# Final Abstract

Final Report Approved on December 23, 2024

## M.L. 2021 Project Abstract

For the Period Ending June 30, 2024

**Project Title:** Biocontrol of Invasive Species in Bee Lawns and Parklands

**Project Manager:** Vera Krischik

**Affiliation:** U of MN - College of Food, Agricultural and Natural Resource Sciences

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**Website:** https://cfans.umn.edu/

**Funding Source:**

**Fiscal Year:**

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

**Appropriation Amount:** $425,000

**Amount Spent:** $424,930

**Amount Remaining:** $70

**Sound bite of Project Outcomes and Results**

The outcome of this project is to reduce insecticides used to manage Japanese beetle (JB) that also kill pollinators. In Northeastern states Japanese beetles are managed by the native soil-inhabiting pathogen called Ovavesicula. Surveys were performed to determine pathogen distribution and ways to distribute the pathogen to new sites.

**Overall Project Outcome and Results**

Research wanted to identify ways to reduce Japanese beetle populations. In Northeastern states, Japanese beetle population decline was correlated to infection by the native soil-inhabiting microsporidian Ovavesicula fungus. Managing Japanese beetle is important, as the beetles eat leaves and kill trees and crops. In addition, Japanese beetles reduces fruit yield that sustains humans, birds, and other wildlife.

Biocontrol is a sustainable pest management tactic that uses good bugs and host specific pathogens, like Ovavesicula, to kill pest insects and reduces insecticide use thereby protecting pollinators. Japanese beetles do not have many beneficial insects that kill them, so we investigated a native soil pathogen that spreads slowly. The Fish and Wildlife Service identify insecticides as a major contributor to bee and butterfly decline.

Before this research started, it was not know if this native pathogen was fond in Minnesota. Now we know that it is. Surveys of 44 sites in 2022 and 34 sites in 2023 revealed by qPCR analysis and spore counting that the pathogen is found mostly at 11 sites in Stillwater.

But why are spores found in some sites and not others? Soil heath (pH, N, P, K, soil type, and fungal biomass) was correlated to spore numbers, so we could understand why spores were more common in some soils than others. This will help understand where to release pathogens for establishment.

Research was performed to increase pathogen spread, which showed that Japanese beetle traps containing spore covered leaves, resulted in spores inside the adults. The adults are released from the traps and carry the spores to sites where they will lay eggs. Previously, spores were poured on the soil which was not efficient.

The pathogen will reduce Japanese beetle numbers, reduce insecticide use, and result in less non-target insecticide effects on good bugs, thereby increasing ecosystem health.

**Project Results Use and Dissemination**

The research and outreach program focused on reducing insecticide use and increasing soil pathogens that kill JB, thereby conserving pollinators. The outreach programs included a table at the Minneapolis Park and Rec Board Monarch Festival for 2 years, and online website on JB management, an online educational program with site visits, and an online Advisory Committee meeting for stakeholders and state agencies, which met online 3 times. Outreach included 8 articles for the nursery industry, golf course, and Master Gardener newsletters, websites, 36 talks, 3 peer-review papers, 3 UMN UROP fellowships, and a Post Doc that received a UAL professorship.

# Environment and Natural Resources Trust Fund

M.L. 2021 Approved Final Report

## General Information

**Date:** December 31, 2024

**ID Number:** 2021-164

**Staff Lead:** Tom Dietrich

**Project Title:** Biocontrol of Invasive Species in Bee Lawns and Parklands

**Project Budget:** $425,000

## Project Manager Information

**Name:** Vera Krischik

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

**Office Telephone:** (612) 625-7044

**Email:** krisc001@umn.edu

**Web Address:** https://cfans.umn.edu/

## Project Reporting

**Final Report Approved:** December 23, 2024

**Reporting Status:** Project Completed

**Date of Last Action:** December 23, 2024

**Project Completion:** June 30, 2024

## Legal Information

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

**Appropriation Language:** $425,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to establish a biocontrol program to manage the invasive Japanese beetle in a way that reduces insecticide use in bee lawns and pollinator restorations and the associated economic and environmental costs to wildlife and humans.

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

## Narrative

**Project Summary:** The proposed research and outreach program is to establish a biocontrol program to manage the invasive, exotic Japanese beetle to reduce insecticide use in bee lawns and parks.

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

The Minnesota Department of Agriculture and the Minnesota Department of Natural Resources list Japanese beetle (JB, Popillia japonica) as a highly destructive, invasive exotic pest (USDA 2017, CAB 2005). Since introduction from Japan in 1916, JB has been defoliating over 300 species of plants

JB damages flowers, fruits, and foliage, which results in decreased food resources for bees and other wildlife. However, the spraying of insecticides on lawns for JB grubs and on plants and flowers for adults probably results in more non-targeted deaths of pollinators than the JB damage itself. Fortunately, JB has a natural biocontrol agent that was discovered in 1988 in Connecticut (Hanula and Andreadis 1988) that could be introduced into MN. This microsporidian (fungal) pathogen (Ovavesicula popilliae) was studied at Michigan State University (MSU) (Perry et al. 2013, Smitley 2011) and was released in four states. Research is needed to survey greater MN for the presence of both Japanese beetles and the pathogen, which was found in one locale in MN. Research is needed to identify, culture, and disseminate the biocontrol pathogen. Until the pathogen can be established, an integrated pest management program that identifies unintended impacts of current insecticides on pollinators needs to be developed.

**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.**

Our proposed solution is to reduce economic and environmental damage caused by the exotic JB through two approaches: one short term and one long term. For long term, we will survey Minnesota for the presence of Japanese beetles and their possible infection by a beneficial pathogen called Ovavesicula popilliae. This pathogen was first described in Connecticut and infects JB tubules and spreads systemically (Andreadis and Hanula 1987). Research shows the fungus kills 25 to 50 percent of JB grubs. After obtaining approval from the Minnesota Department of Agriculture and the US EPA, we propose to establish this fungus statewide using a nursey system to supply volunteers from various organizations like Master Gardeners with infected beetles as well as using JB traps, after testing confirms this as an appropriate pathogen dispersal method.

Since it may take a long time for the natural pathogen to establish, we will develop near term practices as well. We will test four current EPA registered microbial insecticides and three conventional insecticides in the lab and field for efficacy of killing JB adults and grubs and for unintended impacts on pollinator species, like bumble bees. The information will be developed into outreach bulletins posted at our website.

**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?**

The long-term outcomes of the project are to develop a biocontrol program for the invasive JB, which will reduce insecticide applications in urban areas, especially lawns and restorations planted to support pollinators. In the short-term, the goal is to use EPA registered microbial insecticides and other bee friendly insecticides, that conserve pollinators, to control JB. We developed an advisory board from MN Department of Agriculture, Lawn to Legumes program, Golf Course Superintendents Association, Michigan State University researchers, MN Nursery and Landscape Association, and Minneapolis Park and Recreation Board, that have offered park sites for research and outreach.

## 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: Pathogenic biocontrol in bee lawns and parklands

**Activity Budget:** $200,000

**Activity Description:**
Activity 1. Long term management. Survey the state of MN for the presence of O. popilliae, which will be identified through collaborations with Dr. Dave Smitley of Michigan State University. Work with Drs. Raj Mann and Mark Abrahamson of the MDA to authorize the release of O. popilliae at 10 sites; 4 demonstration sites at parks and 6 golf courses with high JB populations. These parks eventually will serve as nursery sites for spreading the pathogen throughout Minnesota. Once the pathogen is established, we will work with outreach groups such as Master Gardeners and Master Naturalists to distribute and monitor JB pathogen establishment. We will continue checking the one positive location for O. popilliae in Minnesota and work with MSU to determine if it is any different than populations from MSU that have been released in other states (CO, KY, AR and KS). Monitor pathogen infection levels at introduction sites (10 sites: 4 parks and 6 golf courses), and monitor JB population levels at pathogen nursery sites and control sites located 5 - 10 km away.

