

# Final Abstract

Final Report Approved on November 13, 2024

## M.L. 2020 Project Abstract

For the Period Ending June 30, 2024

**Project Title:** Bee Minnesota – Protect Our Native Bumblebees

**Project Manager:** Declan Schroeder

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**Funding Source:**

**Fiscal Year:**

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 03h

**Appropriation Amount:** \$650,000

**Amount Spent:** \$568,096

**Amount Remaining:** \$81,904

### Sound bite of Project Outcomes and Results

Our goal was to assess if Minnesota native bee pollinators were at risk of disease transmission (pathogen Spillover) from honeybees. We found that honeybees and bumblebees have distinct virus communities and while they do share the same habitat, this has not resulted in widespread transmission and infection in bumblebees.

### Overall Project Outcome and Results

Our goal is to protect native pollinators from risk of disease transmission and population declines. By screening and possibly neutralizing bee pathogens (if needed) we wish to promote best management practices to maintain honeybee health and prevent pathogen spillover into native bee populations. Native bumblebees, *Bombus* spp., are important pollinators of wild flowering plants and crops such as tomatoes and berries, and are appreciated for their beauty. Unfortunately, five of Minnesota's twenty-three species of native bumblebees are considered vulnerable, endangered, or critically endangered. The potential of pathogen spillover from the western honeybee to other insects is well established. New variants should inevitably emerge following a host expansion, yet to our knowledge no study has shown this within this honeybee to bumblebee transmission system. To investigate the outcome of viral spillover, we sequenced the RNA virus communities of sympatric honeybee (n = 389) and common eastern bumblebee, *Bombus*

impatiens (n = 117), over three years. Distinct virus communities (viromes) occurred within each bee species throughout the study duration, with honeybee viruses forming a minor fraction of the bumblebee virome. Viruses shared by both bees shared over 98 % nucleotide identity, and no bumblebee-specific strains of honeybee viruses occurred, as expected if spillover led to a true host expansion involving bumblebee-bumblebee transmission. We conclude that the honeybee viruses, namely deformed wing virus (DWV), black queen cell virus, and sacbrood virus, were present in the bumblebees due to environmental exposure or dead-end spillover, and not spillover host expansion. We also observed that other potential pathogen spillover species such as Nosema was also present in both bee species but the sequence identity could not speciate the type of Nosema present. Therefore, we could not resolve whether Nosema spillover was occurring in Minnesota bees. Finally, the therapeutic anti-DWV agent proved ineffective in our lab based studies.

### **Project Results Use and Dissemination**

Dissemination of our project outcomes have been and will continue to be posted on our Bee Minnesota website (<https://beeminnesota.umn.edu/>).

We prepared a manuscript on the viral spillover aspect of the project, entitled: "Virome compositions indicate that viral spillover is a dead-end between the western honey bee and the common eastern bumblebee" to Communications Biology (Nature journal) for review. The preprint is available here: <https://www.researchsquare.com/article/rs-4802694/v1>

Dr. Schroeder has presented and continues to be invited to speak on the outcomes of this ENTRF funded work at local, regional and national beekeeping meetings.



## Environment and Natural Resources Trust Fund

M.L. 2020 Approved Final Report

### General Information

**Date:** November 22, 2024

**ID Number:** 2020-003

**Staff Lead:** Michael Varien

**Project Title:** Bee Minnesota – Protect Our Native Bumblebees

**Project Budget:** \$650,000

### Project Manager Information

**Name:** Declan Schroeder

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### Project Reporting

**Final Report Approved:** November 13, 2024

**Reporting Status:** Project Completed

**Date of Last Action:** November 13, 2024

**Project Completion:** June 30, 2024

### Legal Information

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 03h

**Appropriation Language:** \$650,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to protect native bee health by investigating the potential to mitigate against pathogens that may be transmissible between honeybees and wild bees and by promoting best practices to beekeepers and the public. This appropriation is subject to Minnesota Statutes, section 116P.10.

**Appropriation End Date:** June 30, 2024

## Narrative

**Project Summary:** Our goal is to protect native pollinators by screening and neutralizing bee pathogens, and promoting best honey bee management practices to prevent pathogen spillover into native bees.

