M.L. 2016 Project Abstract
For the Period Ending July 30, 2021

PROJECT TITLE:	MITPPC Sub-project 3: Genetic Control of Invasive Insect Species: Phase I
PROJECT MANAGER:	Michael Smanski
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FUNDING SOURCE:	Environment and Natural Resources Trust Fund
LEGAL CITATION:	M.L. 2016, Chp. 186, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $296,655
AMOUNT SPENT: $296,655
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results
We have demonstrated (in lab cages) a powerful new approach to combat invasive insect pests. Genetically engineered male insects would be released to mate with wild females, who would not have offspring. This can crash a wild population, and it is applicable to any sexually reproducing insect.

Overall Project Outcome and Results
With the overall goal of demonstrating our innovative genetic biocontrol approach in the pest insect Spotted Wing Drosophila, we had three specific objectives on this project: (i) demonstrate a proof-of-concept in the model laboratory insect and close cousin to SWD, *Drosophila melanogaster*, (ii) translate what we learned from *D. melanogaster* into the SWD species, and (iii) study the genome sequence of wild SWD so we can precisely design our engineered biocontrol agents to effectively suppress wild SWD populations in Minnesota.

Our outcomes and results for the first objective exceeded project expectations. We succeeded in making the proof-of-concept in *D. melanogaster*, and the engineered insects were 100% incompatible with wild-type flies. We made over a dozen versions. We also added additional genetic control elements to automatically sort the males from females, making the technology more economical to deploy for pest control.

We did not meet our objective two milestones (completing the engineering of SWD), however, we made good progress in that direction. Near the end of the award, we succeeded in making our first transgenic SWD flies, so we should be able to move quickly now in finishing the engineering process.

Our results from the third objective exceeded expectations. While we initially planned to sequence the genome of 20 wild-caught flies, we instead invented a new approach that allowed us to sequence the relevant genes from over 10,000 wild flies. We are using this data in our current engineering efforts with SWD.

This was a high-risk/high-reward project. We were able to overcome a tremendous amount of technical risk on the project so far, and the approach is looking very promising. We plan to continue to make progress towards Objective 2 in our second Phase of this project.

Project Results Use and Dissemination
We have disseminated our results through the normal channels available to academic labs (regional, national, and international conferences and workshops; peer-reviewed publications; patents; etc.). We are most proud of two high-impact publications from this work. The first was published in [Nature Communications](#) in 2020, and the

second is currently undergoing peer review at a top-ranked journal. We will have at least two additional papers submitted in the next year that stem from this project.

PROJECT TITLE: Subproject #4: Dwarf Mistletoe Detection and Management in Minnesota
PROJECT MANAGER: Marcella Windmuller-Campione
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: ML 2016, CH 186, Art. 2, Sec. 6a

APPROPRIATION AMOUNT: $ 433,250
AMOUNT SPENT: $ 433,250
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results

We were able to identify key considerations for the early detection of the invasive American dwarf mistletoe on jack pine, including different detection methods and the need for field-level biology and identification education for foresters and loggers.

Overall Project Outcome and Results

American dwarf mistletoe is an invasive species that infects and kills jack pine, a native tree species of Minnesota. American dwarf mistletoe is not currently present in Minnesota but has been detected in neighboring Canadian provinces. The goal of our project was to utilize Minnesota's native dwarf mistletoe, eastern spruce dwarf mistletoe (ESDM), to explore options for detection and management. Just like American dwarf mistletoe, ESDM results in mortality for its host tree, black spruce. We tested different types of detection methods. Google Earth was able to detect mortality, but we were unable to determine if mortality was caused by ESDM. Winter sampling resulted in higher potential false positives due to snow cover on tree. Summer sampling provided a clear view of the trees but movement within the stands were more difficult. Summer sampling was also used to explore impact of ESDM on forest ecosystems. ESDM is not a binary variable; lower levels of ESDM in black spruce stand resulted in higher tree species diversity and did not negatively impact regeneration.

With this new insight we explored different methods for predicting ESDM at the individual tree level and at the stand level using multiple different datasets. At the landscape level, we identified areas that have greater potential for impact from ESDM and linked those with stand and environmental variables which can provide foresters and natural resource management tools to prioritize management.

An additional part of our project was conducting focus groups and surveys with foresters and loggers within northern Minnesota. We found variable opinions regarding management and knowledge about ESDM and foresters and loggers identified the need for additional information about mistletoe and more data on results of management. We identified the need for training as a key component when considering early detection for the invasive American dwarf mistletoe.

Project Results Use and Dissemination

Results have been shared through talks at local, regional, and national meetings. We shared results through a special symposium: Lake States Lowland, Wet, and Floodplain Forests. Published papers include:

- Influence of eastern spruce dwarf mistletoe on stand structure and composition in northern Minnesota,

- The Difficulty of Predicting Eastern Spruce Dwarf Mistletoe in Lowland Black Spruce,

- Results of a Qualitative Assessment of Northern Minnesota Loggers' and Foresters' Perspectives and Experiences with Dwarf Mistletoe in Black Spruce Stands, and

- Results of a Survey of Minnesota Foresters Regarding Knowledge of and Treatment Practices for Dwarf Mistletoe in Black Spruce Stands in Northern Minnesota .

ML 2016, Ch 186, Sec. 2, Subd. 6a Minnesota Invasive Terrestrial Plants and Pests Center
For the Period Ending June 30, 2022

PROJECT TITLE: Studies of Fungi Associated with the Emerald Ash Borer: Finding effective biocontrol agents and elucidating the role of fungi during ash decline and mortality.
PROJECT MANAGER: Robert Blanchette
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WEBSITE: https://mitppc.umn.edu/project/connecting-fungi-and-emerald-ash-borer
http://forestpathology.cfans.umn.edu/publications.htm
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: ML 2016, Ch 186, Sec. 2, Subd 6a

APPROPRIATION AMOUNT: ~~$472,757~~ $500,000
AMOUNT SPENT: ~~$472,757~~ $500,000
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results
Important new findings have been obtained about the fungi associated with the emerald ash borer (EAB). This knowledge helps better understand the biology and ecology of EAB invasion and provides new biological control agents that can be used to help manage this invasive pest.

Overall Project Outcome and Results
The emerald ash borer (EAB) is an exotic beetle that has been introduced into the United States and is currently causing serious losses of ash trees in Minnesota. To effectively manage this pest, it is essential to understand the biology and ecology of the beetle and associated microorganisms. Our research has identified the diverse fungi that are associated with EAB. These include i) canker causing fungi that work along with EAB to kill trees, ii) aggressive pioneer decay fungi that enter EAB wounds and cause hazardous conditions in ash trees attacked by the beetle and iii) fungi that can kill EAB with potential use as biological control agents. Laboratory and field studies have been done to test the pathogenicity of selected fungi on eggs, larvae and adult EAB. These studies have shown that fungi can kill EAB and several species have been evaluated and are now available for field trials. This method of control for EAB provides an additional tool that natural resource managers will be able to use to control the pest. Methods of spraying and injecting trees have also been tested. Other fungi obtained from EAB galleries produce lesions and pathogenicity studies show that several of these canker causing fungi work in concert with EAB to kill trees. We also have a better understanding of the pioneer species of decay fungi that come into wounds made by EAB. These fungi cause extensive decay and strength losses early in the colonization process resulting in affected ash to become hazardous. These results, which are especially important in the urban landscape, indicate that timely tree removal is needed to avoid hazards produced by EAB associated wood decay fungi. Our research results provide important new findings for integrated pest management that will benefit Minnesotans long into the future.

Project Results Use and Dissemination

Results have been disseminated in scientific publications, presentations and news releases. This includes journal articles on the diverse fungi associated with the emerald ash borer and fungi from EAB that produce cankers in ash trees, as well as presentations on the ovicidal effects of fungi on EAB and other aspects of biological control and management of EAB. Numerous news releases on fungi attacking EAB, protecting Minnesota's ash trees and others have taken place.

ML 2016 Project Abstract
For the Period Ending February 28, 2022

PROJECT TITLE: MITPPC sub-project 2: Understanding the benefits and costs of using goats for invasive plant control
PROJECT MANAGER: Tiffany Wolf
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WEBSITE:
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: ML 2016, Ch. 186, Sec. 2, Subd 6a
$3,750,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota for the Invasive Terrestrial Plants and Pests Center to conduct research to prevent, minimize, and mitigate the threats and impacts posed by terrestrial invasive plants, pathogens, and pests to the state's prairies, forests, wetlands, and agricultural resources. This appropriation is available until June 30, 2023, by which time the project must be completed and final products delivered.

APPROPRIATION AMOUNT: $410,267
AMOUNT SPENT: $410,267
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results
Targeted grazing by goats demonstrates some benefits for the control of invasive *Rhamnus cathartica* and the enhancement of native plant communities. While *P. tenuis* transmission to goats remains a concern during invasive plant management, co-grazing goats with waterfowl may mitigate this seasonal disease risk.

Overall Project Outcome and Results
The use of goats for invasive plant control is increasing, yet few data exist on the effects of goat browsing on invasive species populations or native plant community composition. The cost of this management strategy is also elevated in some regions due to mortality caused by a parasite of white-tailed deer, *Parelaphostrongylus tenuis,* that goats may be exposed to when browsing in areas where infected deer defecate. To address these issues, we used *Rhamnus cathartica* as a target species to quantify the short- and long-term effects of goat browsing for invasive plant control and non-target impacts on associated native plant communities. We found that goats provide temporary suppression of *R. cathartica* abundance but this invasive shrub rebounds following grazing cessation. Native vegetation was similarly temporarily suppressed, but in some cases native plant diversity reached higher levels following grazing treatments. A broader synthesis and meta-analysis of the targeted grazing literature revealed similar patterns for the effects of goats and other livestock used for targeted grazing of invasive or undesired plant populations. Importantly, consumption by goats kills the seed of *R. cathartica*, and other invasive plants with larger seeds, indicating that goats are unlikely to exacerbate invasions by spreading them to new areas. Finally, in evaluating the *P. tenuis* risk to goats, we conducted a retrospective study of *P. tenuis*-associated mortalities of small ruminants in Minnesota over a 19-year period, as well as examined whether co-grazing goats with waterfowl could reduce transmission risk through waterfowl consumption of the gastropod intermediate hosts that harbor this parasite. Overall, we determined that the *P. tenuis*-associated mortality rate of goats is low (<1%), though it is unclear how browsing for invasive plant control might affect this level. Through our co-grazing experiments, we found more gastropods in habitats after goats had browsed alone; however, we did not observe these increases when goats were co-grazed with waterfowl. In addition, waterfowl did not negatively affect overall gastropod abundance or diversity. Thus, waterfowl could reduce *P. tenuis* risk to goats without harming gastropod communities.

Project Results Use and Dissemination

Research highlights were regularly disseminated throughout the duration of our project. Over the course of our research, our project was featured in eight popular press articles within the midwest region, one Minnesota radio station and PBS's Prairie Lawn and Garden show. Our project team leveraged our research in 11 education events targeting primary, secondary, higher education-level students, and community members, and seven presentations to university, local, regional, and national scientific, natural resource management, and public audiences. We've also had four manuscripts published in peer-reviewed scientific journals, with a fifth nearing submission. Project highlights have also been shared on <u>Twitter</u>.

M.L. 2016 Project Abstract
For the Period Ending December 31, 2022

PROJECT TITLE: MITPPC Sub-project 9: Genetic Control of Invasive Insect Species: Phase II
PROJECT MANAGER: Robert Venette
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WEBSITE: https://mitppc.umn.edu/
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a

APPROPRIATION AMOUNT: $3,750,00
AMOUNT SPENT: $3,604,542
AMOUNT REMAINING: $ 145,458

Sound bite of Project Outcomes and Results
The Minnesota Invasive Terrestrial Plants and Pests Center (MITPPC) funded 10 research sub-projects through this appropriation to protect Minnesota lands from the harmful effects of 11 priority invasive species such as common buckthorn, emerald ash borer (EAB), and several knotweeds. Results from these projects were featured prominently by local media.

Overall Project Outcome and Results
Ten MITPPC research teams, funded through this appropriation, made transformative discoveries about 11 priority terrestrial invasive species (TIS). These findings yielded exciting approaches to prevent TIS damage in Minnesota. Collectively, these projects captured the public's attention, as demonstrated by extensive media coverage. The projects were also severely disrupted by COVID-19. Nevertheless, teams made remarkable progress. Here are three highlights:

- <u>Fungi with EAB </u>(Robert Blanchette, lead). This team revealed over 100 fungal species associated with EAB in ash trees. This team was the first to discover that some of these fungi rapidly decompose wood, explaining why trees attacked by EAB can quickly become hazard trees, with falling limbs affecting people's safety and property. Other fungi can infect and kill EAB; these fungi are being pursued as biological control agents.
- <u>Goats to manage common buckthorn</u> (Tiffany Wolf and Daniel Larkin, leads). Goats are widely used to manage common buckthorn, but goats in buckthorn may get infected by a lethal brain parasite. This team discovered that domesticated ducks and geese, pre-released into a buckthorn stand, will consume the snails and slugs that are intermediate hosts for the parasite, and lower chances for goat infection. Goats also digest >99% of buckthorn seeds, so they are unlikely to move buckthorn from site to site. Goats temporarily open sites and lower buckthorn densities, but without other management, buckthorn quickly rebounds.
- <u>Plants to shade out buckthorn</u> (Peter Reich, lead). Young buckthorn plants die if they do not get adequate light. This team demonstrated that native grasses, forbs, shrubs, and trees, if planted when buckthorn is removed/treated, can create enough shade to prevent buckthorn from returning. Elderberry is particularly effective. Buckthorn does not have a long-lived seed bank, so intensive management can provide significant long-term benefits.

All ten projects directly addressed needs from TIS managers across Minnesota.

Project Results Use and Dissemination

Research teams were very active in disseminating their research results to broad audiences. Collectively, these teams published 30 peer reviewed articles in esteemed journals such as Restoration Ecology, PloS Genetics, and Biological Invasions. Research teams made over 70 presentations to professional audiences, educational institutions, and land management groups. Regional and statewide media, ranging from the Duluth News Tribune to KAXE radio in Grand Rapids, to the Mankato Free Press to WCCO, carried stories about our research and extended the reach of our results. The University of Minnesota Extension is incorporating findings into programs to reinforce lessons learned.

Date of Report: August 18, 2023 [for activities completed June 30, 2023; accounts reconciled August 15, 2023]

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2023

PROJECT TITLE: Minnesota Invasive Terrestrial Plants and Pests Center – Phase III

Project Manager: Robert Venette

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Web Address: www.mitppc.umn.edu

Location: Statewide

Total Project Budget:	$3,750,000
Amount Spent:	$3,604,542
Balance:	$145,458

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a

Appropriation Language:
$3,750,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota for the Invasive Terrestrial Plants and Pests Center to conduct research to prevent, minimize, and mitigate the threats and impacts posed by terrestrial invasive plants, pathogens, and pests to the state's prairies, forests, wetlands, and agricultural resources. This appropriation is available until June 30, 2023, by which time the project must be completed and final products delivered.

I. PROJECT STATEMENT:

Funding is requested to accelerate priority research that will protect Minnesota's prairies, wetlands, forests, and agricultural resources from terrestrial invasive plants and pests, including non-native weeds, pathogens, and insects. The Minnesota Invasive Terrestrial Plants and Pests Center (MITPPC) leads research that will provide new tools and techniques to:
  ● predict and prevent the arrival of new terrestrial invasive threats (e.g., pathway analyses for giant hogweed, soybean rust, and Asian longhorned beetle)
  ● detect and rapidly respond to new pest arrivals in the state (e.g., early detection tools for Dalmatian toadflax and brown marmorated stinkbug)
  ● mitigate impacts from well-established threats (e.g., improved integrated pest management for soybean aphid, buckthorn, and oak wilt);
  ● minimize impacts from measures to control invasive threats (e.g., protection of water quality and wildlife habitat);

The MITPPC was established at the University of Minnesota under ML 2014, Chapter 312, Article 13, Section 44. The MITPPC is administratively located in the College of Food, Agricultural, and Natural Resources Sciences. Research and outreach activities of the Center are conducted in close collaboration with state, federal, local and tribal governments, nongovernmental agencies, the private sector, Extension, and other colleges and universities. The MITPPC leverages existing expertise and infrastructure at the University, including Research and Outreach Centers located across the state, and the Departments of Entomology, Plant Pathology, Agronomy & Plant Genetics, Horticultural Science, Applied Economics, Fisheries Wildlife & Conservation Biology, Bioproducts & Biosystems Engineering, Plant Biology, and Ecology Evolution & Behavior.