**Activity Milestones:**

| Description | Approximate Completion Date |
|---|---|
| 3. Approval from MDA to release the pathogen | June 30, 2024 |
| 2. Conduct 40 surveys on pathogen and JB distribution in MN. | June 30, 2024 |
| 1. Receive training in identifying O. popilliae at Michigan State University; establish Krischik lab | June 30, 2024 |
| 4. Release pathogen at approved nursery sites | June 30, 2024 |
| 5. Perform lab trials to determine pathogen efficacy and spread. | June 30, 2024 |
| 6. Quantify number infected grubs and adults after pathogen release. | June 30, 2024 |

### Activity 2: Biocontrol in bee lawns and parklands using IPM

**Activity Budget:** $225,000

**Activity Description:**
The efficacy of new EPA approved microbial insecticides and new conventional insecticides for killing JB will be researched. Also, It will be determined if these insecticides are friendly to bees when used for JB grub control on bee lawns and JB adult control on flowers. EPA registered microbial insecticides for bioassays are GrubGone (Bacillus thuringiensis galleriae, BTG, recently available), a soil-applied fungus Beauveria bassiana, parasitic nemaotdes Steinernema scarabaei (Nemagard, recently available), and bee friendly Acelepryn (chlorantraniliprole) compared to standard neonictoinoids (imidacloprid, MeritG and clothianidin, ArenaG). The effects of these microbial insecticides on Bombus impatens, bumblebees and Osmia, mason bees, will be performed in large tents in the greenhouse with label rates of microbes sprayed over artificial feeding stations containing pollen and nectar that the bees will collect for their nests. We have done these bioassays many times for our research and are proficient and collect viable data on the effects of label rates of insecticides on bee colony health. In addition, we will study whether commercially available JB traps can be used to disseminate BTG, as a model system for dispersing Ovavesicula. We will evaluate the correct timing for applying these insecticides while causing the least amount of pollinator harm.

**Activity Milestones:**

| Description | Approximate Completion Date |
|---|---|
| 1. Determine if the EPA registered microbial pathogens and bee friendly insecticides conserve pollinators. | June 30, 2024 |

| | |
|---|---|
| 2.Understand the efficacy of bee friendly EPA registered insecticides on JB grubs and adults. | June 30, 2024 |
| 3. Determine if JB traps can disseminate pathogens. | June 30, 2024 |
| 4. Outreach: Install demonstration education programs at key parks to promote the IPM and biocontrol | June 30, 2024 |

## Project Partners and Collaborators

| Name | Organization | Role | Receiving Funds |
|---|---|---|---|
| Dr. David Smitely | Michigan State University | Dr. Smitley is a Professor and Past Head of the Department of Entomology at Michigan State University. He has worked for the last 10 years on understanding how the fungal pathogen can be identified and surveyed. A Post Doc trained at MSU will bring the research program back to Minnesota. | 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 research and outreach program will help mitigate decline of native bees and beneficial insects that control pest insects. The USFWS identified insecticides and bee pathogens as a major factor contributing to the decline of the urban dwelling endangered rusty-patched bumblebee, Bombus affinis. Research on the natural occurring pathogen of JB will help manage JB populations and it does not affect pollinators. In park lands, restorations, and bee lawns site specific IPM programs are needed to control pests and conserve good insects. The IPM programs will be posted on two websites: the CUES CFANS college website http://cues.cfans.umn.edu; and the Conservation biocontrol: IPM and pollinators website http://ncipmhort.cfans.umn.edu/.

Outreach programs will be delivered through educational bulletins, websites, blogs, field days, demonstration projects at parks, talks in annual pesticide workshops, and talks in commodity workshops. Master Gardeners, Master Naturalists, and other groups will be identified as participants and will be asked to help with information dissemination.

The Advisory Board will meet by Zoom twice a year to discuss the research and outreach programs. The advisory board consists of members from MN Department of Agriculture, MN Department of Natural Resources, Minneapolis Park and Recreation Board, Golf course Superintendents Association, MN Nursery and Landscape Association, county parks, and NGOs. These members already volunteered sites for research and outreach demonstration projects. The research and outreach program will help mitigate decline of native bees and beneficial insects that control pest insects. All products and demonstration projects will give credit to the ENTRF funding in writing and with the icon of the loon.

Advisory Committee members and Participants contacted for Zoom meeting April 7 + Sept 24 2020
Advisory Committee:
Roberta Groening, Minneapolis Park & Recreation Board
Jeremy Barrick, Minneapolis Park & Recreation Board
Kaitlin Ryan, Minneapolis Park & Recreation Board
Mark Abrahamson, MDA
Raj Mann, MDA
Dan Shaw, MN DNR, Minnesota Board of Water and Soil Resources
Jack MacKenzie, Minnesota Golf Course Superintendents' Association
Jim Calkins, Research Information Director, Minnesota Nursery and Landscape Association (MNLA)
Dan MacSwain, Natural Resource Coordinator, Washington County Public Works Department
Steve Ellis, Be keeper
Laurie Schneider, Pollinator Friendly Alliance

Participants:
Jennifer Vieth, Carpenter Nature Center
Matthew Lagus, UM

Mark Hansen,Christmas Tree Growers
Daniel Whitney, MN Beekeeper
Laurie Schneider, NGO
Mary Meyer, UM
Sarah Foltz Jordan, Xerces Society
Erin Rupp, NGO
Nick Partington, UM
Julia Ponder, UM
Roy, Charlotte, DNR
Sarah Rudolf, MPCA
Patria Hauser, NGO
Sarah Pennington, DNR
Erin Raupp, NGO
Master Gardeners
Master Naturalists

## 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 research and outreach program will help mitigate decline of native bees and beneficial insects that control pest insects. New IPM programs will be implemented that employ microbial insecticides, such as BT galleriae (bacteria specific to JB grubs), Ovavesicula (fungus specific to JB grubs), and chlorantraniliprole, (Acelepryn insecticide friendly to bees). The USFWS identified insecticides in bee habitat as a major factor behind the decline of the urban dwelling rusty-patched bumblebee. MN efforts to increase restorations and bee lawns also need site specific IPM programs to control pests in these restorations. Future grants will be pursued for project continuation.

## Other ENRTF Appropriations Awarded in the Last Six Years

| Name | Appropriation | Amount Awarded |
|---|---|---|
| Understanding Systemic Insecticides as Protection Strategy for Bees | M.L. 2014, Chp. 226, Sec. 2, Subd. 06b | $326,000 |
| Promoting Conservation Biocontrol of Beneficial Insects | M.L. 2017, Chp. 96, Sec. 2, Subd. 08b | $400,000 |