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

Native bumblebees, *Bombus* spp., are important pollinators of wild flowering plants and crops such as tomatoes and berries, and are appreciated for their beauty. Unfortunately, five of Minnesota's twenty-three species of native bumblebees are considered vulnerable, endangered, or critically endangered by the International Union for Conservation of Nature. The global decline in bee populations has been attributed to habitat loss, pesticides, parasites, and pathogens. For some bumblebee species, a leading problem may be infectious diseases. For example, the spread of the bumblebee pathogen, *Nosema bombi*, exacerbated through commercial rearing and distribution of *Bombus impatiens* across the U.S., was associated with declining bumblebee species. Another emerging threat is viral pathogen transmission among pollinator species as they forage on common flowers. For example, there is evidence that Deformed wing virus (DWV), may be transmitted from honey bees to bumblebees if diseased honey bees deposit viruses on flower parts (spillover) and other bees subsequently pick them up when visiting the same flowers. Very little is known about pathogen prevalence in bumblebees in Minnesota.

**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.**

The prevalence of DWV and *Nosema ceranae* and *Nosema bombi* in bumblebees in Minnesota has not been previously examined. While it is suspected that honey bees may play a role in increasing the prevalence of pathogens in bumblebees, we currently have no information on existing pathogen level in Minnesota bumblebees. Minnesota is an important place to examine the relationship between honey bee and bumblebee pathogen levels because as a top honey producing state, there are areas of Minnesota with high densities of honey bee colonies and Minnesota is one of the last states still housing the endangered rusty-patched bumblebee. To first understand and then mitigate further declines in these important pollinators, it is critical to collect baseline data on archetypal pathogens in our local populations of honey bees and bumblebees. Finding a solution or even a cure to bee pathogens is a high priority for our assembled team; therefore, we propose to run an innovative pilot study in an attempt to neutralize DWV. Finally, we will protect our native pollinators by educating beekeepers about the critical "public health" need to keep managed bees as healthy as possible.

**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?**

Minnesota's bee diversity boasts over 450 species and includes both native and non-native, managed and wild species. While we recognize the important role of managed bees in Minnesota, it is critical to provide outreach regarding the importance of our native bee pollinators and how unmindful honey bee management might negatively impact native bee health. We propose an educational campaign that increases understanding of the roles of native and managed bees in Minnesota. Additionally, we will engage backyard beekeepers in the cities of Minneapolis and Rochester to participate as beekeeper citizen scientists via local pathogen and pest sampling campaigns.

## 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: Screen for Deformed Wing Virus and Nosema in bumblebees and quantify potential for virus transmission between honey bees and bumblebees.**

**Activity Budget:** \$475,000

**Activity Description:**

There is potential for pathogens to be transmitted from honey bees to bumblebees while foraging on flowers, but the extent of this transmission in nature has not been explored. In three locations where we manage honey bee colonies (8 colonies per location: Minneapolis, the MSP airport, and in Rochester), we will use a new sequencing assay, first developed in the Schroeder Lab, to quantify the prevalence and abundance of DWV and Nosema in three species of bumblebees collected while foraging. We also will monitor pathogen load in honey bees foraging on the same species of flowers during early, mid, and late summer and throughout the year in our managed honey bee colonies. Furthermore, we will also set out pathogen-free *Bombus impatiens* colonies (reared from wild-caught queens) in the same locations to monitor possible infection over the season, and how the infection affects their health and reproduction.

**Activity Milestones:**

Description	Approximate Completion Date
Screen for DWV and Nosema in three common bumblebee species and surrounding honey bee colonies	October 31, 2023
Monitor potential for virus transmission into <i>B. impatiens</i> colonies and quantify effects on bumblebee health	December 31, 2023

**Activity 2: Explore potential to neutralize DWV in bees.**

**Activity Budget:** \$88,500

**Activity Description:**

Currently, there are no specific treatments for DWV infections in bees. The Schroeder Lab has previously reported on a phenomenon known as Superinfection Exclusion in which infection by one virus variant infers protection against other more virulent variants. Application of this phenomenon in honey bees is controversial and is still the subject of much debate. Another yet not commonly explored option to inhibit virus propagation in bees is the application of a foreign antibody. Here we will run laboratory-based cage and cell culture assays to determine if DWV can be neutralized in honey bees and bumblebees. It was recently reported that an effective treatment for a related virus of DWV, namely Sacbrood virus, was discovered. This treatment is based on a specific antibody raised from egg yolk against the virus which was used to immunize honey bee pupae. This work will be repeated here to determine whether DWV can be neutralized in both honey bees and bumblebees. Antibody synthesis, formulation, and production will be carried out in collaboration with Dr Ben Hause (vaccine production specialist).