The MITPPC relies on a strategic prioritization process to set its research direction. Financial resources are directed towards research that (i) addresses the invasive terrestrial plants and pests which pose the greatest threat to Minnesota and (ii) has the greatest potential to substantially improve management. A more expansive prioritization is nearing completion and will guide the construction of future Requests for Funding.

II. OVERALL PROJECT STATUS UPDATES:

Status as of *January 31, 2017*:
There has been no spending on this appropriation to-date.

Amendment Request (01/30/2017)
We are requesting to adjust the activity 1 completion dates in order to accommodate a spring 2017 RFP.
Amendment Approved (2/06/2017)

Status as of *July 31, 2017:*
A Request for Pre-proposals was issued for terrestrial invasive species research in March 2017. This RFP's timing was aligned with the LCCMR RFP in the hopes of streamlining and clarifying the appropriate funding process for terrestrial invasive species research. The MITPPC received 13 pre-proposals, requesting $6 million. Pre-proposals were received from seven university departments and from three university colleges. Each of the pre-proposals has a deep partnership with an implementing agency or organization. The pre-proposals are currently under internal technical review. Project managers with highly rated pre-proposals will be invited to prepare full proposals. Full proposals will be sent for peer-review by scientific experts outside the University of Minnesota.

Amendment Request (12/15/2017)

The MITPPC is requesting to add a research project to this appropriation. The research proposal, "MITPPC #1, Fungi in Ash Trees: Towards protecting trees from emerald ash borer and new diseases," by Dr. Robert Blanchette, of the UMN Plant Pathology department, seeks a greater understanding of the relationship between fungi associated with emerald ash borer (EAB). This work complements other MITPPC-funded EAB projects and will provide researchers with much needed data on this relationship with a goal of a potential biocontrol for the invading insect.
Amendment Approved (2/1/2018)

Status as of January 31, 2018:
Currently, the MITPPC has five full proposals under consideration. Two research projects have completed work plans and are to be amended into the MITPPC ML 2016 work plan. One of the projects is in external review; the remaining two are in work plan development. Funding of these five projects are estimated to total $2.3 million. The balance of this appropriation will be used to fund the ML 2016 January 2018 RFP.

Status as of *July 10, 2018:*
A second RFP was issued under this appropriation in January 2018. It solicited 12 pre-proposals. Of those, one was not eligible for consideration, as it did not address a priority species. The remaining 11 pre-proposals were reviewed by nine faculty at the University of Minnesota. Three pre-proposals have been invited to develop full proposals. We anticipate external review of those documents to begin later this summer. We anticipate that this process will round out the portfolio of projects funded under the ML 2016 appropriation. Per LCCMR instructions, this report does not contain a narrative update for each project, as they only began work within the last six months.

On April 19, 2018, Robert Venette and Heather Koop met with LCCMR staff to revise reporting procedures for this project. The changes were to be implemented immediately to make reporting as simple as possible. The revised procedures will be tested for 1 year. This document is meant to be the primary vehicle to describe research plans and report significant accomplishments of sub-projects funded by this appropriation. Here will be found a brief narrative of relevant Center-related activities and a table with the current status of each sub-project. Each sub-project will be described with outcomes and activities with corresponding completion dates with enough detail to adequately convey what work is being conducted, why, and the projected impact. A budget for each subproject will be attached to the overall work plan, however budgets for sub-projects will not report on sub-activities. Separate sub-project work plans will not be required. MITPPC will maintain copies of research addenda for each sub-project and make them available to LCCMR staff upon request. Dissemination activity will be reported with each sub-project; overall MITTPC dissemination will be reported in the overall dissemination section of the work plan.

A draft of this report was submitted to LCCMR staff on June 27, 2018. This updated version addresses requested points of clarification.

Amendment Request (8/1/2018)
This update includes three amendment requests. First, MITPPC requests to add three sub-projects to Activity 1 in this work plan:
  o "Sub-project 2 Understanding the Benefits and Limitations of Using Goats for Invasive Plant Control" by Dr. Tiffany Wolf for $445,533
  o "Sub-project 3 Genetic Control of Invasive Insect Species: Phase I" by Dr. Michael Smanksi for $296,655
  o "Sub-project 4 Dwarf Mistletoe Detection and Management in Minnesota" by Dr. Marcella Windmuller-Campione for $455,606
These three projects had developed separate sub-project work plans, which were approved formally by LCCMR staff on 2/19/2018 (Wolf), 2/28/2018 (Smanki), and 4/19/2018 (Windmuller-Campione).

Second, we request changes to the structure of this report that affect how research projects supported by MITPPC are described, how research accomplishments are reported, and how budget activity is summarized. With this report, MITPPC will begin using revised budget templates provided by LCCMR staff and a revised format to this document as suggested by LCCMR staff. These changes will be implemented on a trial basis for one year.

Finally, we request the addition to Activity 1 of an additional sub-project, "Sub-project 5: Developing a Spatially Explicit Bio-economic Dispersal Model to Aid with the Management of Brown Marmorated Stink Bug" by Drs. Senait Senay and Terrance Hurley for $561,348. Pursuant to the administrative changes described above, a separate work plan has not been prepared for this sub-project. A complete, peer-reviewed research addendum for the sub-project is maintained by MITPPC and is available upon request. A synopsis of the project is provided in the updates to Activity 1.

With addition of Subprojects 2-5, the amount of unobligated funding (i.e., the "reserve") is reduced by $1,759,142 from $3,250,000 to $1,490,858.

Amendment Approved (8/6/2018)

Status as of January 31, 2019:
MITPPC activities during this reporting period focused on completing the process of external review, incorporation of comments, and approval of proposal. The addition of three research projects, described below, from this 2018 RFP. Previously approved projects continue to make progress in establishing their research teams, labs, and protocols. Individual project summaries may be found later in this document.

Amendment Request (March 25, 2019)
MITPPC requests a modification of "Sub-project #2, Understanding the benefits and limitations of using goats for invasive plant control." For this sub-project, the research team would like to decrease personnel by $5,456 from $404,888 to $399,432 with a commensurate increase in professional services from $8,000 to $13,456. This change is due to not hiring an undergraduate student during the first year and the increase in plot fees and the subscription price for research software to help with the management of activities one and two.

MITPPC requests the addition to Activity 1 of three research sub-projects to this appropriation. The sub-projects are:
- o "Sub-project 6 Management of Invasive Knotweeds" by Dr. Alan Smith for $579,670
- o "Sub-project 7 Improved Detection and Future Management of Leafy Spurge and Common Tansy using Remote Sensing, Mechanistic Species Distribution Models, and Landscape Genomics " by Dr. David Moeller and Dr. Ryan Runquist for $422,000
- o "Sub-project 8 Using Plants to Control Buckthorn: an Expanded Approach" by Dr. Peter Reich for $560,000.

These research projects total $1,561,670. Currently, $1,490,858 remains in this appropriation. Upon approval of the amendment request, reserves for this appropriation will be $0. We request to make up the difference of $70,812 by using reserves from ML 2015, Ch. 76, Art. 2, Sec. 6a for Sub-project 7 Improved Detection and Future Management of Leafy Spurge and Common Tansy using Remote Sensing, Mechanistic Species Distribution Models, and Landscape Genomics.

Pursuant to the administrative changes described above, separate work plans have not been prepared for the three sub-projects. A complete, peer-reviewed research addendum for each sub-project is maintained by MITPPC and is available upon request. A synopsis of each sub-project is provided in the updates to Activity 1.

With this amendment, we also provide budget amounts for each sub-project.

Amendment Approved (4/2/2019)

Status as of July 31, 2019:
The first round of projects (sub-projects 1-5) continued apace with their work. Noteworthy highlights include: Nearly 500 species of fungi in ash trees attacked by emerald ash borer have been identified; some may be new to science (sub-project #1). Only approximately 0.2% of buckthorn seeds that are eaten by goats are viable after passing through a goat's digestive system (sub-project #2).

The second round of sub-projects' child accounts (sub-projects 6-8) were established for work to commence at the beginning of July 2019.

We are not asking for any amendments to these research sub-projects at this time.

Status as of January 31, 2020:
This appropriation supports eight research sub-projects. All research teams are fully staffed and have begun work in accordance with the timelines described below. Progress to date has been fully satisfactory. Specific sub-project accomplishments are described below, but particularly notable accomplishments are described here (not necessarily for all sub-projects). *Sub-project 1*: More than 1100 fungi have been found in association with emerald ash borer. Some of these fungi seem to be new to science, as their genetics and growth form do not match with previously described species. *Sub-project 2*: Only about 0.2% (2 out of 1000) buckthorn seeds that pass through the gut of a goat are still viable, suggesting goats used for buckthorn management pose little risk of spreading the invasive plant. *Sub-project 6*: Giant knotweed was detected in Minnesota for the first time. *Sub-project 7*: Leafy spurge was collected from 185 populations, of which 69% were new records and two were new county records in Minnesota; common tansy was collected from 187 populations, of which 49% were new records and one was a new county record in Minnesota. *Sub-project 8*: More than 300 Minnesotans have volunteered in a citizen-science project to test the use native plants as a component of buckthorn management, far exceeding expectations and current project capacity. The team considers this situation a welcome problem.

Amendment Request (January 31, 2020)
We are requesting two amendments to sub-projects. The first is a change to outcomes and the second a change to budget.

Sub-project 2, a change to outcomes: Activity 3 outcomes under this sub-project are being revised in response to the logistical obstacles encountered this past field season. This portion of the project is meant to evaluate the role that domesticated waterfowl (i.e., geese and ducks) might play in lowering the abundance of a potentially lethal parasite to goats. The parasite uses mollusks (snails or slugs = gastropods) as intermediate hosts, and goats acquire the parasite when they accidentally consume infected mollusks on plant foliage. Waterfowl prefer to consume mollusks, but are immune to the parasite. The original experimental design was to divide a buckthorn restoration-site into at least two parts, with one part having waterfowl, the other not. Based on the way that the industry partners manage the goats across field sites throughout the season, it became challenging to impossible for researchers to keep individual goats in waterfowl treatment groups for the entire period of time that they are at risk of meningeal worm exposure. In order to address the initial research questions, the following changes to the outcomes and timeline are proposed:

- Describe meningeal worm exposure in goats associated with invasive vegetation management by documenting changes in *P. tenuis* incidence associated with the browsing season (Feb 2022)
- Describe the efficacy of using waterfowl co-grazing to reduce *P. tenuis* exposure risk in goats by using changes in gastropod abundance and diversity as a proxy for risk (Feb 2022)
- Contribute to the gastropod collections at the Bell Museum, which have gaps in coverage of terrestrial gastropod diversity (October 2021)
- Disseminate research findings to ungulate owners through UMN Extension (Feb 2022)
- Publish results in peer-reviewed scientific journals (Feb 2022.)

The changes will not affect the overall budget for this sub-project. The changes overcome practical constraints and provide scientifically credible, practical results.

Sub-project 3, a budget modification: We are requesting one budget amendment to sub-project 3. The request is to decrease equipment/tools/supplies by $7,700 from $40,000 to $32,300 and increase professional and technical services by $7,700 from $20,000 to $27,700. This change will allow the team to contract with the UMN Genomics Center to generate high-quality genome sequence from a single fly, as the team encountered technical problems at the level of sequence library preparation.

Amendment Approved (02/26/2020)

Status as of July 31, 2020:
Projects confronted considerable complications associated with the COVID-19 pandemic. For several months this spring, the University prohibited all travel, dismissed undergraduate students (and undergraduate research assistants), barred non-exempted research, and suspended normal University operations. Research teams were generally resilient, switching to work from home, establishing home-based laboratories, and submitting detailed protocols to resume campus-based research while assuring safety of other researchers, University staff, and the public. All projects supported under this appropriation were affected, but not to the same degree.

All projects have made good progress. Particularly, notable accomplishments during this reporting period include: *Sub-project 1* (Blanchette) has found that emerald ash borer (AB) creates opportunities for dozens of disease-causing fungi. These fungi likely play important, but previously underappreciated, roles in the decline of ash trees attacked by EAB. Approximately 40 of these fungal species may be new to science. *Sub-project 2* (Wolf) has analyzed previously published studies to show that livestock (esp., cattle, sheep, and goats) significantly reduce unwanted vegetation for at least one year after grazing. *Sub-project 8* (Reich) has recruited 123 citizen scientists to conduct 110 experiments on the use of native plants to reduce buckthorn survival and growth. Several sub-project teams are publishing findings in peer-reviewed journals.

Progress toward outcomes for all sub-projects is described under Activity 1 Sub-Project Synopsis below.

Amendment request (09/18/2020)
There are several timeline changes requested to sub-projects due to the effects of COVID-19 on research efforts. None of the changes affect outcomes and all are within the time limits of the appropriation. Projects requesting timeline changes:

Sub-project 2: budget changes
The request is to decrease the line item for laboratory testing $16,030 (from $18,400 to $2,370) and increase personnel by $7,000 (from $399,432 to $406,432), increase transportation by $3,030 (from $10,000 to $13,030); and establish a new line item for publications at $6,000 ($0 to $6,000). Significant testing will now be completed by trained students which accounts for budget reductions for testing and increases for personnel.

Sub-project 3: timeline and budget changes - Smanski
(extension of final outcome [Activity 2] from February 28, 2020 to February 28, 2021 with commensurate changes to intermediate outcomes in Activity 3) An extra year extension to the scheduled completion date is requested because of a combination of (i) technical challenges that have slowed progress on this Outcome, and (ii) COVID-related shutdowns that prevented us from working on this for 2 months, and (iii) current COVID-related precautions which limit the occupancy of our lab, slow all lab projects, and have resulted in changes needed for fly microinjection protocols, which previously required multiple people in tight spaces.

The budget-change request is to decrease equipment/tools/supplies by $10,000 (from $32,300 to $22,300) and increase professional services by $10,000 (from $27,700 to $37,700). The change does not impact what the research will accomplish, but is a change in technique for gene sequencing.

Sub-project 4: timeline change
(extension of final outcome [Activity 3] from November 30, 2021 to November 30, 2022 with commensurate changes to remaining intermediate outcomes.) This MITPPC sub-project was one of the most severely affected by COVID-19. University shutdowns, travel restrictions, and sub-project personnel at high-risk of complications from COVID-19 resulted in a complete loss of the 2019/20 field season. Without the additional year, the project will be unable to make defensible conclusions. The sub-project manager indicates that no additional funding will be needed to complete the work and all outcomes will ultimately be achieved.

Sub-project 5: timeline change
The timeline originally submitted for this sub-project was taken from the research addendum but did not account for delays in the project start time. The completion date for the final outcome (Activity 3, Outcome 3) is extended from September 15, 2021 to June 30, 2022, the previously approved end date of the overall sub-project. Commensurate changes were made to completion dates for intermediate outcomes.

Sub-project 7: timeline and budget change; slight change in method
(extension of final outcome [Activity 4, Outcome 5] from September 30, 2022 to December 31, 2022 with commensurate changes to remaining intermediate outcomes) The budgetary change is in the equipment/supplies/tools line item. We request to move $492 from the line for computer software ($4,500 to $4,008) and increasing supplies for seed collection, et al by $492 ($1,795 to $2,287). Finally, the PI requests to eliminate Act. 2 outcome 4 as it isn't feasible given timeline and needs as a result of inability to work in the labs over winter and spring due to COVID.

Sub-project 8: timeline and budget changes
(extension of final outcomes from December 31, 2022 to March 31, 2023 with changes to one intermediate outcome) The budgetary request is to establish a new line item for mailing costs for $2,500 ($0-$2,500) and decrease lab supplies by $2,500 (from $15,000 to $12,500). Mailing costs are to facilitate correspondence related to the citizen science activity that was not anticipated due to COVID-19.

Status as of January 31, 2021:
All sub- projects continued to make good progress, although many at a pace that was slightly slower than anticipated. While most sub-project leaders were optimistic about their capacity to maintain research timelines going into the 2020 field season, reality proved to be different. Three issues were common: i) research teams were unable to locate summer research assistants as students had been sent off campus; ii) social distancing requirements in buildings limited both the number and time of people who could work in laboratories; and/or, iii) social distancing requirements during travel (one individual per vehicle) effectively limited the number of people who could conduct field work and increased travel costs. In addition, some project managers noted that COVID stress (at home child-care, teaching, family health, etc.) reduced work efficiency.