## Budget Summary

| Category / Name | Subcategory or Type | Description | Purpose | Gen. Ineligible | % Benefits | # FTE | Classified Staff? | $ Amount | $ Amount Spent | $ Amount Remaining |
|---|---|---|---|---|---|---|---|---|---|---|
| **Personnel** | | | | | | | | | | |
| Project investigator | | $0 | | | 0% | 0.6 | | - | - | - |
| Undergraduate research associate | | Help with research and outreach programs | | | 0% | 0.3 | | $12,281 | - | - |
| Research associate 2 | | Perform research and outreach programs | | | 31.8% | 3 | | $151,740 | - | - |
| Post Doc | | Direct and perform research and outreach programs; PostDoc received a permanent job in Nov 2023 | | | 25.4% | 0.6 | | $87,555 | - | - |
| | | | | | | | **Sub Total** | **$251,576** | **$251,576** | **-** |
| **Contracts and Services** | | | | | | | | | | |
| USDA National Standards Lab, Gastonia NC Residue analysis of insecticides | Professional or Technical Service Contract | Funds for USDA Gastonia NC does pesticide analysis for a fee. We have used their services in all our grants to verify the solutions and LC50 used in bioassays. Costs $130/sample x 38 samples=$5,000 We have not used this lab as we decided to use label rates. | | X | | 3 | | - | - | - |
| UM soil testing lab | Internal services or fees (uncommon) | UM soil testing lab will analyze soil for nutrients, organic matter, and texture. $32/sample x 40 samples x 2 seasonal times = $2600 | | | | 0.3 | | $9,000 | $9,000 | - |
| Ward Laboratories uses PLFA phospholipid fatty acid tests for fungal and bacterial biomass | Professional or Technical Service Contract | Ward Laboratories, Kearney, NE, is identified by NSF Long term ecosystem research as a vendor PLFA, phospholipid fatty acid tests for fungal and bacterial biomass. Higher fungal biomass should support Ovavesicula fs and predict better biocontrol sites.$85/sample x 40 samples x 2 seasons; vendors not available in MN | | X | | 0.3 | | $16,200 | $16,200 | - |
| Michigan State University Dr. | Professional or Technical | From 2021 to Nov 2023, the Post Doc processed samples and MSU ran the qPCR on the samples. From Nov 2023 to | | X | | 3 | | $57,439 | $57,439 | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dave Smitley lab | Service Contract | June 2024 the samples were sent to MSU for both processing and running of qPCR. | | | | | | | | |
| | | | | | | | **Sub Total** | **$82,639** | **$82,639** | **-** |
| **Equipment, Tools, and Supplies** | | | | | | | | | | |
| | Tools and Supplies | Lab equipment to identify the pathogens, field equipment to release and survey the pathogens. Lab and field equipment to test the efficacy of the 4 EPA registered insecticides for research and in demonstration projects. Rent UM greenhouse for 3 years. Equipment/Tools/Supplies: Research supplies greenhouse space for research $500/mo x 36mos=$18,000; purchased sod for JB grubs= $4,000; insecticides= $2,000; UM field plot charges=$1,000; Elisa development reagents, buffers, glassware, equipment= $18,000; ultralow freezer to store samples=$3,400;JB traps and collection supplies= $4,000; containers, netting=$5,000; Bombus colonies, $6,600. Total = $62,000 | Establish and maintain the pathogen Ovavesicula and perform research in the lab and field on efficacy and establishment. | | | | | $58,338 | $58,338 | - |
| | Tools and Supplies | Supplies to make demonstration programs at 7 sites, such as parks and bee lawns; poster using commercially (Uline) available poster stands and printed posters. | Supplies to make demonstration programs at 8 sites; costs for posters was much lower as we found a cheap poster stand at Uline for around $150/site; printing of posters is under printing; costs from ULine for poster stands and mylar covers were [odter dtsnd snd plsstic covers | | | | | $1,726 | $1,726 | - |
| | | | | | | | **Sub Total** | **$60,064** | **$60,064** | **-** |
| **Capital Expenditures** | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Purchase one tissue grinder Qiagen Tissuelyser II to grind samples for qPCR analysis. | Purchase tissue grinder to grind Japanese beetles to identify the Ovavesicula pathogen inside the beetles. | X | | | | $11,271 | $11,271 | - |
| | | | | | | | **Sub Total** | **$11,271** | **$11,271** | **-** |
| **Acquisitions and Stewardship** | | | | | | | | | | |
| | | | | | | | **Sub Total** | **-** | **-** | **-** |
| **Travel In Minnesota** | | | | | | | | | | |
| | Miles/ Meals/ Lodging | Instate travel to research sites, rental a UM car for 7mo/yr x 2 yr; we needed to visit survey sites longer than anticipated so the cost was higher; there were no overnights or per diem paidts | Instate travel to research sites, demonstration sites, meetings. Renting UM car, mileage | | | | | $12,940 | $12,940 | - |
| | | | | | | | **Sub Total** | **$12,940** | **$12,940** | **-** |
| **Travel Outside Minnesota** | | | | | | | | | | |
| | Miles/ Meals/ Lodging | Air Fare $336 x2trips; rate $56/diem+$106 lodging=$162/day; Total=$3,973; we made fewer trips to learn the technique. | Outstate travel to receive training on pathogen identification and survey techniques: Training at MSU to learn molecular techniques and field work. Training is only possible at MSU. | X | | | | $3,973 | $3,973 | - |
| | | | | | | | **Sub Total** | **$3,973** | **$3,973** | **-** |
| **Printing and Publication** | | | | | | | | | | |
| | Printing | Costs associated with demonstration site printing of signage; costs mainly for printing of 8 posters for demonstration sites | Educational program for consumers and professional landscape managers to use pollinator friendly management programs for JB. | | | | | $984 | $984 | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Publication | Page costs associated with publishing research in peer reviewed publications. | One peer review publication was published for free as service in kind as I am an editor on the journal. | | | | | - | - | - |
| | | | | | | | **Sub Total** | **$984** | **$984** | **-** |
| **Other Expenses** | | | | | | | | | | |
| | | Mailing samples and supplies | Mail samples to USDA for residue analysis and shipping JB to MSU, $100/box for 14 boxes=$1,400 | | | | | $1,553 | $1,483 | $70 |
| | | | | | | | **Sub Total** | **$1,553** | **$1,483** | **$70** |
| | | | | | | | **Grand Total** | **$425,000** | **$424,930** | **$70** |

## Classified Staff or Generally Ineligible Expenses

| Category/Name | Subcategory or Type | Description | Justification Ineligible Expense or Classified Staff Request |
|---|---|---|---|
| **Contracts and Services** - USDA National Standards Lab, Gastonia NC Residue analysis of insecticides | Professional or Technical Service Contract | Funds for USDA Gastonia NC does pesticide analysis for a fee. We have used their services in all our grants to verify the solutions and LC50 used in bioassays. Costs $130/sample x 38 samples=$5,000 We have not used this lab as we decided to use label rates. | The USDA AMS National Standards Science Lab in Gastonia, NC with Dr. Jonathan Barber is the only lab in the US that analyzes samples for pesticide residue and certifies the result according to EPA Best Management Lab Practices. This is a sole source lab and no other federal, state, or private business offers residue analysis, sample safety, and certification are available. |
| **Contracts and Services** - Ward Laboratories uses PLFA phospholipid fatty acid tests for fungal and bacterial biomass | Professional or Technical Service Contract | Ward Laboratories, Kearney, NE, is identified by NSF Long term ecosystem research as a vendor PLFA, phospholipid fatty acid tests for fungal and bacterial biomass. Higher fungal biomass should support Ovavesicula fs and predict better biocontrol sites.$85/sample x 40 samples x 2 seasons; vendors not available in MN | Ward Laboratories uses PLFA phospholipid fatty acid tests for fungal and bacterial biomass.. Higher fungal biomass should support the Ovavesicula fungal spores. Soil fungal biomass will predict better biocontrol sites. $85/sample x 40 samples x 2 seasonal times=$6,800, vendors not avilable in MN |
| **Contracts and Services** - Michigan State University Dr. Dave Smitley lab | Professional or Technical Service Contract | From 2021 to Nov 2023, the Post Doc processed samples and MSU ran the qPCR on the samples. From Nov 2023 to June 2024 the samples were sent to MSU for both processing and running of qPCR. | From 2021 to Nov 2023, the Post Doc processed samples and MSU ran the qPCR on the samples. From Nov 2023 to June 2024 the samples were sent to MSU for both processing and running of qPCR.<br>Contract to MSU to $49,000. April 2024 $10,000 was added from personnel to cover additional sampes for processing for a total of $59,000. |
| **Capital Expenditures** | | Purchase one tissue grinder Qiagen Tissuelyser II to grind samples for qPCR analysis. | At first tissue grinder/lyse seemed cheaper, but the estimate forgot the ginding tubes. The tissue grinder needed grinding tubes which were separate from the estimate from the motor so $1,100 was added to capital expenses. Totalcost is $11,271<br>**Additional Explanation :** The tissue grinder was less than anticipated. |
| **Travel Outside Minnesota** | Miles/Meals/Lodging | Air Fare $336 x2trips; rate $56/diem+$106 lodging=$162/day; Total=$3,973; we made fewer trips to learn the technique. | The grant requires the Post Doc to go to Michigan State University to the lab of Dr. David Smitely to learn how to identify the pathogen by molecular methods, since using microscopes and morphology has high error rates and takes time. Only in Dr. Smitely's lab can we learn the research. The Post Doc will develop in MN the techniques to identify the pathogen. Also, the Post Doc will learn how to survey the pathogen in the field and perform experiments on efficacy that Dr. Smiley has developed. We need to establish this pathogen in MN and we have a unique opportunity thru collaboration with MSU to do this. Establishing the pathogen in MN will reduce JB numbers and economic and environmental costs. |