**Activity Milestones:**

Description	Approximate Completion Date
Preparation, synthesis, formulation, and testing of DWV specific antibodies in bee tissues	July 31, 2022
Application of DWV antibodies in caged honey bees and bumblebees	May 31, 2023

**Activity 3: Beekeeper and community “public health” education about native and non-native bees in Minnesota.**

**Activity Budget:** \$86,500

**Activity Description:**

Minnesota’s bee diversity boasts over 450 species and includes both native and non-native, managed and wild species. While we recognize the important role of managed honey bees in Minnesota, it is critical to provide outreach regarding the value of our native bee pollinators and how unmindful bee management might negatively impact native bee health. We propose an educational campaign (Bee Minnesota) that increases understanding of the roles of native and managed bees in Minnesota. Additionally, we will engage backyard beekeepers in the cities of Minneapolis and Rochester to participate as beekeeper citizen scientists via pathogen and pest sampling in their honey bee colonies. Everyone will be kept updated as to progress made throughout the project by means of a well-managed and curated website.

**Activity Milestones:**

Description	Approximate Completion Date
Provide education via a bee public health campaign to promote practices to protect native bees	April 30, 2024
Establish and disseminate updated beekeeping best management practices to protect wild, native bees in Minnesota	June 30, 2024

## Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Dr Ben Hause	Tallgrass Biologics	Tallgrass Biologics created antibodies in eggs against DWV and has preliminary evidence supporting its specificity for DWV and therefore its potential application as an oral immunoglobulin's therapy against DWV (Patent filed). Here we will conduct controlled lab-based studies to determine the effectiveness of the antibodies against DWV	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 Bee Lab and Bee Squad 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, M. Spivak, E. Evans, R. Masterman and several Bee Squad team members frequently present to both the public and beekeepers throughout Minnesota. Both E. Evans and R. Masterman are Extension Educators within Minnesota Extension. They will disseminate information using well developed communication channels within the Minnesota Extension system. Where appropriate, communications will acknowledge ENRTF as per ENRTF 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 through Bee Minnesota will create a new body of work that could predict how pathogens spillover into native pollinator communities. The Bee Lab at the University of Minnesota has an active Extension and Outreach program run by Dr. Elaine Evans (native bees, especially bumblebees) who will continue to disseminate results after project completion. Drs. Schroeder and Spivak 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



## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
<b>Personnel</b>										
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 between the team and the Minnesotan company Tallgrass Biologics to effectively deliver on Activity 2 of the proposal.			36.5%	0.36		\$69,192	-	-
Co-investigator		Dr Spivak will advise on the experimental design for surveying and collecting honey bees and bumblebees (Activities 1 and 3) and will assist with data analysis and publication, and with all dissemination of results and outreach.			36.5%	0.03		\$4,046	-	-
Co-investigator		Dr Evans is an UMN Extension Educator and Bee Researcher working on pollinator education and research relating to bee conservation. She will be responsible for bumble bee surveys and rearing to support Activity 1 due to her expertise in native bees, particularly bumble bees. In addition, she will be responsible for developing and delivering content to increase awareness and protection of native pollinators in Activity 3.			36.5%	0.6		\$52,754	-	-
Co-investigator		Dr Masterman the Bee Squad for the University of Minnesota Bee Lab and is also a Minnesota Extension Educator. She will lead the Bee Squad in collecting the honey bee samples for analysis in Activity 1 as well as coordinating the beekeeper citizen science sampling effort in Activity 3.			36.5%	0.6		\$56,501	-	-