Nevertheless, some significant results were obtained in the previous six months; only the most significant findings are reported here. Sub-project 1 has identified a remarkable array of fungi associated with emerald ash borer. Some of these fungi proved to be promising biological control agents for emerald ash borer in laboratory tests; field tests of the best fungi began during the 2020 field season and are in progress. Sub-project 3 has overcome significant technological challenges to genetically transform spotted wing drosophila, a serious pest of soft-skinned fruits. This advancement is a major step towards achieving genetic biocontrol. Efficacy tests are underway. Results from successful proof-of-concept experiments with a closely related fly species have been reported in high-visibility forums and journals, reflecting the revolutionary nature of this science. Sub-project 6 found that knotweeds grown from seed in MN can begin to produce their own seed in as little as seven months, a remarkable finding. The result suggests that some knotweeds may be able to spread much more quickly than previously estimated. Sub-project 8 demonstrated that grasses and wildflowers do suppress buckthorn, when restoration sites have been properly prepared. More than 100 citizen scientists are applying the method across the state.

Amendment request (01/29/2021)
Four amendment requests are described below. Timeline adjustments result from unanticipated impacts from COVID-19.

Sub-project 1: timeline change
The sub-project manager requests an extension of Activity 2, Outcome 1 from September 31, 2020 to June 30, 2021. This extension does not impact outcomes, nor does it change the overall length of the project.

Sub-project 3: budget change
The sub-project manager requests four budget modifications: a) a reduction in personnel by $1,604 from $235,655 to $234,051 because the laboratory was subject to staffing restrictions; b) an increase in Professional/Technical/Service Contracts by $104 from $37,700 to $37,804 to account for a slight increase in work that could be outsourced to a service laboratory; c) an increase in Equipment/Tools/Supplies by $2,500 from $22,300 to $24,800 compensate for an over-adjustment made in the previous reporting period; and d) a decrease in printing by $1,000 from $1,000 to $0 to stay within budget. The investigator is pursuing internal funding sources to cover future publication costs.

Sub-project 5: timeline changes
The sub-project manager requests to alter the timeline for two activities: Activity 1, Outcome 3 from September 30, 2020 to March 15, 2021 and Activity 2, Outcome 3 from October 30, 2020 to February 15, 2021. Both adjustments are within the original timeline of the overall project.

Sub-project 7: timeline and budget change
The sub-project manager requests to change the timeline of Activity 2, Outcome 2 from December 21, 2020 to December 31, 2021 due to an inability to find sufficient undergraduate assistants to date to support the project. These adjustments are still within the original timeline of the overall project. A budget adjustment is requested to collapse the two line items under travel (one for instate, $6,000 and one for out of state, $3,000) for a total of $9,000 in-state travel. In-state travel expenses have been greater than originally estimated but are still within the original total travel budget.

Amendment Approved 2/11/2021

Status as of July 31, 2021:
All sub-projects continue to make good to excellent progress towards achieving specified outcomes. Most teams have adopted strategies to compensate for impacts from COVID-19. Safe travel to research field sites, hiring undergraduate research assistants, and safe use of shared research facilities have been common challenges.

Accomplishments of all sub-projects are described below, but a few achievements are particularly noteworthy. The team associated with sub-project 1, led by Blanchette, published findings on fungi associated with emerald ash borer, and the story was covered widely by several local, regional, and national news organizations. This study is the first of its kind to document that emerald ash borer can carry disease-causing fungi, some of which are harmful to ash trees or EAB itself. Field trials to evaluate the effectiveness of certain fungal strains to kill EAB eggs were initiated this spring. The team associated with sub-project 2, led by Wolf and Larkin, published a report on the role of targeted grazing for invasive plant management. The authors found that targeted grazing reduces undesirable plants and increases species richness, but significant questions remain about permanence of these changes once grazing ends. Sub-project 3, led by Smanski and now complete, fully demonstrated the potential for genetic techniques to provide a form of biological control for spotted wing drosophila. Sub-project 6, led by Smith, completed a genetic analysis for 868 collections of knotweed and found higher levels of diversity than first expected, especially for Japanese knotweed. The findings are important because they suggest the possibility of multiple introductions of the species rather than extensive spread from a small number of introductions. Sub-project 7, led by Briscoe-Runquist and Moeller, developed methods to identify leafy spurge from satellite imagery, a major accomplishment.

The final abstract for MITPPC sub-project 3, "Genetic Control of Invasive Insect Species: Phase I" by Dr. Michael Smanski is provided as a separate document with this report.

Amendment Request (7/29/2021):
We are requesting amendments to one project, Sub-project 7. The sub-project manager asks to alter the timeline for three activities: Activity 2, outcomes 5 and 6 from 12/31/2021 to 11/30/22; Activity 3, outcome 6 from 6/30/2022 to 4/1/2023; and Activity 4 from 6/30/2022 to 4/1/2023 and 5 from 12/31/2022 to 4/1/2023. A placeholder for an additional project update has been inserted to III. Project Activities and Outcomes, Activity 1, MITPPC Sub-project 7. The adjustment to the overall duration of the sub-project is also reflected in the budget summary spreadsheet. These requests are due to difficult growing seasons over the last two years and the desire to have sufficient field data to build the mechanistic model for common tansy. The amendment requests are within the duration of the appropriation. Adequate funding exists because fewer undergraduate assistants were hired than first estimated, and some staff time is being paid from other funds for work on unrelated projects. The PI also requests a modest budget adjustment to decrease travel by $156 (from $9,000 to $8,844) and increase supplies by $156 (from $2,287 to $2,443). The team has concentrated field research closer to the St. Paul campus to reduce travel expenses. Enclosures to adjust temperature and moisture levels in the field were slightly more expensive to build than first estimated.

We are also requesting a small amendment to correct a typographical error in the section II. Overall Project Status Updates (Status as of January 31, 2021). The overview accidentally referred to sub-project 3 as sub-project 2.

Amendment Approved 8/8/2021

Status as of January 31, 2022:
At the start of this reporting period, seven sub-projects were active under this appropriation. In that time, Sub-project 4, "Dwarf Mistletoe Detection and Management in Minnesota" led by Dr. Marcella Windmuller-Campione, concluded. The final abstract for the sub-project is provided as a separate document with this report. This sub-project sought to identify major challenges to the detection and (possible) eradication of American dwarf mistletoe, a parasitic plant that affects jack pine, if it invaded Minnesota. The research team worked with a related mistletoe species, eastern spruce dwarf mistletoe that affects black spruce, as a surrogate. Interviews with foresters and loggers, groups most likely to first encounter American dwarf mistletoe, found significant uncertainty about the impact of mistletoes and the effectiveness of management alternatives. These uncertainties influenced willingness to take management action. More training on mistletoe identification,

ecology, and management will be necessary. Ground-based surveys and aerial-image analyses are feasible but will face significant logistical challenges.

Sub-project 5, "Developing a spatially explicit bio-economic dispersal model to aid with the management of brown marmorated stink bug" was terminated on November 18, 2021 by the MITPPC director, a decision that was supported by the College of Food, Agriculture, and Natural Resource Science (CFANS) administration and LCCMR Director Nash. The project was unable to complete its objectives due to the unanticipated inability of the principal investigators to continue with the project. Fortunately, Activity 1 of the project - Develop a high resolution, spatially explicit, agent-based dispersal model for *H. halys* - was complete and results were published in the peer reviewed publication "Effects of Starvation, Age, and Mating Status on Flight Capacity of Laboratory-Reared Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)" in *Environmental Entomology* Volume 50, Issue 3, June 2021. https://doi.org/10.1093/ee/nvab019. Half of the individuals flew less than 1/6 of a mile in 24 hours; however, one individual flew 4.5 miles in 24 hours. Flight patterns were generally unaffected by starvation, age, or mating status. Please see the amendment request for additional details on the disposition of the remainder of the project.

The remaining sub-projects continue to make good to excellent progress towards achieving stated outcomes. Most teams are in the process of analyzing and summarizing results collected during 2021-field season and/or preparing manuscripts that describe major findings for publication. Three findings are particularly noteworthy. First, the team associated with sub-project 6, "Management of Invasive Knotweeds" is discovering that seeds are more significant in the spread of knotweeds than previously realized. In the past it was presumed that most local spread was through rhizomes. The finding suggests that active management of seedlings might have major benefits for limiting the spread of the spread of the plant. Second, the team associated with sub-project 7, "Improved Detection and Future Management of Leafy Spurge and Common Tansy using Remote Sensing, Mechanistic Species Distribution Models, and Landscape Genomics" has developed a new method to detect leafy spurge from satellite images. The method is being tested in greater Minnesota. Finally, the team associated with sub-project 8, "Using Plants to Control Buckthorn: an Expanded Approach" found that native elderberry species have a similar phenology to buckthorn. Elderberry may be highly effective in competing for light and preventing buckthorn establishment.

Amendment Request (01-31-2022)
There are several minor budget amendment requests:

Sub-project 2: Change to reporting dates
We request the deletion of a January 31, 2023 reporting date. The project is scheduled to end on June 30, 2022.

Sub-project 4: Changes to reporting dates and budget changes
The sub-project manager requests the deletion of the July 31, 2022 reporting date. The project ended November 30, 2021. Changes apply to the workplan (and the description of the Final Report Summary) and the sub-project budget spreadsheet. The spreadsheet incorrectly indicated that the project ran for 4.5 years until November 30, 2022. The correct project duration is 3.5 years.

The sub-project manager also requests budget changes to a) increase personnel by $8,382 from $367,746 to $376,128; b) decrease equipment, tools, and supplies by $3,315 from $5,500 to $2,185; and decrease travel by $27,423 from $82,360 to $54,937. More personnel time for analyzing data and preparing publications was needed than first estimated. Fewer supplies were needed than first estimated. Finally, COVID precautions made aerial surveys for eastern spruce dwarf mistletoe infeasible and limited other field studies. These adjustments close out the budget for this project. The balance of $22,356 remaining (indicated on the bottom of the sub-project budget spreadsheet) is requested to return to the appropriation's reserves, formerly $0. The overall budget summary spreadsheet does not show this amount in the reserve as additional changes to this line item

are requested with this report. We also request a change to the sub-project budget spreadsheet to correct typographical errors. The spreadsheet incorrectly indicated that the project ran for 4.5 years until November 30, 2022. The correct project duration is 3.5 years, ended on November 30, 2021.

Sub-project 5: budget change
The sub-project manager requests changes to a) decrease personnel by $220,999 from $547,628 to $326,629; b) decrease equipment tools supplies by $4,775 from $7,500 to $2,725; c) decrease publications by $4,000 from $4,000 to $0; d) decreases travel by $870 from $870 to $0; and e) decrease other expenses by $1,350 from $1,350 to $0. This adjusts and closes out the budget for this project. The balance of $231,994, shown on the bottom of the sub-project budget spreadsheet, is requested to return to the appropriation's reserves. The reserves now increase from $22,356 to $254,350. Further amendment requests from changes to other sub-projects will affect the amount in the reserve line item. We also request a change to the sub-project budget spreadsheet to reduce the project duration and change the project end date to July 18, 2021. We also request the deletion of the Final Report Summary from the workplan, a change discussed with Director Nash.

Sub-project 7: budget change
The sub-project manager requests changes to a) increase equipment/tools/supplies by $1,398 from $2,443 to $3,841 and b) decrease travel by $1,398 from $8,844 to $7,446. COVID restrictions complicated travel to field sites, so more research was conducted in or near St. Paul. More supplies were needed than first estimated for an experiment on the St. Paul field station.

Sup-project 8: budget change
The sub-project manager requests changes to a) increase professional services contracts by $500 from $27,000 to $27,500; b) increase postage/mailing by $804 from $2,500 to $3,304; and c) decrease travel by $1,304 from $14,000 to $12,696. The adjustment to the service contract was necessary to cover greater than estimated costs for herbicide applications on field plots. The increase in postage/mailing was necessary to send materials to citizen science volunteers. Again, COVID safety requirement limited the amount of travel that could be done for the project.

Sub-project 9: project establishment
We are requesting the addition of sub-project 9, "Genetic invasive insects, Phase 2" to this appropriation" led by Dr. Michael Smanski. The project targets spotted wing drosophila, a major agricultural pest. The project underwent internal and external peer-review and was initially supported with non-ENRTF funds. A full research workplan is available upon request. The proposed six-month project, $60,000 is within the original scope of work but more rigorously investigates the impact of temperature on the effectiveness of genetic biocontrol for this insect. Details, including budget, description, and activity and outcomes are found below and in the accompanying budget spreadsheet. Funds for this project will be from the reserves accrued from the closed projects described above. These changes bring the budget reserve from $254,350 to $194,350.

Amendment Approved (02/18/2022)

Status as of April 26, 2022
The next, regularly scheduled status report is not due until July 31, 2022. However, this interim report is meant to serve three purposes. First, we close sub-project 2, "Understanding the benefits and limitations of using goats for invasive plant control" (led by Dr. Tiffany Wolf) on time and provide a final report summary. Second, we correct the final budget information that was submitted in January 2022 for Sub-Project 4 and Sub-Project 5. (Details are provided with the amendment request.). Finally, we request the addition of a new sub-project.

Sub-project 2 was highly successful and demonstrated that goats are effective at suppressing buckthorn and increasing native plant diversity. However, these effects are short-lived, and buckthorn quickly rebounds when

grazing stops. Fortunately, goats are not likely to spread buckthorn seeds, as most buckthorn seeds are killed when they eaten. Goats in buckthorn patches face a real but small chance of becoming infected with a lethal parasite that is spread by deer. (Deer are not affected.) The life cycle of the parasite is complex and involves infection of snails or slugs as intermediate hosts. Goats may inadvertently consume these intermediate hosts as they are grazing on buckthorn or other wild vegetation and become infected. Ironically, the number of snails and slugs increased after sites were grazed by goats. However, placing domesticated geese or ducks at these sites with the goats prevented these increases and may offer some protection to goats, particularly at sites that need to be grazed for multiple years. Results have been shared broadly in peer-reviewed publications and diverse presentations. The final project abstract accompanies this report.

Lastly, we request the addition of sub-project 10, "Novel Diagnostic Tools for Rapid and Early Detection of Oak Wilt" (led by Dr Abdennour Abbas). Earlier work (funded under an appropriation to MITPPC in ML 2014) led to the discovery of exciting new technologies for pathogen detection, but those technologies encountered significant limitations when applied to complex samples from the field. In this phase of the project, Dr. Abbas will utilize a complementary approach (loop mediated isothermal amplification, also known as LAMP) to detect DNA of the plant pathogen responsible for oak wilt. The project proposal underwent external peer-review. A full research workplan is available upon request. Oak wilt is the second greatest threat from invasive plant pathogens to the state. This research directly addresses the need for more refined tools to detect invasive species. More details about the sub-project are included in the amendment request, in Section III. PROJECT ACTIVITEIS AND OUTCOMES, and in attached budget spreadsheet.

Amendment Request (04/26/2022)
Sub-project 2 is now complete. We are requesting final budget changes to close-out this sub-project. We are requesting that personnel be decreased by $14,044 from $406,432 to $392,388; professional services be decreased by $6,648 from $13,456 to $6,808; equipment/tools/supplies decreased by $1,650 from $6,615 to $4,965; travel expenses decreased by $9,070 from $13,030 to $3,960; and publications decreased by $3,854 from $6,000 to $2,146. COVID restrictions during this sub-project forced a downscaling of the overall project so fewer expenses were incurred than originally estimated. As a result, the total amount allocated to the sub-project is reduced from $445,533 to $410,267. A balance of $35,266 will be returned to the reserve. The budget reserve increases from $194,350 to $229,616.

We are also submitting corrections to the final report for sub-project 4, which stated that the project budget was $433,250. The correct budget was $455,606. Additional personnel changes ($398,484 rather than the reported amount of $376,128) had been incurred that were not reflected in the previous budget submission. The personnel expenses were within the limits of the original allocation to the sub-project. We adjust the sub-project budget worksheet to reflect the correct amount. The final balance for this sub-project is $0. This correction reduces the reserve by $22,356 from $229,616 to $207,260. We also adjust this workplan (as requested on 11 April by LCCMR staff) to correctly report the final budget spent for sub-project 5 (completed previously); the correct amounts had been reported in the budget worksheets and in the final project abstract.