## Non ENRTF Funds

| Category | Specific Source | Use | Status | $ Amount | $ Amount Spent | $ Amount Remaining |
|---|---|---|---|---|---|---|
| **State** | | | | | | |
| Cash | Federal money to the MDA called Specialty Grants to fund state research on plants and pest management. | Future grants will be pursued for project continuation. | Potential | $50,000 | - | $50,000 |
| In-Kind | 55% indirect cost waiver fee=$119,300 x 55%=$65,615 | Cost sharing by PI of salary and fringe | Secured | $65,615 | - | $65,615 |
| | | | **State Sub Total** | **$115,615** | **-** | **$115,615** |
| **Non-State** | | | | | | |
| | | | **Non State Sub Total** | **-** | **-** | **-** |
| | | | **Funds Total** | **$115,615** | **-** | **$115,615** |

# Attachments

## Required Attachments

### *Visual Component*

File: 9f60d5d0-a76.pdf

### *Alternate Text for Visual Component*

Research, outreach education, and demonstration projects to conserve pollinators by using biocontrol with a native fungus to control the exotic Japanese beetle in bee lawns, parks, and restorations....

## Supplemental Attachments

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

| Title | File |
|---|---|
| 2020-164 Krischik Invasive Species Biocontrol of Bee Lawns and Parklands cv | 1f147900-51c.pdf |
| LCCMR APRROVED_2021-164 Krischik Updated Research Addendum (4) March18 | bf2f0320-bfe.docx |
| Background check Certification Form for ENTRF Funding Recipients | 4bc53662-5a6.pdf |
| 2023 Outreach | a6feaff8-89f.pdf |
| 2023 Research | 75ed159b-dbf.docx |
| Research Table | 7e8ac2c0-8c2.docx |
| 1. 2022-2023 maps, JB numbers, spore numbers, infection rate | eeeef516-ef3.pdf |
| 2. 2022-2023  JB spores and Ct Values | 3b9caeda-d04.pdf |
| 3. 2024 Total JB site/yr Jul12 | 6f6183c1-c0e.xlsx |
| 4. 2024 JB site contacts | 41ee125d-2d1.pdf |
| 5. 2024 JB regression for soil and biomass | 1e459177-4a6.xlsx |
| 6. 2024 Research: Paper JB consumer | 738d844f-a52.pdf |
| 7. 2024 Resaerch: Paper Ova host range | 41088c45-d04.pdf |
| 8. 2024 Research: Paper biocontrol | 8f87383e-0ce.pdf |
| 9. 2024 Research: Poster biocontrol JB bee lawns | 17b74b98-e6b.pdf |
| 10. 2024 Outreach: LCCMR JB handout | cdd123e8-263.pdf |
| 11. 2023 Outreach: LCCMR bee lawns ppt | c499e705-920.pdf |
| 12. JB MN Golf Corse Superintendent's Hole Notes 57(5) June 2022 | 4b6d6c2f-e64.pdf |

### *Media Links*

| Title | Link |
|---|---|
| Krischik UM website: Pollinator Conservation and IPM | https://ncipmhort.cfans.umn.edu/ |
| Krischik UM website: UM CFANS CUES | https://pesticidecert.cfans.umn.edu/ |
| Krischik UM website: Consumer lawns infested with grubs | https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grubs |
| 12.2022 Bicontrol of JBMMGolf Corse SUperintendent's Hole Notes 57(5) June 2022 | https://issuu.com/mgcsa/docs/june_2022. |

# Difference between Proposal and Work Plan

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

budget updated to $425,000 limit; activities made clearer

## 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?**

Yes

**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?**

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

| 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 | Amendment Request | • Budget - Capital, Equipment, Tools, and Supplies | At UMN, we will process the Japanese beetle (JB) samples to search for the biocontrol, Ovavesicula pathogen. To do this, we must purchase a Qiagen tissuelyser 2 for $14,535 to grind samples. The processed sample will be sent to MSU to identify the pathogen DNA with an Applied Biosystems 7500 Fast Real-Time PCR System. We will decrease our disposable supplies by $14,535 to cover this added expense. | July 19, 2022 | Yes | July 19, 2022 |
| 2 | Amendment Request | • Narrative<br>• Budget - Professional / Technical Contracts | For the research we want to correlate soil qualities with the distribution of the fungus, so we can predict the best locations to release the fungus for establishment. We need to send samples to the UM Soil Testing lab fat $25 samples x 40 sites x 4 seasonal times =$4,000; fungicide a, herbicides, and neonic $150/sample x 40 samples=$6,000; and other pathogens $100 x 40 sites=$4,000, total $14,000. | September 12, 2022 | Yes | September 13, 2022 |
| 3 | Amendment Request | • Budget - Personnel<br>• Budget - Professional / Technical Contracts<br>• Budget - Travel and Conferences<br>• Budget - Printing and Publication | We want to continue to perform the soil analysis so we moved frunds from travel and printing into soil and fungal biomas analysis. The post Doc received a university postion so her remaning funds were moved to undergraduate salary. | June 29, 2023 | Yes | June 30, 2023 |
| 4 | Amendment Request | • Budget<br>• Budget - Personnel<br>• Budget - Professional / Technical Contracts<br>• Budget - Capital, Equipment, Tools, and Supplies | As in 2023, we need to transfer funds to MSU for qPCR DNA analysis to determine the pathogen presence in Japanese beetles. QPCR machines are very expense and MSU has a machine and technique for the pathogen determination. MSU is a | December 15, 2023 | Yes | January 3, 2024 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | • Budget - Travel and Conferences<br>• Budget - Printing and Publication | collaborator on the research. We need to move $33,000 into the contracts line for MSU. This is the last 6months of the grant. We will move funds from balances that are not needed. | | | |
| 5 | Amendment Request | • Budget - Personnel<br>• Budget - Professional / Technical Contracts<br>• Budget - Capital, Equipment, Tools, and Supplies<br>• Budget - Other<br>• Attachments | The UMN PostDoc received a job at University of Alabama, Huntsville and left early. Her salary savings were transferred to contracts.Funds of $15,000 were moved to contracts with MSU to finish the qPCR research by a PostDOc at MSU. Our collaborator, Dr.Dave Smitley, performs research on the JB soil pathogens Ovavesicula at MSU. Other budget field were adjusted. | May 10, 2024 | Yes | May 20, 2024 |
| 6 | Amendment Request | • Other<br>• Budget - Professional / Technical Contracts<br>• Budget - Capital, Equipment, Tools, and Supplies<br>• Budget - Other | We needed to move funds to cover qPCR supplies and mailing. The MSU contract was decreased by $1561 to match billed costs. The difference was used to pay for shipping ($153 for shipping) and qPCR supplies ($1408 for qPCR) to equal $1561. | June 11, 2024 | Yes | June 24, 2024 |
| 7 | Completion Date | Previous Completion Date: 06/30/2024<br>New Completion Date: 12/31/2024 | Administrative Work Around - LCCMR Staff | June 24, 2024 | Yes | June 24, 2024 |
| 8 | Completion Date | Previous Completion Date: 12/31/2024<br>New Completion Date: 06/30/2024 | Administrative Work Around - LCCMR Staff | June 24, 2024 | Yes | June 24, 2024 |

# Status Update Reporting

## Final Status Update August 14, 2024

**Date Submitted:** December 16, 2024

**Date Approved:** December 18, 2024

**Overall Update**
Research investigated ways to reduce insecticide use on bee lawns to protect pollinations.

Japanese beetle (JB), is a plant pest for which the most insecticides are used in urban areas. UMN collaborated with Michigan State University on surveying and establishing a JB pathogen as a biocontrol agent to kill JB, called Ovavesicula (microsporidia, fungi, spread by spores).