		Additionally, Masterman will collaborate with Dr. Evans on the educational campaign and will lead the dissemination of information to beekeepers in Minnesota in Activity 3.								
Molecular postdoc		Responsible for running the sequencing assays for DWV & Nosema. And carrying out the antibody neutralization experiments.			25.4%	3		\$189,563	-	-
Molecular tech		To support and assist the postdoc in running the molecular assays during the heavy data generation periods in Activity 1 & 3.			31.8%	1		\$45,260	-	-
Bee squad research tech 1		Responsible for co-ordinating the Bee Squad tech team, setting up the field experiments, managing the honey colonies and collecting the managed and wild bees. Also involved in communication to beekeepers and providing resources to community			31.8%	0.3		\$24,686	-	-
Bee squad research tech 2		As as of team assist in setting up the field experiments, managing the honey colonies and collecting the managed and wild bees. Also involved in communication to beekeepers and providing resources to community			31.8%	0.24		\$10,975	-	-
Bee squad research tech 3		As part of a team assist in setting up the field experiments, managing the honey colonies and collecting the managed and wild bees. Also involved in communication to beekeepers and providing resources to community			31.8%	0.24		\$11,496	-	-
Bee squad research tech 4		As part of a team assist in setting up the field experiments, managing the honey colonies and collecting the managed and wild bees. Also involved in communication to beekeepers and providing resources to community			31.8%	0.24		\$13,877	-	-
Bumblebee lab tech		To assist Dr Evans in setting up bumblebee lab and field experiments			31.8%	0.03		\$1,381	-	-
							<b>Sub Total</b>	<b>\$479,731</b>	<b>\$417,702</b>	<b>\$62,029</b>

<b>Contracts and Services</b>										
Tallgrass Biologics	Professional or Technical Service Contract	Tallgrass Biologics have a patented therapeutic method to use antibodies to DWV from chickens. Egg samples will be collected from egg laying hens at the time of first vaccination. Final antibody purification, formulation and production will be carried out by Tallgrass Biologics for use in this study.		X		0.5		\$10,000	\$10,000	-
Design of new website pages	Professional or Technical Service Contract	A professional, accurate and informative website will have the greatest reach for disseminating the outcomes of our research.				0.1		\$2,500	-	\$2,500
							<b>Sub Total</b>	<b>\$12,500</b>	<b>\$10,000</b>	<b>\$2,500</b>
<b>Equipment, Tools, and Supplies</b>										
	Tools and Supplies	Supplies to setup and collection of field data	For the purchasing bee packages, nets, containers, hive equipment, tools, tubes, preservation material etc.					\$8,800	\$8,800	-
	Tools and Supplies	Pathogen screening molecular consumables ( Nucleic acid extraction, molecular grade chemicals, RT-PCR, NGS sequencing etc.) for 2,250 bee samples	Surveying the pathogens in bee material collected.					\$106,614	\$106,614	-
	Tools and Supplies	Bee packages, cages, molecular grade chemistry & plastics - 300 assays	Neutralization assays for the removal of DWV					\$35,300	\$24,938	\$10,362
							<b>Sub Total</b>	<b>\$150,714</b>	<b>\$140,352</b>	<b>\$10,362</b>
<b>Capital Expenditures</b>										
							<b>Sub Total</b>	-	-	-
<b>Acquisitions and Stewardship</b>										
							<b>Sub Total</b>	-	-	-

<b>Travel In Minnesota</b>										
	Miles/ Meals/ Lodging	Setting up and maintenance of sentinel colonies, release and collecting of bees in the field.	Throughout the 3 years, travel to and from three field sites (Minneapolis, the MSP airport, and in Rochester). Surveying floral diversity and bumblebees colony health. Carry out community surveys and outreach activities. To reduce costs, many activities will be co-ordinated.					\$3,680	\$42	\$3,638
							<b>Sub Total</b>	<b>\$3,680</b>	<b>\$42</b>	<b>\$3,638</b>
<b>Travel Outside Minnesota</b>										
							<b>Sub Total</b>	-	-	-
<b>Printing and Publication</b>										
	Printing	Leaflets	An universal and accessible communication tool especially for those communities that do not have easy access to computers and the internet.					\$375	-	\$375
	Publication	Peer reviewed scientific journal papers	We aim to publish our findings in open access journals. These journals charge publication fees.					\$3,000	-	\$3,000
							<b>Sub Total</b>	<b>\$3,375</b>	<b>-</b>	<b>\$3,375</b>
<b>Other Expenses</b>										
							<b>Sub Total</b>	-	-	-
							<b>Grand Total</b>	<b>\$650,000</b>	<b>\$568,096</b>	<b>\$81,904</b>

## Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
<b>Contracts and Services</b> - Tallgrass Biologics	Professional or Technical Service Contract	Tallgrass Biologics have a patented therapeutic method to use antibodies to DWV from chickens. Egg samples will be collected from egg laying hens at the time of first vaccination. Final antibody purification, formulation and production will be carried out by Tallgrass Biologics for use in this study.	Tallgrass Biologics is developing a novel therapeutic method and will all record specific expenses as it relates to this project (detailed in subcontract with the university). If generally ineligible expenses are to be made, this will first be cleared through the University of Minnesota finance office and the them confirmed with LCCMR before purchasing.

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: [7d2b3e04-99a.pdf](#)

#### *Alternate Text for Visual Component*

Overview of the project...

### Supplemental Attachments

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

Title	File
LCCMR Background Check Certification	<a href="#">7cde7d5d-4a8.pdf</a>
Figure 1, Activity 2	<a href="#">e09ed48d-f29.pdf</a>
Nosema Spillover Report	<a href="#">b60f2852-f8a.pdf</a>

#### *Media Links*

Title	Link
Bee Minnesota	<a href="https://beeminnesota.umn.edu/">https://beeminnesota.umn.edu/</a>

## Difference between Proposal and Work Plan

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

Over the past year we automated our molecular virus screening workflow which means that we can reduce our molecular consumable budget for this project.

## Additional Acknowledgements and Conditions:

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

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

N/A

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

Yes, I understand the UMN Policy on travel applies.

**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



## Work Plan Amendments

No Amendments Entered

# Status Update Reporting

## Final Status Update August 14, 2024

**Date Submitted:** November 1, 2024

**Date Approved:** November 8, 2024

### Overall Update

The primary objective of Bee Minnesota was to investigate the true host range expansion or Spillover potential of honey bee pathogens such as Nosema and Deformed wing virus (DWV), whether these may be transmitted from honey bees to bumblebees if diseased honey bees deposit pathogens on flower parts and other native Minnesota bees subsequently pick them up when visiting the same flowers. Very little was known about pathogen prevalence in bumblebees in Minnesota. We successfully completed our three year study concluding that:

- 1) Nosema was detected in both honey bees and bumblebees but the genetic profiles of the Nosema species found in both insect families were too similar for the molecular tools used to definitively prove either way that Spillover was indeed occurring in Minnesota.
- 2) DWV and indeed other honey bee viruses were present in the bumblebees due to environmental exposure or dead-end spillover, and not spillover host expansion. Instead we uncovered a new diversity of bumblebee viruses that could be the likely cause of the decline in bumblebee species across Minnesota, the US and indeed the world.
- 3) The antiviral antibody against DWV did not show any efficacy at lowering the virus load in cell cultures.

### Activity 1

Objective 1: "Screen for DWV and Nosema in three common bumblebee species and surrounding honey bee colonies" - Sequence analysis was completed and the results of the Nosema analysis was submitted (see attached report). We could not conclude that spillover due to host expansion was happening between honey bees and bumblebees. Both species have a "natural" source of Nosema. Given this null result, we will not be submitting this work for peer-review publication.

Sequence analysis of the virus data has now also been completed, and a manuscript for publication has been submitted to Communications Biology (a Springer Nature open access journal). Our preliminary analysis shows no evidence of active honey bee virus infections or Spillover with host expansion from honey bees to bumblebees. Bumblebees that have their own diversity of novel viruses, some of which could be highly pathogenic.

Objective 2 : "Monitor potential for virus transmission into *B. impatiens* colonies and quantify effects on bumblebee health". As reported previously, we were able to release *B. impatiens* colonies into the field over the summer of 2023. We found the diversity of viruses in these bees changed while in captivity and no Spillover with host expansion was observed here as well.

*(This activity marked as complete as of this status update)*

### Activity 2

This activity was previously marked complete.