Lastly, we are requesting the addition of sub-project 10, "Novel Diagnostic Tools for Rapid and Early Detection of Oak Wilt" to be funded in the amount of $190,740 until June 30, 2023. Dr Abdennour Abbas will lead this project. Details, including budget, description, and activities and outcomes are found below and in the accompanying budget spreadsheet. The proposed work will be supported with funds available in the reserve for this appropriation. The budget reserve decreases from $207,260 to $16,520. This amount is reflected on the overall synopsis worksheet in the accompanying budget spreadsheet.

Amendment Approved (06/14/2022)

Status as of July 31, 2022:

At the start of this reporting period, five MITPPC sub-projects (1, 6, 7, 8, and 9) were active under this appropriation. All sub-projects made good to excellent progress towards achieving stated outcomes. Some particularly salient highlights are noted here. Sub-project 1 (Blanchette) ended during this reporting period. This team identified more than 170 fungi that are associated with emerald ash borer (EAB). Fourteen of these fungi were entomopathogens, fungi capable of infecting and killing one or more stages of EAB. Laboratory tests confirmed that several fungal killed eggs or larvae. Field testing with a small subset of fungi continued in Spring 2022. Fungi injected into ash trees near ground level successfully moved at least 4 m (13 ft) into the crown. The efficacy of these fungi is still being determined. This project has identified several fungi for further development as control agents for emerald ash borer. Results from this project also affirm the need to promptly remove EAB killed trees, as decay fungi are likely to make trees hazardous (i.e., decay fungi cause limbs and branches to fall and risk injury to people or harm to property). A final report for Sub-project 1 is included with this update. Sub-project 6 (Smith) completed a genetic analysis of knotweeds in Minnesota and confirmed the presence of Japanese, Giant, and Bohemian knotweeds, as well as a fourth previously undescribed species, *Fallopia compacta*. Herbicide trials indicate Arsenal, Garlon, Habitat, and Milestone may provide good control of knotweeds; the team continues work to find appropriate concentrations to avoid under-/over-dosing plants. Sub-project 7 (Moeller and Briscoe-Runquist) published findings that demonstrate the utility of satellite images from Worldview 2 and Planet-Scope (publicly available) to detect leafy spurge with >96% accuracy. Sub-project 8 (Reich) published findings in the journal *Ecological Engineering* that document the benefits of planting native plants to help with buckthorn control; the studies demonstrate that select native plants can outcompete buckthorn for light. A new sub-project (#10) was approved by LCCMR staff on June 14, 2022. Per previous agreement with LCCMR staff, sub-projects within six months of approval are not required to submit progress reports as most activity is being directed to project organization.

Amendment request (08/12/2022)

We are requesting amendments to four sub-projects: 1, 7, 8, and 9.

Sub-project 1 (Blanchette): We request the following budget adjustments to close this sub-account. These adjustments reflect actual expenses incurred to complete activities and outcome. Fewer than 45 days have elapsed since the close of the sub-project. The description of expenses is still subject to change but is the best available currently. We ask to decrease personnel by $18,355 from $442,529 to $424,174. Slightly less labor was needed to complete the project than was initially estimated. We also ask to decrease professional services by $6,391 from $20,871 to $14,480 because more fungal genes were sequenced in-house than outsourced to a commercial laboratory as originally planned. We ask to increase supplies by $3,997 from $27,000 to $30,997 to account for the additional DNA extraction kits and sequencing reagents that were needed to work with fungal DNA in house. We ask to decrease travel by $6,494 from $9,600 to $3,106, as less travel was feasible during the project due the effects of the pandemic. The total amount dedicated to the sub-project changes from $500,000 to $472,757; the project will return $27,243 to the reserve. The description of the sub-project in this report is modified to reflect these actual expenditures. A mistaken, additional reporting date for this sub-project (January 31, 2023) is deleted from this report as the sub-project has ended.

Sub-project 7 (Moeller and Briscoe Runquist) requests budget amendments to reflect actual expenses incurred and refined estimates of future expenditures. The drought of 2021 had a substantive, negative impact on progress. These amendments will allow greater involvement of the Researcher (Briscoe Runquist) than was originally planned at this stage to still complete the project on time in April 2023. The request is to increase funds for personnel by $9,947 from $303,893 to $313,840; decrease professional services by $5,447 from $27,500 to $22,053 (genetic sequences were analyzed from fewer samples than first estimated); increase equipment and supplies by $1,076 from $3,841 to $4,917 (for additional materials needed to propagate plants in a common garden in 2022); decrease publication fees by $4,500 bringing that line item to 0 (other funding opportunities have come available to cover those costs and will be detailed as contributions to the project in a

future report); and decrease travel by $1,076 from $7,446 to $6,370 (as a result of reduced travel due to the pandemic).

Sub-project 8 (Reich) requests a modest budget amendment to cover a slight overage in mailing costs associated with the citizen science project component of the research project. The request is to increase postage by $615 from $3,304 to $3,919 and decrease travel by $615 from $12,696 to $12,081. Slightly less travel has been possible than was first estimated because of the pandemic.

Sub-project 9 (Smanski) requests extension of both outcomes from 9/30/2022 to 12/31/2022. The slight time extension is due to unexpected difficulties in hiring staff to work on the project. Critical staff are now in place and work has progressed. The time extension has no budget implications.

Amendment Approved (09/13/2022)

Status as of January 31, 2023:

The five active sub-projects supported by this appropriation (MITPPC Sub-projects #6-10) continue to make steady progress. Sub-project 9 (led by Dr. Michael Smanski) completed work and submitted a final report. All remaining sub-projects are on pace to achieve stated outcomes by June 30, 2023. Detailed descriptions of accomplishments over the previous six months are provided below. However, results from three sub-projects are particularly significant and are highlighted here. Sub-project 9 tested an approach to genetic biocontrol of spotted wing drosophila, a significant invasive pest in Minnesota, in laboratory environments. This team made three major discoveries: (1) ambient temperatures affected the efficacy of the technology, (2) designed gene mis-expression caused flies to die, and (3) resistance genes in wild flies can quickly lower the effectiveness of the technology (but releasing an engineered fly that targets a different gene can quickly restore the effectiveness of the technology). Sub-project 8 (led by Dr. Peter Reich) has determined that viable buckthorn seeds do not accumulate beneath buckthorn plants, so there is little to no "seed bank" for this invasive species. "Seed banks" complicate the management of many invasive plants by extending the time after management in which plants might easily re-establish themselves from seed. This finding, submitted for publication in the prestigious journal Biological Invasions, should provide additional confidence to land managers about recent management recommendations for this invasive shrub. Sub-project #10 (led by Dr. Abdennour Abbas) confirmed detection of the oak wilt pathogen with loop-mediated isothermal amplification (LAMP). The finding was a major technological breakthrough towards the development of a robust in-field test for oak wilt. The technique also utilizes nanotechnology advancements previously funded by MITPPC.

Amendment Request (02/13/2023)

We are requesting minor amendments to four sub-project budgets:
- Sub-project #6 (Smith) requests to increase personnel by $35,000 from $425,481 to $460,481; to create a professional services line of $25,214; to decrease equipment/tools/supplies by $35,000 from $62,096 to $27,096; to decrease travel by $25,214 from $29,525 to $4,311. This request is due to loss of key personnel, a change in vendors for the carbohydrate analysis, and the need to hire additional assistants for bioinformatic analyses. Significantly less travel is needed than originally planned because field testing of herbicides occurred primarily on the St. Paul campus instead of sites around Minnesota. Fewer supplies are needed than originally estimated because the carbohydrate analysis can no longer be conducted by an on-campus cooperator as originally planned. These changes will allow the project to complete carbohydrate analyses, a critical element to determine the timely application of herbicide for knotweed.

- Sub-project #7 (Moeller) requests to decrease personnel by $145 from $313,840 to $313,695; increase equipment/supplies/tools by $145 from $4,917 to $5,062. These minor adjustments reflect actual expenses incurred on the project, not estimates, and will see the project through to its conclusion. Please note that this project will remain open until April 2023 to accommodate the writing and publication of project findings.
- Sub-project #8 (Reich) requests to decrease personnel by $2,204 from $504,000 to $501,796; increase postage and mailing by $140 from $3,919 to $4,059; increase equipment/supplies/tools by $503 from $12,500 to $12,503; and increase travel by $2,061 from $12,081 to $14,142. These minor adjustments reflect actual expenses incurred on the project, not estimates, and will see the project through to its conclusion in June 2023.
- Sub-project #9 (Smanski) requests to reduce personnel from $60,000 to $55,100. The change reflects actual expenses incurred on the project. These expenses are final. As a result, $4,900 is returned to the reserve, bringing the reserve total from $43,763 to $48,663.

We are also asking to extend the completion of sub-project 8 (Reich) from 3-31-2023 to 6-31-2023 to accommodate the preparation and submission of peer reviewed manuscripts resulting from the research.

Amendment Approved 3/16/2023

Status as of June 30, 2023:

Four research sub-projects (#6-8, and #10) remained active through June 30, 2023. Each has now closed. All teams were working diligently on final analyses of data, interpretations of findings, and preparations of manuscripts. They have made very good progress. Sub-projects #6 and 8 contended with significant staffing changes. Details of accomplishments since the last report are provided below. Sub-project #6 (Smith) is working on at least nine manuscripts. One of the most salient recent findings is that seed production by knotweed species is far more extensive than previously thought. Wind dispersal of seed, particularly in winter over frozen ground, may be contributing to long-distance spread of the plant more than was previously recognized. Sub-project #7 (Moeller and Briscoe-Runquist) is working on at least two manuscripts that document genetic heterogeneity within tansy and leafy spurge in Minnesota. These studies suggest that both plants have adapted to the unique growing conditions in Minnesota. Sub-project #8 (Reich) is working on at least two additional manuscripts as well. They recently published the major finding that common buckthorn does not have a long-lived seed bank. This finding supports the value of more aggressive treatments in the short-run that will provide greater long-term benefits. Sub-project #10 (Abbas) has at least one additional paper in process. In short, this team has developed an assay that provides sensitive detection of the oak wilt pathogen. All teams have committed to completing publications, largely supported by base funding to faculty researchers.

Amendment Request (08/18/2023)

We request amendments to three sub-project budgets:
- Sub-project #1 (Blanchette) requests to amend the final report previously submitted. The correct total expenditure is $500,000 (which was originally budgeted), due to an increase in personnel by $27,243 from $472,757. These changes reflect actual expenses for work incurred during the sub-project period, as identified during the final budget reconciliation. The total $27,243 is taken from the reserve.
- Sub-project #3 (Smanski) requests to amend its final report, previously submitted. Additional charges were added to the sub-project that resulted in a change in personnel from $234,051 to $234,324 (an increase of $273; change in professional services from $37,804 to $21,196 (a decrease of $16,608), and a change in equipment/tools/supplies from $24,800 to $40,197 (an increase of $15,397). These changes reflect actual expenses for work incurred during the sub-project period, as identified during the final

budget reconciliation. The total spent is an overall decrease from $296,655 to $295,717. The difference of $938 is returned to the reserve.

- Sub-project #5 (Senay), previously closed, requests an amendment of personnel expenditures from $326,629 to $326,579. The change reflects actual expenses for work incurred during the sub-project period, as identified during the final budget reconciliation. The difference of $50 is returned to the reserve.
- Sub-project #6 (Smith) requests to decrease personnel by $85,340 from $460,481 to $375,141; decrease professional services by $25,214 from $25,214 to $0; decrease equipment/supplies/tools by $5,899 to $21,197; decrease publications by $2,476 from $4,000 to $1,524; and increase other by $15,982 from $58,568 to $74,550. The latter increase is due to the decision to undertake the carbohydrate analysis internally rather than through an external contract. These changes reflect actual expenses incurred, not estimates, and will close out this research sub-project. A total of $102,947 will be returned to the reserve.
- Sub-project #8 (Reich) requests to decrease personnel by $1,120 from $501,796 to $500,676; to increase postage and mailing by $19 from $4,059 to $4,078; to increase equipment/tools/supplies by $111 from $12,503 to $12,614; and finally, to increase travel expenses by $990 from $14,142 to $15,132. These changes reflect actual expenses incurred, not estimates, and will close out this research sub-project. The changes have no impact on the reserve.
- Sub-project #10 (Abbas) requests to decrease personnel by $23,180 from $150,240 to $127,060; to increase equipment/tools/supplies by $17,604 from $24,500 to $42,104; to decrease professional services by $11,904 from $13,000 to $1,096; decrease travel by $2,623 from $3,000 to $377. The increase in equipment/tools/supplies comes from a decision to not use outside professional services to conduct specific tests. These changes reflect actual expenses incurred, not estimates, and will close out this research project. A total of $20,103 will be returned to the reserve.

The reserve previously held $48,663. With these amendments, a balance of $96,795 (reflected on the budget spreadsheet) will be added to the reserve, leaving a balance of $145,458.

Amendment Approved 1/1/2024

Overall Project Outcomes and Results:

Ten MITPPC research teams, funded through this appropriation, made transformative discoveries about 11 priority terrestrial invasive species (TIS). These findings yielded exciting approaches to prevent TIS damage in Minnesota. Collectively, these projects captured the public's attention, as demonstrated by extensive media coverage. They were also severely disrupted by COVID-19. Nevertheless, teams made remarkable progress. Here are three highlights:

- Fungi with emerald ash borer (EAB) (Robert Blanchette, lead). This team revealed over 100 fungal species associated with EAB in ash trees. This team was the first to discover that some of these fungi rapidly decompose wood, explaining why trees attacked by EAB can quickly become hazard trees, with falling limbs affecting people's safety and property. Other fungi can infect and kill EAB; these fungi are being pursued as biological control agents.
- Goats to manage common buckthorn (Tiffany Wolf and Daniel Larkin, leads). Goats are widely used to manage common buckthorn, but goats in buckthorn may get infected by a lethal brain parasite. This team discovered that domesticated ducks and geese, pre-released into a buckthorn stand, will consume the snails and slugs that are intermediate hosts for the parasite, and lower chances for goat infection. Goats also digest >99% of buckthorn seeds, so they are unlikely to move buckthorn from site to site. Goats temporarily open sites and lower buckthorn densities, but without other management, buckthorn quickly rebounds.

- Plants to shade out buckthorn (Peter Reich, lead). Young buckthorn plants die if they do not get adequate light. This team demonstrated that native grasses, forbs, shrubs, and trees, if planted when buckthorn is removed/treated, can create enough shade to prevent buckthorn from returning. Elderberry is particularly effective. Buckthorn does not have a long-lived seed bank, so intensive management can provide significant long-term benefits.

All ten projects directly addressed needs from TIS managers across Minnesota.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Accelerate research on high priority terrestrial invasive species
Description:
By using funds from the 2014 General Fund appropriation, the MITPPC conducted a rapid prioritization in the spring of 2015 to identify immediate research needs among state agencies with primary responsibility for the management of terrestrial invasive plants and pests on public and private lands. The resulting Request for Proposals resulted in four projects that were funded under an ML 2014 ENRTF appropriation.

A more expansive research prioritization was initiated in May 2015 to systematically evaluate threats posed by a wider array of terrestrial invasive plants, pathogens, and insects/arthropods. The more expansive prioritization will be used to allocate the remaining research funds from the M.L. 2014 and M.L. 2015 ENRTF appropriations.

Upon the completion of the expansive research prioritization, MITPPC will prepare a request for proposals and work-plans to conduct research to address identified priority invasive species. Proposals will be sent for peer review to ad hoc scientific reviewers in the field of research, which will allow for rapid turnaround of proposals to expedite work to be completed. The ad hoc scientific reviewers will make recommendations to MITPPC's Director on the suitability of the project for funding. Final award decisions will be made with the consent of the Associate Dean for Research and/or the Dean (both College of Food, Agricultural and Natural Resources Sciences, University of Minnesota). A small portion of funds (not to exceed 30% of this allocation) will be held to conduct research on emerging issues that might not have been addressed during the prioritization or to conduct cross-cutting research that addresses more than one priority species.

These selected proposals are to be considered sub-projects with respect to this work plan. Detailed sub-project work plans and budgets will be submitted to LCCMR for review and approval. The details about each sub-project work plan will be included as attachments to this document. Regular activity updates and budget updates will be provided by sub-project leaders and MITPPC to LCCMR. This overarching work plan and budget will be updated accordingly to include general progress of the Center and a synopsis of activities completed by each sub-project. The budget updates for this overall work plan will provide summaries of expenditures (by budget line item) for each sub-project. Detailed sub-project reports and associated budget updates will be prepared by investigators in cooperation with the MITPPC Director and Associate Director. MITPPC will provide LCCMR with updates to this overall work plan and each sub-project as a single packet.