For the qPCR research we surveyed in 2022 44 sites and in 2023 34 sites for 2 dates and collected 80,000 JB/year. JB were preserved for spore counting and for qPCR analysis to identify Ovavesicula. Soil health and fungal biomass parameters were correlated to JB and pathogen numbers.

Outreach programs were designed to educate professionals, consumers, and park visitors on ways to reduce pesticide use. Our outreach programs included a table at the Monarch Festival for 2 years, an online educational program with site visits, and an online Advisory Committee meeting for stakeholders, state agencies, Master Gardeners, Golf Courses and Nursery Industry that met 3 times. Outreach included websites, 36 talks, 3 peer-reviewed papers, 8 articles for the nursery, golf course, and Master Gardener newsletters. Research supported 3 UMN UROP fellowships and a Post Doc that received a UAlabama professorship

**Activity 1**
Activity 1. Long term management. The goal is to survey the TwinCities for the presence of the JB specific Ovavesicula pathogen.

For 2 summers we surveyed in 2022 44 sites and in 2023 34 sites for 2 dates and collected 80,000 JB/year. Pathogens were found in 11 sites around Stillwater. Experiments showed that spores spread on leaves in traps can inoculate adults and can disseminate pathogens. We found only 4 grub infested sites (MPRB) that permitted us to release pathogens and not use insecticides.

Data analysis showed that spore counts and JB % infection (0.001) and qPCR machine values and JB % infection (0.018) were highly correlated, which means both qPCR analysis and spore counting quantify pathogens.

Soil samples were collected for 2 years for analysis for soil health (pH, N, P, K, and soil type) and biomass (fungal, bacterial) to understand the best soils for pathogens. Data showed that % pathogen was correlated to % bacteria (0.02), total biomass (0.025) and % protista (0.015). JB numbers were correlated to % moisture (0.01) and negatively correlated to % clay (0.003).

An online workshop available for all, had 40 who learned to reduce insecticide use.

All products acknowledge ENRTF.
*(This activity marked as complete as of this status update)*

**Activity 2**

Activity 2. Short term management. The goal is to use EPA registered biorational insecticides until JB biocontrol by the pathogen can increase.

The Ovavesicula pathogen is widespread in the East Coast where JB populations are low and correlated to Ovavesicula numbers. The efficacy of EPA approved biorational and conventional insecticides for killing JB were researched though LC50 bioassays. Bioassays showed that the bee friendly biorational insecticides chlorantraniliprole (Acelepryn), Beauveria, and Bacillus thuringiensis galleriae killed JB, but also killed painted lady butterfly larva. Scott's GrubEx (chlorantraniliprole, Acelepryn) does not harm bees visiting flowers in bee lawns. Consequently, biocontrol by the Ovavesicula pathogen is very important as most insecticides used to manage JB kill some pollinators. Outreach included websites, 36 talks, 3 papers. Research supported 3 UMN UROP fellowships and a Post Doc that received a UAL professorship.

In addition, research showed that commercial JB traps with lures can be filled with leaves covered with cellulose and pathogen spores. The spores were found on JB adults and in the adult's gut. The adults move the pathogen spores with them to oviposition sites in the turf, increasing pathogen spread to new sites. Using JB traps offers a better way to distribute pathogen spores.
*(This activity marked as complete as of this status update)*

**Dissemination**

The research and outreach program will help mitigate decline of native bees, butterflies, and beneficial insects that control pest insects. The USFWS identified insecticides and bee pathogens as a major factor contributing to the bee decline.

For 2 summers, we surveyed in 2022 44 sites and in 2023 34 sites for 2dates and collected 80,000 J/year. JB trapping established population numbers to establish JB population size and through dissection and morphological determination and qPCR analysis. However, qPCR analysis is faster.

In the JB online education program, we found out that 95% of the homeowners were using organic milky spore insecticide, that does not work. Instead, homeowners were educated to use bee friendly Scott's GubEx.

The outreach programs included a table at the Monarch Festival for 2 years, an online educational program with site visits, and an online Advisory Committee meeting for stakeholders, state agencies, Master Gardeners, Golf Courses and Nursery Industry that met online 3 times. Outreach included 8 articles for the nursery industry, golf course, and Master Gardener newsletters. Outreach included websites, 36 talks, 3 peer-review papers. Research supported 3 UMN UROP fellowships and a Post Doc that received a UAL professorship.

All products acknowledge ENRTF.

# Additional Status Update Reporting

## Additional Status Update August 14, 2024

**Date Submitted:** June 11, 2024

**Date Approved:** June 24, 2024

**Overall Update**
We performed research and outreach on  biocontrol of Japanese beetle (JB) using a soil inhabiting pathogen to reduce insecticide use in bee lawns, parks, and golfcourses. We have data on fungal and bacterial biomass and soil  health parameters from JB infested sites. This will permit us to predict sites preferred by JB. Since the last update the 2023 data has been received and we are correlating these soil and biomass parameter to JB population size. We are almost complete looking at the presence of the fungus at 30 parks and golfcourses for 2023. The 2022 dat is complete. Since the last update, we have looked at JB insecticides and found that they kill nontarget species such as painted lady butterflies. Since the last update, we made an online consumer JB educational project. We visited the consumer sites and collected beetle grubs. We send information back the consumers on how to manage JB and European Chafer grubs with insecticides that have fewer non-target effects. Please read the dissemination section to learn more about the outreach projects.

**Activity 1**
This activity is almost complete.

**Activity 2**
This activity is almost complete.

**Dissemination**
The research and outreach program will help mitigate decline of native bees and beneficial insects that control pest insects. The USFWS identified insecticides and bee pathogens as a major factor contributing to the decline of the urban dwelling endangered rusty-patched bumblebee, Bombus affinis. Research on the natural occurring pathogen of JB will help manage JB populations and it does not affect pollinators.

Outreach programs were delivered through educational bulletins, and websites. Information on the IPM programs were created and posted online at https://ncipmhort.cfans.umn.edu/ipm-krischik-lab-research/ipm-case-study-2021-2024-lccmr-biocontrol-bee-lawns-parks-and-landscapes.

We created a demonstration project online at https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grub.We worked with Master Gardeners and collected grubs on their lawns. They participated in an online course with a pre and post video survey. https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grub..Survey results showed that the video changed their management and they learned new information, and will stop using tactics that do not work.

We set up double sided IPM signs at 6 parks and golf courses promoting JB management with reduced risk insecticides to conserve pollinators.

We had an advisory committee meeting every year to update our collaborators on our resaearch and outreach projects.

# Status Update Reporting

## Status Update June 1, 2024

**Date Submitted:** June 11, 2024

**Date Approved:** June 24, 2024

**Overall Update**

The long-term outcomes of the project are to develop a biocontrol program for the invasive JB using a JB specific soil pathogens, which will reduce insecticide applications in urban areas and conserve pollinators. For two summers, we surveyed Japanese beetle (JB) populations at 30 golf courses and parks. We used qPCR analysis and spore counting to determine which sites were infected with the soil pathogen. Pathogens were found in 11 sites around Stillwater, the UM St. Paul campus, and two sites in Roseville. Experiments showed that using JB spores can inoculate adults with spores. We identified 4 sites at the MPRB to release pathogen spores.

In the short- term, the goal is to use EPA registered microbial insecticides. Bioassays showed that the biorational insecticides chlorantraniliprole (Acepepryn), Beauveria, and BT killed JB, but also killed painted lady butterfly larvae and bumblebee adults.

The advisory board met online three times with members from the MN DA, MN DNR, Lawn to Legumes program, Golf Course Superintendents Association, MNLA, and eight parks and golf courses.

We created a demonstration project online at https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grub.We setup double sided IPM signs at 6 parks and golfcourses.

**Activity 1**

Activity 1. Long term management.  Survey the state of MN for the presence of the JB specific Ovavesicula pathogen. For two summers, we surveyed Japanese beetle (JB) populations at 30 golf courses and parks. We used qPCR analysis and spore counting to determine which sites were infected with the soil pathogen. Pathogens were found in 11 sites around Stillwater, the UM St. Paul campus, and two sites in Roseville. Experiments showed that using JB spores can inoculate adults with spores. We identified 4 sites at the MPRB to release pathogen spores.