*(This activity marked as complete as of this status update)*

### Activity 3

We submitted a manuscript to Communications Biology in July 2024, preprint can be found at (<https://www.researchsquare.com/article/rs-4802694/v1>). The main message of this Open Access paper and the final conclusion of the Bee Minnesota project was that viruses shared by both bees (honey bees and bumblebees) shared over 98% nucleotide identity, and no bumblebee-specific strains of honey bee viruses occurred, as expected if spillover led to a true host expansion involving bumblebee-bumblebee transmission. We conclude that the honey bee viruses,

namely deformed wing virus, black queen cell virus, and sacbrood virus, were present in the bumblebees due to environmental exposure or dead-end spillover, AND NOT spillover host expansion. Honey bees bare limited threat (due to pathogen spillover) to our keystone bumblebees, in particular to the common eastern bumblebee - *Bombus impatiens*.

We are waiting for the final reviewing process to be completed, so we have not spend or publication fee. Similarly, our preferred method of communication was through the Bee Minnesota website, so we did not spend our printing budget. *(This activity marked as complete as of this status update)*

**Dissemination**

See activity 3 update above

# Status Update Reporting

## Status Update April 1, 2024

**Date Submitted:** November 1, 2024

**Date Approved:** November 8, 2024

### Overall Update

We are in the final stretch in the detection and surveillance of honey bee viruses and the Nosema parasite in native Minnesotan bumblebees. To date, we have completed our analysis on the possible spread of Nosema from honey bees to bumblebees. Sequencing of the final bumblebee collection is still ongoing. We are attending honey bee workshops and conferences, ensuring that we reach a broad range of groups from backyard beekeepers to the bee pollinator research community. We are still on track to complete and meet all our research milestones and objectives.

### Activity 1

Objective 1: "Screen for DWV and Nosema in three common bumblebee species and surrounding honey bee colonies" - Sequence analysis is still ongoing. We needed to re-sequence the last batch of our samples. That said, the Nosema analysis (see attached report) has been completed. We could not conclude that spillover was happening between honey bees and bumblebees. Both species have a "natural" source of Nosema. Given this null result, we will not be submitting this work for peer-review publication. We are awaiting the final sequence results for the honey bee virus data, with the objective of submitting a manuscript for publication in a high-impact virology journal. Our preliminary analysis shows no evidence of active honey bee virus infections in bumblebees.

Objective 2 : "Monitor potential for virus transmission into *B. impatiens* colonies and quantify effects on bumblebee health". As reported previously, we were able to release *B. impatiens* colonies into the field over the summer of 2023. We are currently processing and sequencing this final sample set.

*(This activity marked as complete as of this status update)*

### Activity 2

This activity was previously marked complete.

*(This activity marked as complete as of this status update)*

### Activity 3

We will update the Bee Minnesota website with the final summary reports. Here (<https://beeminnesota.umn.edu/findings-publications>) is a link to the webpage. Dr Schroeder gave two talks at Michigan's "SEMBA Honey bee conference" in Michigan in March 2024. He was also invited to give a talk at the Mead Nebraska meeting in July 2024.

### Dissemination

See Activity 3

# Status Update Reporting

## Status Update October 1, 2023

**Date Submitted:** October 9, 2023

**Date Approved:** January 17, 2024

### Overall Update

We continue to make progress in the detection and surveillance of honey bee viruses and the Nosema parasite in native Minnesotan bumblebees. Moreover, our new sequencing technology have allowed us to discover new virus infections in bumblebees. To date, all of our samples have been collected and we are in the process of analyzing and finalizing the last set of sequence data. Our engagement activities, through holding unique bee health events, creating newsletters and publicizing preliminary results on our Bee Minnesota website, have ensured that we reach a broad range of groups from backyard beekeepers to the bee pollinator research community. We are on track to complete and meet all our research milestones and objectives.

### Activity 1

Activity 1 has two objectives. Objective 1: "Screen for DWV and Nosema in three common bumblebee species and surrounding honey bee colonies". We sequenced the last batch on samples and are in the process writing the final report, with the objective of submitting a manuscript for publication in a high-impact virology journal. We found no evidence of active honey bee virus infections in bumblebees. This was observed in bumblebees collected over two years at all three field stations. This was despite all the honey bees collected, collected off the flowers with the bumblebees or in our sentinel colonies, all having active infections of at least three honey bee viruses, one notably being the highly virulent Deformed wing virus. Moreover, our sequencing also found that Nosema was indeed present in both honey bees and bumblebees. However, the could not conclusively identify the source or direction of transmission.