The Center will initiate and/or accelerate coordinated, applied research according to the prioritized list of pest and plant species that threaten Minnesota's prairies, urban and rural forests, wetlands, and agricultural resources as identified through this assessment process. Depending on the net impacts associated with each species, research may include new control methods including bio-control and technology, development of integrated pest management tools that minimize non-target impacts of control, early detection of and/or rapid response to new threats, and establishment prevention. The Center infrastructure is vital to improving Minnesota's capacity and response time to preventing and limiting introduction of new terrestrial invasive

species. All research projects will include an analysis of any consequences related to the management of prioritized species to the State's non-target flora, fauna or our soils, water and climate.

Workforce development and training experts in invasive species management is also critical. A core component of each project will be funding of graduate students and postdoctoral associates to work with existing faculty.

Existing faculty with less than 12-month appointments may include in the budget up to 25% of their time in their role as the project leader. Providing salary through these awards will secure faculty time and intellectual effort in the projects, assuring that we are attracting the resources to provide project design, effort, and mentoring of the graduate students and post-docs in their research development. We do not anticipate hiring any new faculty for the projects.

The Center will support multiple projects by research teams, each comprised of a UMN faculty member from one of the participating departments, one graduate student and one postdoctoral associate. Estimated funding per project will be $180,000-210,000 per year, for three to four years. We expect this to result approximately five projects in two separate phases, depending upon the priority identified by the annual risk assessment planning. It is expected that per project expenses for established invasive species will be higher as compared to prevention strategies.

Outcome	Completion Date
1. Request for proposals released	March 1, 2017
2. Initial research projects selected and launched (est. 5 projects, ranging from 3-4 years each)	June 30, 2017
3. Research findings for projects completed	May 31, 2023

Status as of *January 31, 2017*:
There has been no spending under this appropriation to-date. We are requesting the change in outcome completion dates to accommodate an RFP announcement this spring to better align with LCCMR's FY 18 RFP.

Status as of *July 31, 2017:*
A Request for Proposals was issued in March 2017; 13 pre-proposals were received. All of the pre-proposals addressed at least one of the top 15 research species in each taxa (plant, insect, pathogen.) Pre-proposals are currently under internal review.

Status as of January 31, 2018:
The MITPPC is currently in the final stages of the research project deliberation phase. Two projects have workplans ready for LCCMR review; one project is in external review; and the remaining two are completing their workplan development. We anticipate that the five research projects to be amended into the overall MITPPC workplan within the next several weeks.

Status as of July 10, 2018:
This report will no longer provide a separate overview of Activity 1. Because the work plan has one activity, the activity summary is redundant with the overall project status and updates. This section will emphasize accomplishments of sub-projects.

Status as of *January 31, 2019:*
No longer provided (see above).

Status as of *July 30, 2019:*
No longer provided (see above).

Status as of *January 31, 2020:*
No longer provided (see above).

Activity 1 Sub-Project Synopsis

MITPPC Sub-project 1: Studies of Fungi Associated with the Emerald Ash Borer: Finding effective biocontrol agents and elucidating the role of fungi during ash decline and mortality.
Project Manager: Robert Blanchette
Description: Fungi play an unclear role in the damage caused by emerald ash borer (EAB). EAB may move fungi that harm trees and contribute to tree decline. Other fungi may attack and kill EAB. Management of EAB to date has focused on the insect itself. This project is meant to find new alternatives to manage EAB by finding and using fungi as biological control agents and/or to keep infested trees healthy by identifying tree-disease-causing fungi. In the course of this project, researchers will also provide the first formal survey in Minnesota for *Hymenoscyphus fraxineaus* a fungus that is devastating ash trees in Europe, as part of efforts for early detection of new invasive threats. The goals of this project are to: 1) identify the fungi associated with EAB from sampling hundreds of trees; 2) test canker causing fungi obtained from the galleries for their ability to contribute to dieback and decline of ash, evaluate the role of decay fungi found in the galleries for their potential to cause serious wood degradation and hazardous conditions in ash, and evaluate the detrimental role of canker and wood decay fungi entering EAB wounds in trees before being chemically treated to control the beetle and 3) identify and test entomopathogenic fungi found associated with beetles or galleries for their biocontrol potential.

Summary Budget Information	ENRTF Budget:
	~~$472,757~~ $500,000

Outcomes, Activity 1, Identify fungi associated with EAB and monitor their distribution	Completion Date
1. Assemble preliminary lists of fungi in Minnesota ash trees that are (a) associated with emerald ash borer (EAB) and might kill the insect; (b) associated with EAB and cause tree disease; and (c) not associated with EAB and cause decline diseases.	12/31/2019
2. Assemble expanded lists of fungi in Minnesota ash trees that are (a) associated with emerald ash borer (EAB) and might kill the insect; (b) associated with EAB and cause tree disease; and (c) not associated with EAB and cause decline diseases.	12/31/2020
3 Provide final list of fungi in Minnesota ash trees that are (a) associated with emerald ash borer (EAB) and might kill the insect; (b) associated with EAB and cause tree disease; and (c) not associated with EAB and cause decline diseases.	12/31/2021
4. Assess distributions of fungi in relation to the distribution of EAB and other environmental conditions	6/30/2022
Outcomes, Activity 2, Identify fungi associated with EAB and monitor their distribution	
1. Determine ability of fungi associated with emerald ash borer to cause disease in live trees	6/30/2021
2. Determine the ability of fungi associated with emerald ash borer to rot wood	12/31/2021
Outcomes, Activity 3, Identify and test entomopathogenic fungi for their biocontrol potential of EAB	
1. Determine ability of isolated fungi to infect and kill emerald ash borer in the laboratory.	12/31/2020
2. Determine ability of fungi to kill emerald ash borer in the field	12/31/2021
3. Identify the most effective approach for infecting emerald ash borer adults with fungi	6/30/2022

Status as of *July 10, 2018:*
Sub-project is in early phases of organization. No update is required per LCCMR instruction.

Status as of January 31, 2019:
Good progress has been made on all aspects of the project. Samples of ash infested with EAB have been obtained from over 50 sites throughout Minnesota for our studies to better understand the microorganisms associated with the beetle. Cultures have been obtained and we have identified three important groups of fungi residing in EAB larval galleries; canker causing fungi, pioneer species of wood decay fungi, and entomopathogens (pathogens of insects). This work is providing new information on the microbes associated with EAB and the biology and ecology of these organisms. In addition to isolations of beetle galleries, isolations from guts of EAB and frass (excrement) as well as ash bark have been made and cultures obtained. We have also completed the sequencing of the genomes for four fungal isolates from EAB galleries which we have identified to known entomopathogenic species (two *Beauveria* and two *Purpureocillium* isolates). The team has not encountered any obstacles during the investigations and work has exceeded expectations. Outreach activities have included a Midwestern presentation at the Upper Midwest Invasive Species Conference on the "Fungi associated with the Emerald Ash Borer: the role of fungi during ash mortality and finding effective biocontrol agents" (also see Dissemination).

Status as of July 31, 2019:
Good progress is being made on the project, outcome deadlines have been accomplished and research is on track. We have obtained a preliminary list of fungi associated with EAB and continue to expand the list as new identifications are made. From the total of 1,169 isolates, we have 280 that are potential canker causing fungi, 97 wood decay fungi and 94 entomopathogenic fungi. Phylogenetic analyses are being done on fungal groups of interest (decay, canker causing and new species) that need additional characterization. In additional studies, we have also identified fungal isolates from living ash bark and EAB frass. 120 seven foot ash trees (a donation from Schmidt Nursery, Oregon) are being planted in a University field plot for inoculations to test for pathogenicity of the canker forming fungi. Infested logs were placed in rearing chambers and emergence of adults has taken place. Scale up for obtaining more adult beetles is underway. The project team has secured additional support in the form of 500 Gb of free sequencing (~$7k value) by the US Department of Energy Joint Genome Institute Community Science Program. This will allow a deeper understanding of the fungal and bacterial communities present in the ash-EAB system, including those which aren't culturable. We are developing additional insect pathogenicity assays that are able to test multiple modes of potential biocontrol including both larval and adult parasitism and accounting for mortality as well as sub-lethal effects that may help suppress EAB populations.

Status as of January 31, 2020:
Excellent progress continues to be made on all aspects of the investigations. Following the culturing and identification of 1130 fungal isolates from EAB galleries, important studies have been initiated in the last six months using some of the most relevant canker, decay and entomopathogenic fungi. Pathogenicity testing of canker fungi is underway in the lab and field to determine their role in tree mortality. Decay potential is being tested of decay fungi in soil microcosms, which will yield data on understanding how rapidly EAB attacked trees may create hazards. We are also a step closer to understanding the pathogenicity of entomopathogens that may be used as biocontrol agents. Entomopathogens applied by spraying a spore suspension onto ash log segments have successfully colonized larvae that developed from reared eggs placed on the bark. No major obstacles have been encountered on the project. A presentation entitled 'Canker, decay and entomopathogenic fungi associated with emerald ash borer galleries' and a poster, 'Links between ash fungal endophytes and emerald ash borer gut communities' were presented at the 87th annual meeting of the Mycological Society of America, in Minneapolis on August 10-14, 2019.

Status as of July 31, 2020:

Excellent progress continues to be made on all aspects of the investigations despite disruptions by the covid-19 pandemic. The results of our broad investigation to elucidate the diversity of fungi associated with the emerald ash borer (EAB) galleries has been drafted in a manuscript and is being submitted to a scientific journal for publication. Among the large fungal diversity identified in this new niche created by EAB, there are three important functional guilds; canker (30% of all fungi), decay (8%) and entomopathogenic fungi (8%). Each appears to be playing an important role in ash tree being affected by EAB. Studies on each of the respective guilds is continuing to determine the role of these fungi. Several laboratory and field experiments are testing the pathogenicity of the canker fungi. Wood decay studies are evaluating the degradation capacity of the decay fungi and studies on the effect of entomopathogenic fungi on EAB have been completed. Among the various fungi isolated EAB galleries, 42 taxa did not match described species using DNA sequencing and many of these fungi appear to represent new species. We are also a step closer to understanding the pathogenicity of entomopathogens that may be used as biocontrol agents. Entomopathogens applied by spraying a spore suspension onto ash log segments have successfully colonized larvae that developed from reared eggs placed on the bark. We have begun selecting sites and developing methods for a field trial using our entomopathogenic isolates. No major obstacles have been encountered on the project.

Status as of *January 31, 2021:*
Excellent progress continues to be made on this project. We have identified a comprehensive group of fungi that are associated with the emerald ash borer (EAB) including fungal pathogens of ash that cause lesions around EAB galleries and contribute to tree death, wood destroying fungi that weaken ash trees and entomopathogenic fungi that attack EAB. A publication entitled, "Fungi associated with galleries of the emerald ash borer", is currently under review for publication. Two field trails have been completed demonstrating the pathogenicity of several species of canker causing fungi isolated from EAB galleries. Wood decay experiments have also been initiated to evaluate the decay capacity of the early colonizing fungi that enter wounds made by EAB and how they contribute to hazardous tree conditions. Laboratory and field experiments have also been initiated to test the pathogenicity of entomopathogens on EAB for potential use as biological control agents. Egg inoculation assays have been completed with *Beauveria bassiana, Lecanicillium longisporum* and several other fungi. Field inoculation studies using mature ash trees were also set up to evaluate the effect of spraying *Beauveria bassiana* and *Purpureocillium lilacinum* on EAB larvae. Adult emergence and larval mortality will be determined. In addition, molecular studies also are being done to determine the microflora within and on EAB, in frass and infested and non-infested phloem. Additional support for expanding the molecular characterization of microbial communities in EAB and infested ash was obtained with a 5-year National Science Foundation Dimensions of Biodiversity grant. This grant begins in 2021.

Status as of July 31, 2021:
Good progress has continued on all aspects of the project's activities. All work is on schedule and significant results have already been obtained. An extensive study of the fungi associated with the emerald ash borer (EAB) from three different regions of Minnesota has been published. This paper provided important new information on the canker and decay causing fungi as well as the entomopathogens found in galleries of EAB. The results were reported in numerous newspaper articles and radio and television news reports. Laboratory studies testing the pathogenicity of several entomopathogenic fungi on EAB eggs has been completed. The results show that several isolates appear to have greater pathogenicity to eggs than others. The best isolates are being used in field trials. New field trials for 2021 have begun with tree trunk spraying with fungal spore suspension to target freshly laid eggs. In addition, other trials focusing on tree trunk spraying with fungal spore suspension to target emerging adults and also larvae under the bark have been initiated. Green and purple funnel traps and green panel sticky traps with lures (3z-hexenol and EAB lactone pheromone) to attract EAB adults have been deployed in ash trees at different locations to collect adults for isolation and environmental sequencing of the fungi on the adult insects. EAB are being collected from the traps every 3-4 days. EAB adults are also being reared in growth

chambers and adults emerging from these logs will be used in laboratory tests to determine the ability of these fungi to infect or kill EAB eggs or larvae.

Status as of January 31, 2022:
Good progress is being made on all aspects of the project. Fungi associated with the emerald ash borer consist of canker causing fungi, decay fungi and entomopathogens. Results from studies on the canker causing fungi have been completed and published. Investigations on the decay fungi are continuing with laboratory studies testing 13 different fungi on ash wood blocks and ash sapwood disks in two different microcosms. These replicated studies will be completed in the next few months. Several entomopathogens have been isolated and identified and laboratory studies have selected strains for field trials. Four different field trials were established to evaluate the effectiveness of these insect pathogens to attack EAB eggs and larvae. All field trials were harvested in November and logs taken into the laboratory where they are being analyzed. Additional laboratory studies have also been completed to evaluate other strains on attacking EAB eggs.

Status as of July 31, 2022:
Our research has provided a great deal of important new information on the fungi associated with the emerald ash borer (EAB). Accomplishments were obtained for all activities that were established for the project. We have a large group of different entomopathogens were identified that we have subsequently used on egg, larvae and adult stages of EAB. Laboratory egg inoculation studies showed that several species are very effective in attacking eggs and could be used as an ovicidal treatment. Field studies using EAB eggs applied to trees and subsequent inoculation appeared to be only partially effective and additional study is needed to test treatments that would ensure entomopathogen survival during dry summer months. Trials also were initiated to test the entomopathogenic fungi on adult EAB. This has provided information that will be useful for future field trials using auto-dissemination traps. Entomopathogenic fungi have also been successfully injected into trees and when injected in the trunk at ground level they can be recovered 4 meters up into the crown. Results from wood decay assays using fungi obtained from EAB galleries has demonstrated the decay capacity of aggressive pioneer fungi that colonize EAB attacked trees. Our results indicate that prompt removal of attacked ash trees is needed to avoid hazardous tree conditions that occur rapidly by these decay fungi. Overall, all activities in the project have had successful outcomes that provides new findings on how fungi interact and control EAB.

Final Report Summary:
The emerald ash borer (EAB) is an exotic beetle that has been introduced into the United States and is currently causing serious losses of ash trees in Minnesota. To effectively manage this pest, it is essential to understand the biology and ecology of the beetle and associated microorganisms. Our research has identified the diverse fungi that are associated with EAB. These include: i) canker causing fungi that work along with EAB to kill trees, ii) aggressive pioneer decay fungi that enter EAB wounds and cause hazardous conditions in ash trees attacked by the beetle and iii) fungi that can kill EAB with potential use biological control agents. Laboratory and field studies have been done to test the pathogenicity of selected fungi on eggs, larvae and adult EAB. These studies have shown that fungi can kill EAB and several species have been evaluated and are now available for field trials. This method of control for EAB provides an additional tool that natural resource managers will be able to use to control the pest. Methods of spraying and injecting trees have also been tested. Other fungi obtained from EAB galleries produce lesions and pathogenicity studies show that several of these canker causing fungi work in concert with EAB to kill trees. We also have a better understanding of the pioneer species of decay fungi that come into wounds made by EAB. These fungi cause extensive decay and strength losses early in the colonization process resulting in affected ash to become hazardous. These results, which are especially important in the urban landscape, indicate that timely tree removal is needed to avoid hazards produced by EAB associated wood decay fungi. Our research results provide important new findings for integrated pest management that will benefit Minnesotans long into the future.