We worked with Master Gardeners and collected grubs on their lawns. They participated in an online course with a pre and post video survey https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grubs  Survey results showed that the video changed their managment and they learned new infomation, and will stop using tactics that do not work.

**Activity 2**

Activity 2. Short term management. In the short- term, the goal is to use EPA registered microbial insecticides. Bioassays showed that the biorational insecticides chlorantraniliprole (Acepepryn), Beauveria, and BT killed JB, but also killed painted lady butterfly larva a.The efficacy of new EPA approved microbial insecticides and new conventional insecticides for killing JB will be researched. Also, It will be determined if these insecticides are friendly to bees when used for JB grub control on bee lawns and JB adult control on flowers. Bioassays showed that the biorational insecticides chlorantraniliprole (Acepepryn), Beauveria, and BT killed JB, but also killed painted lady butterfly larva and bumblebee adults.

**Dissemination**

The research and outreach program will help mitigate decline of native bees and beneficial insects that control pest insects. The USFWS identified insecticides and bee pathogens as a major factor contributing to the decline of the urban dwelling endangered rusty-patched bumblebee, Bombus affinis. Research on the natural occurring pathogen of JB will

help manage JB populations and it does not affect pollinators.

Outreach programs were delivered through educational bulletins, and websites. Information on the IPM programs were created and posted online at https://ncipmhort.cfans.umn.edu/ipm-krischik-lab-research/ipm-case-study-2021-2024-lccmr-biocontrol-bee-lawns-parks-and-landscapes.

We created a demonstration project online at https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grub.We worked with Master Gardeners and collected grubs on their lawns. They participated in an online course with a pre and post video survey. https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grub..Survey results showed that the video changed their management and they learned new information, and will stop using tactics that do not work.

We set up double sided IPM signs at 6 parks and golf courses.

# Status Update Reporting

## Status Update December 1, 2023

**Date Submitted:** December 15, 2023

**Date Approved:** January 3, 2024

**Overall Update**

The long-term outcomes of the project are to develop a biocontrol program for the invasive Japanese beetle (JB), which will reduce insecticide applications and aesthetic and economic Japanese beetle (JB) damage. We surveyed and collected JB at 33 sites in year 1, summer 2022, and 38 sites in year 2, summer 2023. QPCR analysis was performed to determine the presence of the pathogen in the beetles and the pathogen distribution in MN. First year data showed that the pathogen was located around Stillwater, MN in 8 sites.Second year data is being processed. We counted spores in a second set of beetles in 2022 and 2023 to determine qPCR validity.

The short- term outcomes of the project are to develop IPM programs that use EPA registered microbial insecticides and other bee friendly insecticides, that conserve pollinators, to control JB. We found that Beauveria fungus and BTG bacteria effectively kill adults. The organic insecticide containing pyrethrins kill fewer JB compared to the synthetic pyrethroid called bifenthrin, which is degraded by light within a few days. We found that both microbial insecticides BTG and Beauveria kill painted lady larvae at rates that are stated on the label.

**Activity 1**

In summer 2023 we surveyed 30 consumer sites that had high scarab grub populations in their lawns from JB (in MN since 1990) or European chafer (EC, in MN since 2020) . Over 96% of the grubs (n=320) found were EC that feed on lawns with dryer soils compared to JB. We received ground JB solutions from MSU and will use this to establish 30 nursery sites in Spring 2023 at golf courses sites that were surveyed and found not to contain Ovavesicula pathogen spores. Ovavesicula is a native US pathogen first discovered in CT and kills only JB grubs and not invasive European chafer. The homeowner program, https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grubs, was accomplished with the help of MN Master Gardeners, by placing an article in their newsletter that led to a JB site at the UMN Extension website on our homeowners JB survey program. Over 130 homeowners registered for our online educational program that consists of a training video, educational bulletin, and pre and post surveys to determine if participants used less insecticides and had better management.

In addition, we set up display posters on managing JB thru IPM to reduce pollinators at 8 parks and beelawns.

**Activity 2**

We performed experiments to determine if JB traps, that are very effective in catching male and female beetles, can be used to disseminate the pathogen, rather than the current method of spilling spores on the soil surface near JB grubs. Over 3,000 samples were collected and are currently being analyzed by qPCR to determine if spores can be found in the adult gut or on the adult when they are exposed to spores in JB traps. Using traps to disseminate pathogens, rather than pouring spores on the ground, may be a better method of transmission. JB adults can potentially increase the number of spores as the adults probably move the spores to sites when they defecate and lay eggs. However, it is not know how spores are transmitted. to new sites and this is the reason why we are researching transmission.

We are analyzing soil for potassium, nitrogen, organic matter, compaction, and percent moisture (UMN soil testing lab, St Paul, MN) and fungal and bacterial biomass (Ward Labs, Kearney NE) to determine what soil parameters are correlated with JB adult numbers to predict JB spore release sites. Data shows that sites with higher fungal biomass support more JB adults.

**Dissemination**

Outreach programs were delivered with 8 educational posters at parks and bee lawns (Washington Co Conservation District bee lawn, Twin Cities Seed bee lawn, Lake Elmo Park bee lawn, MPR rose garden, MPRB Hiawatha Golf Course, UMN demonstration garden, UMN MAES plots), a booth and poster at the MPRB (MN Park and Rec Board) Monarch's Festival), online education website (https://ncipmhort.cfans.umn.edu/) , blogs on the Stimpmeter, emailed by the MCGSA (MN GC Superintendents Association), UMN Turf Field Days (2 in 2022 and 2 in 2023), Master Gardeners newsletter, an UMN Extension webpage, online JB educational program (https://ncipmhort.cfans.umn.edu/consumer-lawns-infested-grub), and 2 JB management online bulletins (https://ncipmhort.cfans.umn.edu/ipm-bmp-cultural-control/turf-best-management-practices). We published 4 articles in commodity journals: in the MCGSA Hole Notes and in the MNLA (MN Nursery and Landscape Association) Scoop in 2022 and 2023.

We provided talks on the JB biocontrol and bee lawn IPM to: 5 Master Gardener groups, 9 gardens clubs, 5 MNLA workshops, 3 MDA pesticide certification workshops, a USDA biocontrol workshops, and a National Entomology meeting.

The Advisory Board met online in August 2022 and 2023 to discuss the research and outreach programs.

# Status Update Reporting

## Status Update June 1, 2023

**Date Submitted:** June 29, 2023

**Date Approved:** June 29, 2023

**Overall Update**

Japanese beetles (JB) damage plants as adults and turf as the larval (grub) stage. Ongoing research concerns the distribution and utilization of a native pathogen, Ovavesicula, that lives in soil. JB makes contact with the pathogen when grubs are in the soil and the pathogen effectively weakens and kills both grubs and adults. The Krischik Lab at the University of Minnesota is collaborating with Michigan State University, where the fungus has been studied for 10 years, and was eventually released in five states. At 6 years post inoculation, JB grub numbers have dropped by 50%.

In summer 2022, we collected 77,326 JB from 44 sites, sampling each site 4 times from July 5 through Sept (Map 1, blue). The fewest JB recovered at one sites was1 and the most were 3,951. The Northwestern side of the Metro had the fewest JB. Ovavesicula was found around Stillwater at 8 sites (Map 2). For the 44 sites, 36 sites had 0 spores and 0 qPCR values and 8 had both, which shows excellent techniques. Soil samples were analyzed for various parameters and fungal biomass. In Fall 2023 the pathogen will be released at 30 sites. Maps are found in attachments.

**Activity 1**

2023 Research is in progress.

In summer 2023, we collected JB from 52 sites, sampling each site 4 times from July 5 through Sept. At each site for 4 time periods, JB are dissected and spores are counted and JB are processed and undergo qPCR analysis for spore presence. In Fall 2023 we will release the pathogen at 30 sites to encourage its spread as a natural form of JB biocontrol. Through the Master Gardener online newsletter we requested locations with grub infestations and we received over 146 emails with complaints of grubs destroying lawns. We will visit 30 sites to make sure that the grub infestations are large and are JB and not European chafer. We will release pathogen spores at 30 sites to create a nursery for JB to move spores around the Twin Cities metro areas. We will collect soil samples for biomass (Wards fungal and bacterial biomass lab) and soil chemical analysis (UM soil testing lab).

