study. The bees themselves are unique to this study so its NOT part of the USDA study. As this collection is not part of the USDA study, it's not appropriate to specify the USDA grant in the narrative. We rephrased the Activity two, milestone one heading to read as follows "Collection of MN native bees for only this project"
- 6) The USDA grant is not "contributing" to this award. What it does allow is for Dr Cariveau and his postdoc to advise and provide bees without charge to this grant. The budget has "\$0" amount assigned to their time effort.
- 8) Agreed, we moved the expense to the correct section.
- 9 & 10) In the Narrative, we do state the "problem" that our "proposal seeks to address" in the last sentence: "How many other invasive bee viruses are we missing? Without knowing the viral landscape, we cannot be prepared to mitigate their impact on our bees and thus pollination services." Therefore the ENTRF funds are being using to... Activity One "Sampling, cataloging and extraction of viruses from sentinel native MN bees" and Activity Two "Screening for viruses in native bees by applying genomic sequencing technologies". We respectfully disagree with the statements that suggest that our proposed solution or outcomes to the problem is unclear. The confusion might lie with the assumption that we are going to find a solution to the pathogens discovered. This is a small project that first seeks to identity the players involved in bee declines. Future projects will be proposed to identify solutions. This is a low risk high reward strategy. It's too high risk to request a larger amount of funding to proposed mitigation strategies before parametrizing the true problem.
- 11) Agreed, as per Comment ID one, we now added an agency report plus preprint that will be made publicly available upon submission.

- 12) Budget has been updated
- 14 & 15) No changes required to be made.
- 16) Agreed, moved Co-PI and Co-PI's postdoc to Non-ENRTF section.
- 17 & 20). Agreed. I used the suggested text in the updated Narrative.
- 17 & 21). Agreed, the outcomes text has now been updated.
- 19) I uploaded a revised Research Addendum, because of the suggested changes (comment ID 12) to the Suppl. Attachment link. I could not replace the current version as upload.

### 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?

N/A

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

No

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

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF? N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing \$10,000 or more or large-scale stream or wetland restoration?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care, treatment, education, training, instruction, or recreation to children")?

No