MITPPC Sub-project 2: Understanding the benefits and limitations of using goats for invasive plant control
Project Manager: Tiffany Wolf
Description: Common buckthorn, *Rhamnus cathartica*, is a common invasive shrub in many parts of Minnesota and is problematic for a number of reasons. Millions of dollars are spent annually in the state to control it. In many areas, labor costs or site accessibility limit the practicality of cut-treat methods of control. As a result, goats have been touted as a management alternative. These generalist grazers can be trained to eat buckthorn. The long-term effectiveness of the approach has not been tested, nor have the impacts to native plants. In addition, placing goats in shrubby/forested landscapes may expose them to brain parasites, specifically *Parastrongyloides tenuis*. The parasites occur in snails and slugs (intermediate hosts) and can be eaten accidentally while goats graze on vegetation. Geese and other fowl are not susceptible to the parasite, so having fowl graze on snails and slugs may increase the safety of the site for goats. The intent of this research is to evaluate the effectiveness and sustainability of a buckthorn management alternative. The specific goals of this research is to 1) assess the efficacy of goat browsing for invasive species control, 2) determine the effects of goat browsing on native plant abundance and community composition, and 3) assess whether goose grazing can be utilized to lower the incidence of *P. tenuis* in goats by reducing gastropod abundance.

Summary Budget Information	ENRTF Budget:	$410,267

Outcomes, Activity 1, Use *Rhamnus cathartica* as a focal species to assess the benefits of goat browsing for invasive species control	Completion Date
1. Quantify effects of goat browsing with respect to both short-term (defoliation) and long-term (population growth rate) metrics.	10/31/2021
2. Provide demographic data that can be used to target the most vulnerable life stages of *R. cathartica.*	2/28/2022
3. Disseminate research findings to practitioners through University of Minnesota Extension and peer-reviewed scientific journals.	2/28/2022
4. Provide information about the study, including research findings, to the general public and taxpayers through blog posts, social media, and formal journalism.	2/28/2022
Outcomes, Activity 2, Quantify the effect that goat browsing has on native plant species abundance and diversity	
1. Determine the short-term and longer term effects of goat browsing on native plant abundance and community composition.	2/28/2022
2. Disseminate research findings to practitioners through University of Minnesota Extension and peer-reviewed scientific journals.	2/28/2022
3. Provide information about the study, including research findings, to the general public and taxpayers through blog posts, social media, and formal journalism.	2/28/2022
4. Provide best management practices for practitioners to use when determining which sites are most conducive to management using goat browsing to be distributed through University of Minnesota Extension.	2/28/2022
Outcomes, Activity 3, Assess whether goose grazing can be utilized as a management strategy to lower the incidence of *P. tenuis* in goats	
1.Describe meningeal worm exposure in goats associated with invasive vegetation management by documenting changes in *P. tenuis* incidence associated with the browsing season	02/28/2022
2.Describe the efficacy of using waterfowl co-grazing to reduce *P. tenuis* exposure risk in goats by using changes in gastropod abundance and diversity as a proxy for risk	02/28/2022
3. Contribute to the gastropod collections at the Bell Museum, which have gaps in coverage of terrestrial gastropod diversity.	10/30/2021

4. Disseminate research findings to ungulate owners through University of Minnesota Extension.	2/28/2022
6. Publish results in peer-reviewed scientific journals.	2/28/2022

Status as of *July 10, 2018:*
Sub-project is in early phases of organization. No update is required per LCCMR instruction.

Status as of January 31, 2019:
We embarked on our first field season this year, examining the effects of goat browsing on the invasive shrub common buckthorn and native plant community response. Preliminary results show that goats most heavily defoliate buckthorns of intermediate size, largely ignoring small plants and being unable to reach the canopies of buckthorns that are 4+ meters tall. Goats also killed a significant proportion of buckthorn germinants and seedlings through trampling. Additionally, when goats ate buckthorn berries, only a very small percentage of seeds passed through their digestive tracts intact.
We also prepared for experimental work next year that will examine whether co-grazing goats with ducks and geese reduces the exposure of the goats to a potentially deadly parasite, meningeal worm. This preparation included exploring which methods for gastropod and deer pellet collection would be most appropriate in assessing the level of meningeal worm infection risk on the landscape.

Preliminary results of our work have been disseminated through a poster presentation at the University of Minnesota, College of Veterinary Medicine's Points of Pride research symposium, as well as outreach presentations to the public and social media posts.

Status as of July 31, 2019:
Our major accomplishment over the winter was completing a study on viability of common buckthorn (*Rhamnus cathartica*) seeds following goat digestion. While stakeholders are enthusiastic about using goats for buckthorn control, there is concern about goats potentially spreading buckthorn through "endozoochory." Encouragingly, we found that only 2% of buckthorn seeds survived goat digestion, and of those 2%, only 11% were viable, compared to 63% viability in control (undigested) seeds. These numbers align with values from the scientific literature, where we found that seeds >4mm in length (buckthorn is ~5 mm) have low survival of goat digestion. Overall, it appears that goats present low risk for dispersing buckthorn seeds between sites. Our findings also provide a rule of thumb for other plant species based on seed length, an easy to measure characteristic. We will soon submit a manuscript on these results to a peer-reviewed journal with a large land manager audience. Other accomplishments include compiling data for a meta-analysis on the use of livestock for plant management, performing preliminary analyses of data from last field season, conducting late-winter deer pellet surveys, preparation of a land manager survey, setting up ducklings with 4H student rearers, and drawing blood from goats to examine their meningeal worm antibody levels. A minor obstacle is that the immunological test for meningeal worm exposure is still in development, so serum samples will be archived until the test is available. Finally, Dr. Marchetto presented on meningeal worm and our study to the UMN Vet School's small ruminant club.

Status as of January 31, 2020:
In the past 6 months we completed our second field season. In addition to the data on short-term effects of goat browsing on common buckthorns and plant community composition collected last season, we were able to record longer-term metrics, such as buckthorn overwinter growth and survival and changes to plant community composition one year after goat browsing treatments. While we ran into some obstacles with finding landowners with suitable properties who were willing to host the experiment to see if waterfowl co-grazing could reduce the exposure of goats to meningeal worm parasites, we were able to deploy the experiment at the Rosemount Research and Outreach Center and collect preliminary data on the effects of waterfowl additions on the abundance and diversity of the intermediate hosts of the parasite (snails and slugs). A manuscript describing

the proportion of buckthorn seeds destroyed after goats eat the fruit is currently in revision with the *Natural Areas Journal*. Preliminary results were also disseminated at the Conservation Sciences Seminar Series at the University of Minnesota.

We propose to amend Activity 3 outcomes in response to the logistical obstacles we encountered this past field season. Based on the way that our industry partners manage the goats across field sites throughout the season, we have found it challenging to impossible to keep individual goats in waterfowl treatment groups for the entire period of time that they are at risk of meningeal worm exposure.

Status as of July 31, 2020:
Our greatest accomplishment in the last 6 months was submitting a manuscript for a meta-analysis we conducted to determine what is currently known about the overall effects of using livestock for targeted and prescribed grazing projects, where the explicit goal is the reduction of an undesirable plant species and/or increases in plant community diversity and richness. We conducted this work to address questions we frequently field from land managers about whether or not they should use goats to control invasive plants on the properties they manage. We found that, across studies, using livestock for vegetation management did lead to a significant reduction in undesirable plant species both immediately and one year after grazing. We also found that plant community richness and abundance showed a small, significant increase after targeted grazing. However, since most studies reported total species richness, without differentiating numbers of native and non-native species, this effect could be driven by increases in invasive or otherwise undesirable species. Once our experimental study is complete, we will be able to fill in conspicuous gaps in current knowledge, such as effects of goat browsing on native vs. non-native species, the extent of actual mortality vs. mere defoliation, and effects in an under investigated context (a woody target invasive species being managed in urban/suburban environments, as opposed to more common scenarios like herbaceous target species being managed in rangelands). In April, our manuscript about potential dispersal of buckthorn seeds following consumption by goats was published in the *Natural Areas Journal*, which spawned a number of outreach opportunities, including a Natural Areas Association webinar and radio and print interviews.

Status as of January 31, 2021:
Our major accomplishments over the past 6 months were the completion of a successful third field season despite the pandemic, completion of an epidemiological study of meningeal worm cases using UMN Veterinary Diagnostic Lab case reports, and progress towards communication goals. We continued to record demographic data (growth, survival, and reproduction) for over 600 individually marked buckthorn individuals across 4 field sites. We also continued to monitor plant community composition at the same 4 sites. Additionally, we repeated our waterfowl addition experiment at the Rosemount ROC to explore whether co-grazing with waterfowl might reduce gastropod abundance and thus, *P. tenuis* risk to goats. While we were unable to collect goat blood samples to assess meningeal worm exposure this year due to the pandemic, a study of historical data from 2001-2019 case reports confirmed anecdotal evidence we have received from companies using goats for plant management that 1) meningeal worm incidence peaks in late fall/early winter, and 2) there is wide variation in meningeal worm incidence across years. We also determined that higher summer temperatures may be a risk factor for increased *P. tenuis* incidence, but summer precipitation was not—both were hypothesized as factors that could lead to greater exposure through enhanced gastropod activity. Lastly, we have continued to communicate our results by attending and speaking at the virtual Upper Midwest Invasive Species Conference and submitting a revision of our targeted grazing meta-analysis manuscript to Restoration Ecology.

Status as of July 31, 2021:
The last six months have been busy with data analysis, population modeling, and manuscript writing. We have a preliminary population model for buckthorn, which uses information about plant size, growth, survival, and reproduction derived from our 2018-2020 field sampling to estimate how quickly buckthorn populations will

increase with and without goat browsing. This model also allows us to identify stages of the buckthorn life cycle (in particular, size stages of buckthorn individuals) for which management interventions could have the greatest impact. In terms of research dissemination, we recently published a review and meta-analysis of targeted grazing in invasive plant management, which is now online at Restoration Ecology. We also have a manuscript near submission that describes the results of the waterfowl addition experiments of Activity 3 (working title: Mitigating disease risk for goats used to manage invasive plants: can co-grazing with waterfowl reduce exposure to meningeal worm?). Another manuscript is in preparation. This work stems from one of our COVID-19 pivot plans from last year (~50% done; working title: Epidemiological investigation of meningeal worm mortalities in small ruminants and camelids examined by a Minnesota veterinary diagnostic laboratory over twenty years). Finally, Dr. Marchetto gave a presentation about terrestrial gastropods and the waterfowl addition experiment results at the Gathering Partners Conference in May 2021.

Status as of January 31, 2022:
Our major accomplishments in the past six months include completing data collection, finalizing analysis of the full plant community composition data set, writing and submitting a manuscript, and submitting our terrestrial gastropod samples to the Bell Museum of Natural History. Data were collected from 3 sites where goat browsing occurred in 2020, providing important buckthorn growth and survival information as well as long term goat browsing effects data on plant community composition. Both an original and revised manuscript of the waterfowl addition experiment were submitted to EcoHealth (titled Can co-grazing waterfowl reduce brainworm risk for goats browsing in natural areas?). We are also on the cusp of submitting a manuscript for the historical meningeal worm necropsies project (Epidemiological investigation of meningeal worm mortalities in small ruminants and camelids examined by a Minnesota veterinary diagnostic laboratory over a nineteen year period) and a manuscript examining effects of goat grazing on native plant communities (Responses of native plants to targeted grazing of invasive common buckthorn, *Rhamnus cathartica*). In addition, we submitted 37 terrestrial gastropod samples to the Bell Museum, comprising 23 species, 3 of which were new to the collection and 11 of which were recorded in a new county.

Final Report Summary:
The use of goats for invasive plant control is increasing, yet few data exist on the effects of goat browsing on invasive species populations or native plant community composition. The cost of this management strategy is also elevated in some regions due to mortality caused by a parasite of white-tailed deer, *Parelaphostrongylus tenuis,* that goats may be exposed to when browsing in areas where infected deer defecate. To address these issues, we used *Rhamnus cathartica* as a target species to quantify the short- and long-term effects of goat browsing for invasive plant control and non-target impacts on associated native plant communities. We found that goats provide temporary suppression of *R. cathartica* abundance but this invasive shrub rebounds following grazing cessation. Native vegetation was similarly temporarily suppressed, but in some cases native plant diversity reached higher levels following grazing treatments. A broader synthesis and meta-analysis of the targeted grazing literature revealed similar patterns for the effects of goats and other livestock used for targeted grazing of invasive or undesired plant populations. Importantly, consumption by goats kills the seed of *R. cathartica*, and other invasive plants with larger seeds, indicating that goats are unlikely to exacerbate invasions by spreading them to new areas. Finally, in evaluating the *P. tenuis* risk to goats, we conducted a retrospective study of *P. tenuis*-associated mortalities of small ruminants in Minnesota over a 19-year period, as well as examined whether co-grazing goats with waterfowl could reduce transmission risk through waterfowl consumption of the gastropod intermediate hosts that harbor this parasite. Overall, we determined that the *P. tenuis*-associated mortality rate of goats is low (<1%), though it is unclear how browsing for invasive plant control might affect this level. Through our co-grazing experiments, we found more gastropods in habitats after goats had browsed alone; however, we did not observe these increases when goats were co-grazed with waterfowl. In addition, waterfowl did not negatively affect overall gastropod abundance or diversity. Thus, waterfowl could reduce *P. tenuis* risk to goats without harming gastropod communities.

MITPPC Sub-project 3: Genetic Control of Invasive Insect Species: Phase I
Project Manager: Michael Smanski
Description: Spotted wing drosophila, *Drosophila suzukii*, is devastating small fruit producers in Minnesota. Growers are turning to the use of broad spectrum insecticides applied on a calendar basis to control the insect. The result has been a dramatic increase in insecticide use and an equally sharp rise in production costs. This research is meant to explore a revolutionary management alternative, genetic biocontrol. The project builds upon previous research that leverages the latest genetic tools to engineer 'species-like' barriers to reproduction. It is anticipated that this approach will offer a safe, robust, and easily scalable control method that directly addresses several challenges of sterile insect techniques (SITs). The goal of Phase I is to complete a proof of concept in a closely related model insect, *Drosophila melanogaster*, and transition the technology to *D. suzukii,* and perform a genome-wide analysis of sequence diversity in invasive *D. suzukii* populations.

Summary Budget Information	ENRTF Budget: ~~$ 296,655~~ $295,717

Outcomes, Activity 1, Proof of concept of engineering approach in the model insect, *D. melanogaster*	Completion Date
1 Completion and validation of *D. melanogaster* synthetic species	3/30/2018
2 Completion and validation of *D. melanogaster* strains with female lethality and species like barriers to reproduction (= self-sorting incompatible male system [SSIMS]).	6/30/2018
3 Testing the biocontrol insects in laboratory mating experiments to learn how effective the technology is in flies.	3/31/2019
Outcomes, Activity 2, Engineer SSIMS strains of invasive agricultural pest *D. suzukii*	
1 Test the engineered *D. suzukii* in controlled laboratory mating experiments to learn if the applied insect biocontrol agent works as well as our model species.	2/28/2021
2 Identification of additional target genes to make the technology more effective	2/28/2020
Outcomes, Activity 3, Assessment of genetic diversity in local seasonal populations of *D. suzukii*	
1 We will demonstrate that new state-of-the-art genome sequencing technologies can provide high-quality data from a single animal.	9/30/2020
2 Sequence the genomes of 20 wild-caught *D. suzukii* to learn the location of the identical regions that can be targeted by our technology to maximize its efficacy.	9/30/2020

Status as of *July 10, 2018:*
Sub-project is in early phases of organization. No update is required per LCCMR instruction.

Status as of January 31, 2019:
We have made significant progress towards developing a first-of-its-kind biocontrol approach to combat Spotted Wing Drosophila (SWD), using Sterile Male Accelerated Release Technology (SMART) flies. In the past 6 months, we have demonstrated that all of the engineered genetic components are functional in a closely related laboratory species of fruit fly. We have developed protocols for creating and rearing transgenic SWD. Lastly, we have performed an environmental analysis of the DNA sequence diversity in wild SWD. From this we have learned that early season flies from fruit farms located 100+ miles apart are similar genetically and could be controlled with the same engineered SMART flies. We are on schedule to meet our two year project goals.