Objective 2 : "Monitor potential for virus transmission into *B. impatiens* colonies and quantify effects on bumblebee health". We were able to release *B. impatiens* colonies into the field over the summer of 2023. Last of the released bees have been collected and we are currently processing and sequencing this final data set.

### Activity 2

Activity 2 has two objectives. Objective 1: "Preparation, synthesis, formulation, and testing of DWV specific antibodies in bee tissues" and Objective 2 "Application of DWV antibodies in caged honey bees and bumblebees". This was a challenging activity as COVID19 and its subsequent impact on the molecular consumables supply chain meant that this work was significantly delayed. Nonetheless, we completed the efficacy testing on the DWV-antibodies on the clearance rate of DWV in honey bee immortal cell lines chronically infected with DWV. The results were unexpected as rather than clearing the infection, the antibodies caused a significant increase in DWV replication (see figure 1, attached).

Consequently, we abandoned the cage experiments (Objective 2) and we are working with Tallgrass biologics to seek federal funding to further explore the unexplained outcome of elevated DWV production in the presence of DWV-antibodies.

*(This activity marked as complete as of this status update)*

### Activity 3

In Activity 3, we seek to communicate our observations using multiple forums. We use the Bee Minnesota website to publish preliminary data obtained in this project. Here (<https://beeminnesota.umn.edu/findings-publications>) is a link to the webpage that shows that our approach can uncover new viruses infecting bees. We will use this platform throughout the final months of this project to communicate directly with the general public. Dr Schroeder was also invited to give talks at Michigan's "SBGMI 2023 Live Virtual Summer Mini-Conference" and at " Message from the bees" held at the Bell Museum in the summer of 2023.

**Dissemination**

See Activity 3 above

# Status Update Reporting

## Status Update April 1, 2023

**Date Submitted:** April 1, 2023

**Date Approved:** April 17, 2023

### Overall Update

It is widely accepted that pathogen spillover represents an important cause of biodiversity decline. For wild bee species such as bumblebees, lab generated data point towards viral spillover from managed honey bees as a potential cause of bumblebee decline. We completed our pathogen survey for year one of our two year dataset from honey bees and bumblebees collected from flowers in the field and found distinct bee species-specific virus diversity. No evidence of spillover was observed. Moreover, we found new viruses not previously found in MN bumblebees.

We were the first to uncover at least two new, likely invasive, viruses not previously known to infect MN bumblebees. Uncovering the true viral landscape in bumblebees raises the question of how many new and invasive viruses we are missing in our MN native bees. Future research needs to expand the survey to include other MN native bee species.

### Activity 1

We completed our bioinformatic analysis for the samples collected in 2021. Diversity maps were created which clearly and definitively show that the well known honey bee pathogen, DWV, was only found in honey bees. Bumblebees had a very different pathogen profile. In fact, one insect virus called Negevirus, which was previously been isolated from mosquitoes and phlebotomine sand flies, was also found to infect bees in Europe and more recently bees in Colorado and New Jersey. This is the first report of negevirus found in bumblebees in Minnesota. Moreover, we were able to describe a brand new virus never described before. Taken together, virus spillover from honey bees to bumblebees was not observed but a previously undescribed bumblebee viral diversity was uncovered.

### Activity 2

Preliminary efficacy data of anit-DWV IgY on DWV infection suggests that anit-DWV IgY can be used as a virus mitigant. We observed a dose dependent, at least one log reduction in DWV production in chronically DWV infected honey bee cell lines. We are currently testing whether the anit-DWV IgY molecule can be delivered by feeding adult honey bees with anit-DWV IgY seeded patties.

### Activity 3

Our website has been updated with the bee pathogen diversity chart shown. We are also planning a "What are the Bees Telling Us?" event. The aim to create a fun and informative evening focused on pollinators. Inspired by the mythological role of bees as messengers, the Bee Lab's lead scientists will share what bees are teaching them about disease and healing, The event is scheduled to be held at the Bell Museum on May 11th, 2023.