**Activity 2**

2023 Research is in progress

In progress research on using JB traps to disseminate JB fungal pathogens from phoresy or feces.

Current practices used by MSU (Michigan State University) are to pour spores on the ground where grubs are found. We are using JB traps that are excellent at collecting JB, which we will contaminate with fungal spores. The JB adults are released from the JB traps with spores on their body (phoresy) or the spores in their guts (ingestion) from feeding on leaves infected with the fungal pathogen in the traps. Using JB traps will allow the inoculation of more JB adults then pouring spores on the soil surface, which wastes the spores.

In progress research on insecticides used to control JB and their non-target effects.

The biorational insecticides chlorantraniliprole (Acelepyrn), Beauveria bassiana fungus and BTG bacteria all kill painted lady butterfly larvae in 96hr LC50 bioassays. We will test these biorational insecticides on bumble bees. Painted lady butterfly larvae and bumble bees can forage and live in bee lawns. We are trying to determine which insecticides kill JB grubs, but do not have non-target effects on butterflies and bees.

**Dissemination**

2023 Outreach in progress

1. “Think IPM” poster at 8 bee lawns (MPRB Lyndale Gardens and Hiawatha GC, Twin Cities Seed, Washington County

parks (3), UM campus, Stillwater Pollinator G
2. Provide articles in Golf course (MGCSA) Stipmeter and Master Gardener newsletters on the research progress and JB management and pollinator conservation.

2022 Outreach
1. Website developed with information on biocontrol of JB grant activities, JB management information.
and JB biocontrol at https://ncipmhort.cfans.umn.edu/

2. Over 44 talk to professionals and consumers on JB biocontrol and management.

3. Outreach poster and participation at Monarch Festival field at Lake Nokomis (09/10/22)
For this event we put together a table displaying bulletins and t“Think IPM” posters that identified ongoing research on JB and informed the public about pollinator conservation, integrated pest management (IPM), and natural biocontrol methods. The large double-sided posters included a QR code for a direct link to further in-depth information included on our lab’s website. JB trap displays and additional information were also made available for viewing/reading. Krischik Lab researchers were present to answer community questions and increase the accessibility of our research.

4. “Think IPM” poster displayed at Lyndale Park Gardens.

# Status Update Reporting

## Status Update December 1, 2022

**Date Submitted:** January 12, 2023

**Date Approved:** January 23, 2023

**Overall Update**

Substantial research progress on biocontrol of invasive Japanese beetle (JB) in parks and landscapes has been made. The distribution and abundance of the JB pathogen, Ovavesicula, is being evaluated by collecting adult JB and counting spores in their guts and analyzing samples for the pathogen's DNA. Adult JB and soil were collected at 40 sites in the Twin Cities at least 2 times from July to September 2022. Adult beetles are being dissected and spores counted in the guts. Other samples of adult JB are being processed in our lab and the samples sent for qPCR analysis to Michigan State University (MSU) to be analyzed using their methods on their qPCR machine for pathogen identification. The JB pathogen Ovaveiscula is not evenly distributed in MN, so we are identifying soil characteristics in sites that correlate with the highest number of spores. Currently, soil samples are being analyzed for organic matter, texture, pH, nitrates, K, and P by the University of Minnesota soil testing lab. Fungal biomass is an important index of soil health and soil is being analyzed for fungal biomass, species, and ratios that are indicators of soil health. Better biocontrol release sites can be identified by this method.

**Activity 1**

Activity 1. Pathogenic biocontrol in bee lawns and parks. Long term management. Survey the state of MN for the presence of O. popilliae, which will be identified through collaborations with Dr. Dave Smitley of Michigan State University. Through collaborations with parks, golf courses, and cemeteries,we established 40 sites in MN to look for the distribution and abundance of the pathogen. Samples were collected from each site at least 2 times from July to September 2022.The distribution and abundance of the JB pathogen, Ovavesicula, is being evaluated by collecting adult JB and counting spores in their guts and analyzing samples for the pathogen's DNA. Soil samples are being analyzed for different parameters (organic matter, texture, pH) that can then be used to predict what are the better release sites for the pathogen. Soil is being analyzed for fungal biomass, species, and ratios that are indicators of soil health. In 2023, we will collect a second year of data. We have published one paper in a peer review paper on this research, edited 2 extension bulletins, created a website to, provided talks, and provided demonstration sites at parks to share this information with professionals and consumers. In progress.

**Activity 2**

Activity 2. Biocontrol in bee lawns and parklands using IPM. Site specific short term management. Previous biorational control methods were shown to be ineffective controlling JB. Milky spore disease that was widely used in the 1950s to manage JB is no longer effective. Imidacloprid, a conventional neonicotinoid insecticide that was widely used on turf for JB grubs, is now being banned due to its harmful effects on pollinators

Research investigated 2 biorational insecticides that can kill JB adults, but not pollinators. Research was started with Beauveria bassiana fungus (Mycotrol) and Bacillus thuringiensis galleriae bacteria (BeetleGone), that are both EPA registered and commercially available. Bacillus thuringiensis galleriae bacteria kills beetles by lysing their guts. The fungus Beauveria is eaten with leaves and takes over the insect, killing it from inside. We found that it both can kill JB adults on foliage. We can recommend their use to professionals and consumers to control JB adults. In 2023, we need to test other available biorational insecticides for managing JB and protecting pollinators.

In 2023, we will study novel methods, such as using JB traps, to help spread the Ovavesicula biocontrol pathogen In progress.

**Dissemination**

Multiple distribution methods were developed to provide outreach on the research to professionals and consumers in MN. First, we have created a new website (Pollinator Conservation, http://ncipmhort.cfans.umn.edu/ ) with information on the research that is updated as we collect data. Secondly we updated 2 UMN extension bulletins on JB to explain the research, and to suggest alternative insecticides to control JB that conserve bee, butterflies, and beneficial insects. Thirdly, we have written articles for the commodity journal of Golf Course Superintendents (HoleNotes journal and the Stipmeter newsletter) so they could learn about the benefit of the research and provide collaborations for the research by using their sites for JB and soil collection. Fourthly, we have organized an Advisory Committee (20 plus members from state agencies, commodity groups, consumer groups and UM educators) and had an online meeting to discuss the project and the benefits of using biocontrol rather than insecticide to manage JB. By using biocontrol, insecticide use is decreased with benefits to environmental and human health. Fifthly, we have set up displays at 3 Minneapolis and Park Board sites: Monarch Festival , Rose Garden, and Hiawatha Golfcourse. Researchers provided over 15 local and interstate talks. In progress.

# Additional Status Update Reporting

## Additional Status Update August 18, 2022

**Date Submitted:** September 12, 2022

**Date Approved:** September 13, 2022

**Overall Update**

Substantial grant progress was made in research as we collected beetles and soil from 40 sites on 4 seasonal dates. Also, bioassays on the effects of microbial insecticides that are pollinator friendly, but kill adult beetles are in progress. Greenhouse space with proper equipment was setup for research on the use of traps to disseminate the fungus to beetles. Collaborations with Michigan State University are in progress and the PostDoc on the grant has visited the MSU lab to learn of the techniques. Equipment was purchased and setup at UMinnesota to process the samples, which will then be transported to MSU for DNA identification on their qPCR machine. Substantial progress in outreach and dissemination was made as well. An Advisory Committee meetings was held with state agencies, consumer, and commodity groups to learn of the grant's research and outreach activities to further support collaborations and dissemination of the research. Participation in field days for the golf course superintendents and for the Minneapolis Park and Recreation Board was accomplished. Dissemination of the grant's research is being accomplished thru a website, bulletins, online course, and field day.