Status as of July 31, 2019:
In the past 15 months, we have demonstrated that all of the engineered genetic components are functional in a closely related laboratory species of fruit fly (Activity 1). We are several weeks out from combining these together in a single strain (through controlled breeding) to create our first SMART fly. We have developed protocols for rearing SWD, collecting eggs, and performing recombinant DNA microinjections (Activity 2). We

have not yet succeeded in making our first transgenic SWD, but we have reached out to experts in this area and have identified elements of our protocol that can be improved. Lastly, we have performed an environmental analysis of the DNA sequence diversity in wild SWD (Activity 3). From this we have learned that early season flies from fruit farms located 100+ miles apart are similar genetically and could be controlled with the same engineered SMART flies. We have also developed a method to obtain whole genome sequence data from a single fly. We will leverage this ability in the future to characterize any genetic resistance that we encounter during pre-field or field trials.

Status as of January 31, 2020:
We have made substantial progress towards developing a first-of-its-kind biocontrol approach to combat Spotted Wing Drosophila (SWD), using Sterile Male Accelerated Release Technology (SMART) flies. In the past 6 months, we have produced and characterized the first complete example of our SMART technology in a closely related laboratory species of fruit fly (Activity 1). We have completed data collection for our second season of field work to monitor single nucleotide polymorphisms (SNPs) in wild SWD populations (Activity 3). We have analyzed data associated with our single-fly whole genome sequencing aim (Activity 3) and learned about a technical roadblock that likely occurred during library preparation, which limits our ability to identify genome-wide mutations. We have not yet succeeded in making our first transgenic SWD (Activity 2), but this will be the focus of the remaining period of funding. To (i) overcome the technical roadblock associated with single-fly whole genome sequencing (Activity 3), and (ii) address unanticipated changes to our personnel budget, we are submitting a budget re-allocation request along with this end of year report. We have submitted one publication relevant to this project in the past six months, which is currently under peer review.

Status as of *July 31, 2020:*
In the past reporting period, we have made substantial progress towards developing a first-of-its-kind biocontrol approach to combat Spotted Wing Drosophila (SWD), using Sterile Male Accelerated Release Technology (SMART) flies. We have demonstrated the complete system in a closely related laboratory species of fruit fly (**Activity 1**). We have experienced technical difficulties that have slowed progress towards transitioning to SWD, but are making continual (albeit slow) progress towards overcoming these difficulties (**Activity 2**). We have not yet succeeded in making our first transgenic SWD. Lastly, we completed a second year of field collection of wild SWD to complement our population genetics study from year one (**Activity 3**). We are a few weeks away from obtaining the complete dataset to analyze the two-year longitudinal study. This work was presented at a national virtual symposium in a seminar titled "Synthetic Biology in Insects" in April 2020. We have been invited to contribute to a closed-door workshop on genetic control of insect vectors in July 2020, where we will present on technology development for agricultural applications. Two manuscripts have been submitted and are in review.

Status as of January 31, 2021:
Despite ongoing restrictions on operations in research labs due to the Covid-19 pandemic, we made substantial and exciting progress on this project in the past six months. We completed the largest-to-date populations genetics analysis of Spotted Wing Drosophila (SWD) to provide essential information for designing genetic biocontrol approaches. We also succeeded in genetically manipulating SWD in the lab for the first time. Together, these advances pave the way for us to transition our new technology, which was published in Nature Communications for the model insect *Drosophila melanogaster* in September 2020, into this agricultural pest and invasive species. In addition to the publication in Nature Communications, we had a second related publication in PloS Genetics during this 6-month period. Lastly, PI Smanski was invited to discuss our genetic biocontrol technology in an international seminar series organized by the Foundation of the National Institute of Health (link).

Final Report Summary:
With the overall goal of demonstrating our innovative genetic biocontrol approach in the pest insect Spotted Wing Drosophila, we had three specific objectives on this project: (i) demonstrate a proof-of-concept in the model laboratory insect and close cousin to SWD, *Drosophila melanogaster*, (ii) translate what we learned from *D. melanogaster* into the SWD species, and (iii) study the genome sequence of wild SWD so we can precisely design our engineered biocontrol agents to effectively suppress wild SWD populations in Minnesota.

Our outcomes and results for the first objective exceeded project expectations. We succeeded in making the proof-of-concept in *D. melanogaster*, and the engineered insects were 100% incompatible with wild-type flies. We made over a dozen versions. We also added additional genetic control elements to automatically sort the males from females, making the technology more economical to deploy for pest control.

We did not meet our objective two milestones (completing the engineering of SWD), however, we made good progress in that direction. Near the end of the award, we succeeded in making our first transgenic SWD flies, so we should be able to move quickly now in finishing the engineering process.

Our results from the third objective exceeded expectations. While we initially planned to sequence the genome of 20 wild-caught flies, we instead invented a new approach that allowed us to sequence the relevant genes from over 10,000 wild flies. We are using this data in our current engineering efforts with SWD.

This was a high-risk/high-reward project. We were able to overcome a tremendous amount of technical risk on the project so far, and the approach is looking very promising. We plan to continue to make progress towards Objective 2 in our second Phase of this project.

MITPPC Sub-project 4: Dwarf Mistletoe Detection and Management in Minnesota
Sub-Project Manager: Marcella Windmuller-Campione
Description: Dwarf mistletoe, *Arceuthobium americanum*, is a jack pine-killing, parasitic plant that does not yet occur in Minnesota but is as close as Manitoba. Once it becomes widespread in a stand, it is nearly impossible to control. However, given the relatively limited dispersal capacity of the plant, eradication seem highly feasible if infection can be detected early. This project is designed to assess the sensitivity of aerial detection methods to detect small infestations and to evaluate workable silvicultural methods to eradicate it. As the real species of interest does not occur in Minnesota, this project uses the closely related Eastern dwarf mistletoe, *A. pusillum*, as a surrogate to test the methods. The primary goal of the proposed project is to increase detection and management options for dwarf mistletoe. Specific objectives are to: 1) develop efficient and effective detection methods, 2) assess the effectiveness of current management policies and the cost of implementation, and 3) revamp a spatially based model of mistletoe spread and volume loss in dwarf mistletoe impacted forest systems

Summary Budget Information	ENRTF Budget:$455,606

Outcomes, Activity 1, Develop efficient and effective detection methods for dwarf mistletoe	Completion Date
1. Identify and sample 40 black spruce stands prior to harvest	9/30/2020
2. Protocol and efficiency rating for sampling *A. pusillum* developed	1/31/2022
3. Analysis of type of vector of dwarf mistletoe analyzed	4/30/2022
4. BHM (Bayesian Hierarchical Model) for mistletoe detection validated	7/31/2022
Outcomes, Activity 2, Assessment of *A. pusillum* treatment methods and their implementation effectiveness	
1. Identify 30 black spruce stands	9/30/2018
2. Forester and logger survey designed and administered	6/30/2019

Outcomes, Activity 1, Develop efficient and effective detection methods for dwarf mistletoe	Completion Date
3. Field sampling of 30 sites completed	9/31/2021
4. Data analyzed and shared from survey and field sampling	12/31/2021
Outcomes, Activity 3, Develop a detection and eradication plan for dwarf mistletoe in jack pine stands in Minnesota	
1. Formation of *A. mericanum* steering group composed of scientists, practitioners, and forest health specialists from Minnesota and Manitoba	12/31/2018
2. Regular meeting of the steering group to aid in the development of the management plan	11/30/2021
3. Management plan and risk map completed	11/30/2022

Status as of *July 10, 2018:*
Sub-project is in early phases of organization. No update is required per LCCMR instruction.

Status as of January 31, 2019:
The project has made progress in all three activities. Activity 1 highlights include the development, testing, and sampling using a ground-based sampling design for dwarf mistletoe on 11 different forest stands. This resulted in more than 3,000 tree samples! Preliminary results were presented at the National Society of American Foresters meeting in fall 2018. For Activity 2, sampling of pre-harvest black spruce stands is on-going. Currently seven stands have been sampled with additional sampling dependent on weather conditions. The second part of Activity 2 includes using a mix of interviews, focus groups, and surveys of loggers and foresters to understand current knowledge and management of dwarf mistletoe. Ten interviews with loggers were completed. The use of interviews of loggers is a modification from the originally proposed mail survey due to concerns over survey fatigue with loggers as a result of being recently surveyed for other studies. The main accomplishment in Activity 3 is the re-establishment of the dwarf mistletoe network within the Great Lakes region both in the US and Canada. We have established contacts with natural resource managers in Manitoba. We are fortunate to have great working partnerships with state, private industrial, and county land management agencies. This has allowed us to stay on progress. The main limitation which is out of our control will be weather. If winters are mild, there will be reduced harvesting in black spruce stands. This may delay our ability to collect post-harvest data.

Status as of July 31, 2019:
Data from the first field season is actively being summarized. Preliminary results on the sampling design, structure, composition, and implications of different levels of eastern spruce dwarf mistletoe was presented at the Heart of the Continent Workshop in Duluth, MN which includes Minnesota and Canadian natural resource managers (Activity 1 & 3). Stands have been selected for sampling for summer 2019 to continue testing detection methods (Activity 1). Nine black spruce stands were sampled prior to harvest during the winter of 2018/19 (Activity 2). All in-person interview with loggers have been completed (n=10) and analysis is on-going. Three focus groups were conducted in Bemidji and Duluth with loggers (n=34). Initial feedback from the focus group includes interest in potential future presentations on dwarf mistletoe. Analysis is on-going. An online survey was developed and delivered to federal, state, county, industry, and tribal foresters (n=194). This was done in partnership with supervising staff in multiple agencies to try to ensure a greater response rate. After first wave, response rate is 41%. (Activity 2). Canadian scientists have expressed interest in discussing and collaborating on jack pine dwarf mistletoe (Activity 3).

Status as of January 31, 2020:
Over the last six months, data has been rolling in for Objective 1 and Objective 2. Some of the highlights includes over 5,000 individual trees sampled across 30 black spruce stands, over 100 foresters who responded to an online survey (a 58% of response rate) on dwarf mistletoe management, multiple focus groups with loggers and

foresters, the development and testing of two different models on dwarf mistletoe detection, and the successful defense of graduate student, Raychel Skay. The collection of this data will allow the research team to be able to answer the questions – how do can we better detect dwarf mistletoes and how are natural resource professionals (foresters and loggers) managing dwarf mistletoe? The utilization of field data collection, modeling, surveys, and focus groups will provide a holistic assessment of dwarf mistletoe. The project team has had a productive six months with all the objectives staying on target to meet deadlines.

Status as of July 31, 2020:
Activity 1 and Activity 2 saw large accomplishments. For Activity 1, winter sampling was completed so comparisons between on-the-ground sampling completed during the winter and summer can be assessed. Modeling of eastern spruce dwarf mistletoe and variables associated with it is moving forward and preliminary results have been shared through local and regional presentations. Data collection is complete for focus groups, interviews, and surveys for foresters and loggers for Activity 2. Analysis is near completion and results are being summarized into a Department of Forest Resources staff paper. A new MS student has been hired on to assist with pre- and post-harvest assessment of dwarf mistletoe and will begin this work in Fall 2020. We have received positive feedback from stakeholders who are extremely interested in the results of this work.

Status as of January 31, 2021:
COVID-19 has resulted in a longer timeline than initially anticipated due to logistics related to sampling. We have made great progress on aspects related to Activity 1 and Activity 2. In Activity 1, we have had our first publication and the first publication related to the impacts of mistletoe on the stand dynamics of black spruce forests. There was a successful sampling season for Activity 1 and validation will be started in 2021 on the current model to predict mistletoe, as well as a state level model of the relationship among environmental and stand level characteristics to mistletoe risk. This will be used in our discussion for Activity 3. Activity 2 is nearing the submission of staff paper on the two surveys completed on the knowledge that loggers and foresters have related to mistletoe. These surveys have supported the work we are completing in all of the activities, linking social science to vegetation science. Finally, Activity 2, sampling was delayed due to COVID-19 but will be able to be completed during the 2021 field season. Activity 3 is under progress and hope to host online meetings with information gained from Activity 1 and 2.

Status as of July 31, 2021:
Within the last project period, the team has been able to move multiple pieces towards completion or close to completion. Within Activity 1 and 2, the final season of data collection started in May 2021. Data collection includes stands that have been harvested with the goal of assessing post-harvest structure, composition, and the presence of mistletoe. Report writing is on-going for the focus groups and surveys from Activity 2. We are working on pre-planning with the Sustainable Forests Education Cooperative for a multiple day workshop on lowland conifers which will be offered in Fall of 2021. This workshop will be for natural resource managers from across Minnesota to discuss lowland conifer management and hear recent research regarding lowland conifer management including the results of our work. Additionally, there will be a pine systems workshop where we will be able to discussion jack pine dwarf mistletoe.

Status as of January 31, 2022:
This is our last progress report and there has been significant progress across all of the activities and significant work beginning to wrap-up and distribute our results and conclusions. During this last progress period, we have successfully published one peer reviewed article that includes a bench-marking quantifying the previous eastern spruce dwarf mistletoe model; we found that the model predicted mistletoe correctly approximately 50%. A large limitation with the previous model relates to mistletoe being considered a binary variable. We have also published a staff paper on results from surveys and focus groups with logging professional and foresters. This staff paper is currently being converted to a peer review publication. Finally, our work has been shared and part of the discussion in two symposiums – wet forests which included lowland conifer ecosystems and pine forests

which included jack pine ecosystem. These two symposiums brought natural resource managers from across the state and the broader region.

Final Report Summary (submitted between November 30, 2022 and January 15, 2023):
American dwarf mistletoe is an invasive species that infects and kills jack pine, a native tree species of Minnesota. American dwarf mistletoe is not currently present in Minnesota but has been detected in neighboring Canadian provinces. The goal of our project was to utilize Minnesota's native dwarf mistletoe, eastern spruce dwarf mistletoe (ESDM), to explore options for detection and management. Just like American dwarf mistletoe, ESDM results in mortality for its host tree, black spruce. We tested different types of detection methods. Google Earth was able to detect mortality, but we were unable to determine if mortality was caused by ESDM. Winter sampling resulted in higher potential false positives due to snow cover on tree. Summer sampling provided a clear view of the trees but movement within the stands were more difficult. Summer sampling was also used to explore impact of ESDM on forest ecosystems. ESDM is not a binary variable; lower levels of ESDM in black spruce stand resulted in higher tree species diversity and did not negatively impact regeneration.

With this new insight we explore different methods for predicting ESDM at the individual tree level and at the stand level using multiple different datasets. At the landscape level, we identified areas that have greater potential for impact from ESDM and linked those with stand and environmental variables which can provide foresters and natural resource management tools to prioritize management.

An additional part of our project was conducting focus groups and surveys with foresters and loggers within northern Minnesota. We found variable opinions regarding management and knowledge about ESDM and foresters and loggers identified the need for additional information about mistletoe and more data on results of management. We identified the need for training as a key component when considering early detection for the invasive American dwarf mistletoe.

MITPPC Sub-project 5: Developing a spatially explicit bio-economic dispersal model to aid with the management of brown marmorated stink bug
Project manager: Senait Senay
Description: The brown marmorated stink bug, *Halyomorpha halys*, feeds on many economically important plants (e.g., apples, soybean, corn, and more than 300 other plant species). The insect was first detected in Minnesota in 2010, but it has not yet invaded everywhere. Previous research supported by MITPPC is meant to characterize where temperatures might allow the insect to build to large densities currently and under future weather conditions. This project builds on those efforts by developing tools to forecast where the species might move (i.e., disperse) next from centers where it is known to be established. These tools will also be used to measure the economic value of intensifying management efforts around these infested locations to slow spread and protect plants. This project supports MITPPC's goals to provide managers with better tools to know the future distribution of invasive pests and to evaluate the socioeconomic impacts of invasive species and their management alternatives. The specific objectives of this project are to 1) develop a high resolution, spatially explicit, agent-based dispersal model for *H. halys*, 2) develop a bio-economic modeling framework to assess economic resource vulnerability to the risk of *H. halys* invasion, and 3) measure the economic value of this new information by comparing how management effectiveness compares with and without this information.