### Dissemination

Please see activity 3 above

# Status Update Reporting

## Status Update October 1, 2022

**Date Submitted:** October 7, 2022

**Date Approved:** October 21, 2022

### **Overall Update**

We continue to make steady progress in this project. We have now overcome many of the unforeseen delays caused by COVID. For example, we received all the molecular chemistry needed to screen all of the honey bee and bumblebee samples collected in 2021. Our preliminary assessment of the data suggests that honey bees are not transferring their viruses to bumblebees.

### **Activity 1**

We successfully collected all of the honey bees and bumblebees for 2022. These have now been scheduled for nucleic acid extraction and sequencing. We sequenced all of the 2021 samples. Bioinformatics on this 2021 data is still in progress but we anticipate our final analysis to be completed in 2023. Preliminary analysis indicates that the honey bee virome is different to that of the bumblebee virome.

### **Activity 2**

Honey bee cell cultures have now been treated with the anti-DWV IgY molecules. Results are not in yet but we anticipate to obtain efficacy data in the spring of 2023.

### **Activity 3**

We successfully launched our website this summer (<https://beeminnesota.umn.edu/>). As we further analyze and interpret our data in 2023, we will arrange stockholder meetings, produce leaflets and update our website as appropriate.

### **Dissemination**

Please see Activity 3 above



# Status Update Reporting

## Status Update April 1, 2022

**Date Submitted:** April 22, 2022

**Date Approved:** May 4, 2022

### Overall Update

Our primary object is to protect native pollinators from risk of disease transmission and population declines. By screening and neutralizing bee pathogens, we wish to promote best management practices to maintain honey bee health and prevent pathogen spillover into native bee populations. We are well on our way to meeting Activity 2 as the IgY anti-DWV neutralizing antibodies was successfully delivered by Tallgrass Biologics. Similarly, the establishment of the three honey bee apiaries required for Activity 1 was completed and subsequent sampling of pollinators for year one was a complete success. Finally, we completed our storyboard for the creation of our project website (Activity 3). We anticipate the launch of our website to take place over the summer of 2022. The only note of caution is that we have and currently still are being negatively impacted by COVID19 supply chain related delays. This has specifically impacted the timely procurement of molecular consumables for the lab-based neutralization and sequencing assays. The true impact of these delays will be assessed over the coming months, i.e., over the next reporting period.

### Activity 1

For Activity 1, our objective was to screen for Deformed Wing Virus and Nosema in bumblebees and quantify potential for virus transmission between honey bees and bumblebees. To date we:

- 1) purchased honey bee packages, established honey bee colonies in three apiaries and collected a total of 168 samples (56 pooled honey bee samples x 3 sites, for months May to November 2021). These samples will give us baseline data for the pathogens in honey bee colonies over that sampling season.
- 2) collected a total of 315 individual bumblebees from flowers at various distances from the afore mentioned three apiaries, representing eight native species, with the majority being *Bombus impatiens*. We were also able to collect 202 individual honey bees co-occurring on the same flowers as the bumblebees.

All samples are in the process of being prepared for pathogen extraction and sequencing. As outlined in our Summary, we have and currently still are being negatively impacted by COVID19 supply chain related delays. We anticipate however that we can recover and will make-up time over the coming 6 months.

### Activity 2

A key objective in delivering Activity 2 was the creation, synthesis, and delivery of the IgY anti-DWV extracts from Tallgrass Biologics. This was achieved in the Fall of 2021. However, our neutralization treatment tests on honey bee cell cultures have been hampered by the COVID19 related supply chain delays in the procurement of molecular consumables. Many items are on 6-9 month back orders and these are required to run the cell culture neutralization assays. We anticipate however that we can recover and assays will be completed over the coming 6 months.

### Activity 3

The Bee Minnesota team continues to engage with backyard beekeepers in and around the cities of St Paul, Minneapolis and Rochester. As an example, Dr Schroeder will be speaking to Honey Bee Club of Stillwater (<http://honeybeeclubofstillwater.blogspot.com/p/club.html>) this coming April 25th 2022. Our focus over this reporting period was to establish a storyboard for our project website. We have been working our UMN Extension team and we anticipate a launch date in the summer of 2022.

### Dissemination

Our dissemination activities are described in Activity 3. We are in the early stages of our project. As we start producing

data, this will consequently provide us with new insights that can be disseminated through the platforms as described in this project.