**Activity 1**

We have established 40 sites to collect beetles and soil (from Alexandria to Chaska to Stillwater to Burnsville) and have sampled them 4 times in 2022. The samples are stored in the freezer for analysis. The PostDoc on the grant visited our colaborator's lab, Dr. Dave Smitley of Michigan State University, and learned how to identify the pathogen thru morphology and DNA qPCR analysis. Equipment and supplies were purchaased from the grant, and a lab setup at the University of Minnesoata to process the samples for DNA. The samples will be taken by the PostDoc to MSU for DNA identification using MSU's qPCR machine as they cost $45,000 new and we cannot purchase one on the grant. Also, we do not have the exact same machine to use at UMN. Data from our collaborators from MN samples demonstrated that the soil pathogen is present tin MN. Other samples from around the US, show that the pathogen is native and endemic, and therefore research to release it is appropriate as it is native. So far the pathogen has been released in 4 other states from MSU colonies.

**Activity 2**

We tested the efficacy of microbial compared to conventional insecticides on Japanese beetle adults. Based on research in this proposal, new IPM programs will be implemented that employ microbial insecticides The EPA registered microbial insecticides that will be studied, are GrubGone (Bacillus thuringiensis galleriae, BTG, recently available), a soil applied fungus (Beauveria bassiana), bee friendly Acelepryn (chlorantraniliprole) compared to standard neonicotinoids (imidacloprid, MeritG and clothianidin, ArenaG) and pyrethroids (Talstar). So far we have performed standard LC50 tests on BTG batceria, Beauveria fungus, and mixtures of the 2 on Japanese beetle mortality. We want to know if mixtures suppress or enhance the action of the microbes. We have plans for future bioassays with a fungicide (chlorothalonil), herbicide (glyphosate) on Beauveria survival and spore production. We have read papers that suggest that soil fungi are inhibited by fungicides and herbicides. Beauveria serves as a model system for the Ovavesicula pathogen. We have plans to receive fungal spores and use them in experiments on adults to determine various ways the fungus affects the adults. This information is vital to use, because you want to perform future experiments on the use of Japanese beetle traps to disseminate Beauveria and Ovavesicula

**Dissemination**

From Sept 2021 to Aug 2022, we worked with professional landscape managers of parks and greenspace to provide technical information on how to manage pests with site specific IPM programs that use biorational insecticides that

conserve pollinators and good bugs that kill pest insects. We developed a website on IPM and pollinator conservation for different greenspace, new bulletins with undated information on managing Japanese beetles, and provided talks at meetings and workshops to consumers and professionals. In addition, we developed an online course on using biocontrol insecticides to reduce pest insects and what biorational insecticides we kill specific pests to reduce pesticide use and conserve pollinators. We had a zoom meeting with members of the Advisory board to tell them of our goals and progress. From this, 3 state agencies have contacted us for technical information to deliver to their clients. We are working on establishing a collaboration with the Minneapolis Park and Rec Board and Washington County Parks to provide a demonstration sign about IPM and biocontrol of Japanese beetles ad participate at field days at those sites. We have 3 upcoming talks to scientists and managers on the research program.

# Status Update Reporting

## Status Update June 1, 2022

**Date Submitted:** June 24, 2022

**Date Approved:** July 5, 2022

**Overall Update**
The proposed research and outreach program is to establish a biocontrol program to manage the invasive, exotic Japanese beetle to reduce insecticide use in bee lawns and parks.

For outreach, the LCCMR sponsored website was updated with information about Japanese beetle (JB) biocontrol with the Ovavesicula fungal pathogen and recommendations for beneficial insect friendly current management, https://ncipmhort.cfans.umn.edu/ipm-case-studies/ipm-case-study-2021-2024-lccmr-biocontrol-bee-lawns-parks-and-landscapes and https://ncipmhort.cfans.umn.edu/ipm-case-studies/ipm-case-study-japanese-beetle.

Two UMN Extension bulletins were updated:
https://ncipmhort.cfans.umn.edu/sites/ncipmhort.cfans.umn.edu/files/2022-03/2021-Japanese-Beetle-Management-in-Minnesota.pdf and https://ncipmhort.cfans.umn.edu/sites/ncipmhort.cfans.umn.edu/files/2022-03/2021-Managing-turf-insects-in-turf-and-bee-lawns.pdf

An Advisory Committee of 20 people was initiated with members from state agencies (MDA, MDNR, BWSR) parks (Carpenter Nature Center, MPRB, Washington County Parks, commodity groups, UM, businesses, NGO.

For the research, 30 plus golf courses and parks were visited for bimonthly JB collection and provided cooperator letters and an online JB survey, https://ncipmhort.cfans.umn.edu/biocontrol-japanese-beetle-2022, an article in MGSA Hole Notes magazine, https://issuu.com/mgcsa/docs/june_2022, and a blog in MGSA Stimpmeter.

The Post Doc learned the qPCR technique at MSU and is creating a UM lab.

**Activity 1**
For the research a Post Doc was hired to perform the qPCR research to identify the pathogen with MSU methods and a Researcher for bioassays.

Activity 1.1. Surveys on pathogen and JB distribution in MN.
For the research, 30 plus golf courses and parks were visited for cooperation in collecting by UM bimonthly JB collection and provided cooperator letters and an online JB survey, https://ncipmhort.cfans.umn.edu/biocontrol-japanese-beetle-2022, an article in MGSA Hole Notes magazine, https://issuu.com/mgcsa/docs/june_2022, and a blog in MGSA Stimpmeter.

Activity 1.2. Obtain approval from MDA to release the pathogen, perform lab and field studies to determine efficacy and spread.
The Ovavesicula fungal pathogen is native to the US and determined in 1987 by Andreadis in CT and in 2013 by Petty in AR. Also, Krischik has send JB samples to MSU for the past 3 years and the fungus was determined in samples form a Stillwater golf course and the UM Saint Paul campus (2/4 sites). Consequently, the fungus is spread over the US. This research was discussed with MDA and the DNR

Outcome 1.3. Quantify number infected grubs and adults.
In progress, learning MSU

**Activity 2**
In summer 2021 the JB research could not start until UM processed and sent the appropriate approvals, which were not received by the Entomology Business Manger Andrea Little until Sept 15 2021. By September 15 JB adults were no longer flying and available, so research was not possible. Consequently, no research could be performed in summer of 2021.

In 2022 a JB research paper was written about the use of microbial pathogens as biocontrol agents and as insecticides and is currently under peer review for publication.

In order to perform the research, we obtained greenhouse space, insecticides, supplies, and rearing systems for maintaining JB adults. A lab manager and Post Doc was hired to perform the research.

Outcome 2: Short term management, bioassays on JB and pollinators, develop IPM program.
Research will begin in August 2022 when JB adults start flying.

Outcome 2.1. Bioassays for adults, grubs, and bees.
Research will begin in August 2022 when JB adults start flying.

Outcome 2.2. Determine if JB traps can disseminate microbial BTG pathogens.
Research will begin in August 2022 when JB adults start flying.

**Dissemination**
For outreach and research dissemination, the LCCMR sponsored website was updated with information about Japanese beetle (JB) biocontrol with the Ovavesicula fungal pathogen and recommendations for beneficial insect friendly current management, https://ncipmhort.cfans.umn.edu/ipm-case-studies/ipm-case-study-2021-2024-lccmr-biocontrol-bee-lawns-parks-and-landscapes and https://ncipmhort.cfans.umn.edu/ipm-case-studies/ipm-case-study-japanese-beetle.

Two UMN Extension bulletins were updated with project information
https://ncipmhort.cfans.umn.edu/sites/ncipmhort.cfans.umn.edu/files/2022-03/2021-Japanese-Beetle-Management-in-Minnesota.pdf and https://ncipmhort.cfans.umn.edu/sites/ncipmhort.cfans.umn.edu/files/2022-03/2021-Managing-turf-insects-in-turf-and-bee-lawns.pdf

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Demonstration projects at public gardens, parks, and golf course are being developed. A brochure has been created about the project, https://ncipmhort.cfans.umn.edu/biocontrol-japanese-beetle-2022. The MN arboretum and the MSPR board were contacted.