Summary Budget Information	ENRTF Budget:	~~$329,354~~ $329,304

Outcomes, Activity 1, Develop a high resolution, spatially explicit, agent-based dispersal model for *H. halys*	Completion date
1. Dataset and documentation on local and long distance *H. halys* dispersal parameters from existing literature and data calibrated for Minnesota.	05/30/2019
2. Harmonized gridded landscape layer representing the spatially heterogeneous pattern of *H. halys* survival generated from a spatial analysis of various *H. halys* suitability related geographic information.	10/2/2019
3. High-resolution selectively recoded survival landscape dataset generated by employing high-resolution remotely sensed data to encode configuration of landscape features within urban and semi-rural high-suitability hotspot areas of the landscape data from output 2 above.	3/15/2021
4. New flight distance data generated via a tethered-flight experiment.	12/27/2020
5. Parallelized-MdiG and a spatially-explicit, Minnesota-wide *H. halys* dispersal model specification and simulation for years 2010-2050.	12/31/2020
Outcomes, Activity 2, Develop a bio-economic modeling framework to assess economic resource vulnerability to the risk of *H. halys* invasion	
1. A spatially explicit compilation of data on the value of rural and urban resources susceptible to *H. halys* invasion risk,	7/31/2019
2. Standardized data on the cost of various *H. halys* prevention, containment, and mitigation techniques.	9/2/2019
3. Data on the impact of invasion produced from literature review and incorporated into the bio-economic modelling framework for undertaking risk analysis and determining efficient management trade-offs.	2/15/2021
4. Modifications to the MdiG program so that it incorporates economic risk analysis and allows for multi-scenario modeling.	8/1/2021
5. Generation of selected invasion-intervention scenarios, including efficient management scenarios that can be used to inform decision-makers choices regarding prevention, containment, and mitigation of *H. halys* invasion.	10/26/2021
Outcomes, Activity 3, Undertake a Value of Information (VoI) analysis by comparing the predicted resource outcomes of the proposed detailed *H. halys* invasion-intervention scenarios with results from less parametrized invasion and intervention scenarios	
1. Full scale invasion-intervention scenario simulations from Activity 1 and 2 across the entire time (2010-2050) and spatial extent (Minnesota) of varying spatial resolution and model specifications.	1/30/2022
2. Evaluate the value of information of selected modeling components to characterize the benefits gained from utilizing higher-resolution landscape information and incorporating complex dispersal assumptions in the modeling framework.	3/28/2022
3. Results formatted and stored at the IAA platform GEMShare for easy access and further analysis.	6/30/2022

Status as of *July 10, 2018:*
No activity. This project has not yet been approved by LCCMR staff.

Status as of January 31, 2019:
Sub-project is in early phases of organization. No update is required per LCCMR instruction. Project was approved by LCCMR staff on August, 1, 2018.

Status as of July 30, 2019:
The project commenced in October 2018. We held a half-day project kick-off meeting on February 22, 2019. Project team members from UMN, MITPPC and collaborators from MDA and MNDoT attended the meeting. Researchers presented their proposed methods. There were Q & A sessions to address any concerns by stakeholders. The meeting created a common understanding among stakeholders about project outcomes. One data access pipeline is completed and two are under development. Out of the six major datasets that are required to build the Minnesota-wide BMSB survival likelihood data-layer, three are already developed. We collected preliminary empirical BMSB dispersal distance data through the flight experiment being undertaken at the UMN. We acquired and standardized four out of the eight datasets needed for modeling human mediated BMSB dispersal (Activity 1). Preliminary spatial data on prioritized crop hosts of BMSB is being processed. We have completed literature review on BMSB host plants of economic importance for Minnesota. Data on the cost of various BMSB treatment options is being compiled (Activity 2). To facilitate efficient comparisons of different invasion scenarios, developing an easily deployable version of the modelling platform is necessary. To that end, a Docker environment is being set up to containerize MDiG the individual based model that is used to build the dispersal platform (Activity 3).

Status as of January 31, 2020:
The *Halyomorpha halys* tethered flight experiment concluded one of the planned three experiments—effect of starvation on *H. halys* adult flight. Results show that starved adults (both male and female) fly marginally longer distances than do fed adults. Dispersal data required for parametrizing the *H. halys* dispersal model has been collected. These are spatially explicit occurrence information, including data from the literature on distance, direction and frequency of flights. Data are now being analyzed to derive appropriate dispersal parameters for the model. Other information include, a list of reported mechanisms of dispersal for *H. Halys*, in order to inform the human mediated dispersal-modelling component. Various datasets that will be used to generate a survival layer both for self-mediated and human-mediated dispersal are either collected or generated, about 75% of the data needed is already accessed (Activity 1).
Spatial data layers of production information for top *H. halys* host crops in Minnesota have been generated and imported into the Project workspace. *H. halys* impact data has been processed for different target crops identified to be at high risk. Preliminary interviews have been undertaken to construct bio-economic scenarios that will be simulated to assess the costs and effects of different management decisions on *H. halys* dispersal (Activity 2).

The total size of the data generated or accessed for this project so far is about 3.5 TB. All outputs due to be delivered by this reporting period either are completed or will be completed by the January 31st mark.

Status as of July 31, 2020:
All three planned *Halyomorpha halys* tethered flight experiments, which are testing the effect of starvation, post-emergence age, and reproductive status of males and females on dispersal distance were concluded. A paper is currently being prepared for publication to report the findings of the tethered flight experiment. A workflow was developed to generate a gridded growing degree-day (GDD) dataset from the daily US climate data, PRISM. Experimental population data on BMSB from the MITPPC funded project "Early detection, forecasting and management of BMSB" has been used to calibrate GDD calculations for BMSB. The gridded GDD dataset along with the Minnesota wide elevation, BMSB climate suitability and land use datasets was used to generate the spatially explicit survival layer for the dispersal simulation. Sentinel II imagery required to generate vegetation indices and classify landscape features to enhance the survival layer is downloaded through another project and the data now is accessible for this project through the GEMS center. The project met most of its outcomes with the exception of the completion of the satellite image analysis. An additional 3 month extension is required to complete this task as the COVID19 lockdown made it difficult to access workstations where the image analysis was supposed to take place physically. The fact that the team now does not have to handle the sentinel imagery acquisition has saved some time limiting the required extension to finalize this task (Activity1).

Spatial data on host crops and other land cover has been compiled for the bio-economic risk analysis, analyzing damage scenarios is pending the dispersal simulation outcome of activity 1, which is not going to be available until the next reporting period (Activity2).

Status as of January 31, 2021:
Data on flight behavior of *H. halys* are now ready to parametrize various dispersal simulation scenarios. A paper is submitted for publication to report the findings of the tethered flight experiment. The graduate researcher has successfully defended his MSc. Thesis. A 17-county simulation area was used to run *H. halys* dispersal simulation for the years between 2010 and 2025 (10 replicates on a 10m resolution survival landscape). It took 48 hrs to complete a single run, which indicated the necessity to have the parallelized version of MdiG (the individual based modelling software that is being used to run the simulation) that runs on a high performance computing environment (HPC), if fit-to-purpose dispersal modelling over a larger area and higher resolution landscape is to be achieved. We are also on track to have the GPU-based simulation system setup and ready for simulating various bio-economic scenarios in a time-efficient manner for the use of the value of information analysis early 2021 (Activity 1). In the past six months, we have enriched our *H. halys* host, damage and impact information further. The 10m resolution simulated dispersal area maps have been up-scaled to 30m resolution layers to match the CDL crop layer data base that is going to be used as input for the bio-economic risk assessment. Work has started to model the bio-economic risk of *H. halys* invasion for the 17- County pilot simulation area. (Activity2). Five out of the six proposed landscapes for the Value of Information (VoI) analyses are processed, and one out of the four dispersal scenarios that are proposed for the VoI is completed (Activity 3).

Status as of July 31, 2021:
Activity 1: The *H. halys* flight studies were accepted for publication and were used to develop the Minnesota-wide brown marmorated stink bug (BMSB) dispersal simulations. These simulations describe rates and patterns of future BMSB spread through flight under different, plausible circumstances, for example, accounting for the age, sex, or feeding status of the insects. To describe human-mediated *H. halys* dispersal, different models were developed (some based on distance to known infestations, others on population density) and preliminary results were generated for Minnesota cities. Statewide maps were prepared at 10m, 250m, 500m, and 1km scales to describe where BMSB might permanently survive if it were to arrive. These survival layers are used with the state-wide dispersal simulations. Our early projections indicate that it could take up to 70 years for BMSB to spread into all suitable areas within the state if the insect only moved by flight. *Activity 2*: A preliminary analysis was done on the economic risk to Minnesota corn, soybean, apple, and grape production based on a 17-county pilot spread study completed for the last reporting period. From this pilot study, results showed the potential area at risk and value of that production (when pricing information was available) was 6% ($30M), 5% (~$8M), 4%, and 0.25% for corn, soybean, apple, and grape. *Activity 3*: Most of the planned tasks for this activity do not start until September, but the finalized 250m, 500m, and 1km survival landscapes will serve as key inputs for the value of information experiment.

Status as of January 31, 2022:
This project was closed on November 18, 2021 by the MITPPC director. The project was unable to complete its objectives due to the unanticipated inability of the principal investigator to continue with the project. Fortunately, one of the project's components was completed, Activity 1, Develop a high resolution, spatially explicit, agent-based dispersal model for *H. halys*, results of which were published as "Effects of Starvation, Age, and Mating Status on Flight Capacity of Laboratory-Reared Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)" in Environmental Entomology Volume 50, Issue 3, June 2021.
https://doi.org/10.1093/ee/nvab019

MITPPC Sub-project 6: Management of Invasive Knotweeds
Sub-Project Manager: Alan Smith
Description:

Highly destructive knotweeds are beginning to invade Minnesota residential, industrial and natural areas. Their escape from cultivation is facilitated by hybridization and seed production, previously thought insignificant. Knotweeds are challenging to manage, requiring several years of treatment. Their well-known resiliency triggers excessive and unnecessary herbicide applications and ecological disturbances during physical removal. Lack of species identification, carbohydrate use patterns and optimal herbicide use results in unnecessary environmental damage and spread to new sites. Knotweed taxonomy is confusing and confounded by frequent interspecific and intergeneric hybridization among the *Polygonaceae*. Knowing the genetic makeup and species composition of knotweed populations in Minnesota is crucial to its management because knotweed species respond differently to control measures. The lack of knowledge on Minnesota knotweed invasion is conspicuous and leading to an intractable knotweed invasion necessitating costly removal, loss of biodiversity, degradation to the environment and damage to property. Our goal is to provide essential information to understand and manage the knotweed invasion. Activity 1 will determine the genetic structure, species and extent of hybridization and introgression in knotweeds. These data provide essential information on knotweed composition and mode of spread in Minnesota. Activity 2 will determine herbicide sensitivity and potential resistance, measure key invasive phenotypes (reproductive strategy; sexual vs. clonal, biomass allocation and adventitious rooting efficiency) for a Genome-Wide Association Study (GWAS) of invasiveness. These data are fundamental to knotweed management, ultimately reducing further spread and economic and environmental damage by laying the groundwork to understand the evolution of knotweed invasiveness.

Summary Budget Information	ENRTF Budget: ~~$ 579,670~~ $476,723

Outcomes, Activity 1 Determine the genetic structure, species composition, and extent of hybridization and introgression in knotweeds.	Completion Date
1. Sample knotweed populations (U.S., European, Native Japanese and other Polygonaceae members as likely sources of introgression), assessed by DarT-seq to determine the species of *Fallopia* and genetic composition in Minnesota populations.	12/30/2020
2. Determine level of diversity through genetic structure analysis among and within populations and identify species or hybrids.	10/31/2022
3. Measure the level of interspecific and intergeneric introgressions within the Polygonaceae in Minnesota knotweed populations.	10/31/2022
Outcomes, Activity 2: Determine herbicide sensitivity and potential tolerance, measure key invasive phenotypes (reproductive strategy; sexual vs. clonal, biomass allocation and adventitious rooting efficiency) for recommending best management practices and a Genome-Wide Association Study of invasiveness.	
1. Determine male and female fertility of individuals through morphological analysis, controlled crosses and measured seed set.	12/31/2021
2. Characterize seed viability of wild-collected and controlled cross-produced seed, and seedbank dynamics of select Minnesota populations.	11/31/2022
3. Determine asexual vs. sexual spread.	10/31/2022
4. Optimize herbicide use and measure herbicide sensitivities.	6/30/2023
5. Genome-Wide Association Study of genotype, genetic structure associated with intergeneric and interspecific introgressions vs. phenotypes associated with invasiveness (reproduction strategy, biomass allocation, carbohydrate distribution and herbicide sensitivities).	8/31/2022
6. Characterize nonstructural storage carbohydrates and then determine their distribution in crowns and rhizomes, and seasonal fluctuations.	6/30/2023

Outcomes, Activity 1 Determine the genetic structure, species composition, and extent of hybridization and introgression in knotweeds.	Completion Date
7. Recommend best practices for management integrating genotype and phenotype measurements.	6/30/2023

Status as of July 31, 2019:
This sub-project was approved on April 2, 2019 and is in the early phases of organization. No update is required per LCCMR instruction.

Status as of January 31, 2020:
Maximizing research impact and having stakeholder input is fundamental to this research. To facilitate input an in-person and Webex-enabled meeting with knotweed researchers and stakeholders was held. Research activities were discussed as well as stakeholder experience in management and population locations. Toward understanding knotweed genetic diversity for Activity 1, samples for DNA analysis were collected throughout Minnesota. Preliminary results from Diversity Arrays Technology (diversityarrays.com) shows both clonal and highly polymorphic individuals. Genotyping knotweed populations will determine the species present and the risk of pre-existing or development of resistance to management practices. For activity 2., experiments to measure key invasive characteristics were initiated, including assessing seed viability from several geographically separate populations. Results conclusively demonstrate the potential for seed dispersal of knotweeds in Minnesota, suggesting potential for high diversity among and within populations and movement of seed as a dispersal mechanism.

Pollen viability staining revealed a majority of viable pollen among much less frequent non-viable pollen and will be used as an efficient measure of male fertility. Controlled crossing experiments to measure female fertility were unsuccessful. We are trialing other methods for controlled pollination. Competency for adventitious rooting varied from among Japanese, Bohemian and giant knotweeds. These experiments will be repeated to better inform managers that spread via removed shoots is possible, especially early in the growth phase. Greenhouse and field plots were planted to establish perennating individuals to identify and measure carbohydrate movement throughout the season and optimize herbicide management.

Status as of July 31, 2020:
For Objective 1, conducting a genetic structure analysis of the Minnesota knotweed complex, we continue to collect samples in 2020. DNA extractions showed good quality and quantity and were used for DarTseq analysis that discriminated among Bohemian, Giant and surprisingly Japanese populations. Collaborators will be shipping samples from their collections of western North America, Poland and Japan. Phenotyping of live knotweed material is beginning with competency for adventitious rooting, followed by male and female fertility assessments and biomass production. Germinations experiments showed seed was viable, could be a dispersal mechanism and a means to increase genetic variation. We successfully developed a technique to allow broad, meaningful herbicide screening of the invasive knotweed complex in the greenhouse and have completed one trial and anticipate completion of the repeat by September 2020. This enables us to develop the key treatments to test herbicide applications *in situ* and provides the basis from which to develop I_{50} trials to determine if the two genotypes and one hybrid invading Minnesota may differ in their ability to be controlled with herbicides. We have collected and processed what will be our tech standard for carbohydrate analysis and have acquired the necessary CHO standards and HPLC columns to begin lab runs to validate this technique. The lateral spread plot experiment and plots of the two species and the hybrid have successfully been established on the St. Paul campus, enabling comparative phenology, growth and development observations plus providing plant materials for the various ongoing research efforts.

Status as of January 31, 2021:
Sampling for genetic structure analysis of the Minnesota knotweed complex is completed. Leaves were collected from 206 populations for a total of 1,057 samples. An additional 239 samples from Europe, Japan, and the Western-half of the US were acquired from collaborators that will be analyzed for comparison with Minnesota populations. In a pilot study, DNA extractions from 879 samples showed adequate quality and quantity of DNA for optimization of DarTseq genotyping (2/879 or 0.2% were inadequate and required re-extraction). The single nucleotide polymorphisms discriminated among Bohemian, Giant and Japanese populations. During leaf sampling, rhizomes were collected, grown on the St Paul campus showing variation for key invasive traits including competency for adventitious rooting, male and female fertility, and biomass production. For measurement of female fertility, seed was collected from natural populations and St Paul campus grown plants. Seeds showed surprising variation in viability. A subset of seed-derived plants flowered after approximately 7 months and showed a 50/50 segregation for male/female flowers. The short juvenile phase and production of male and female plants from seed has significant implications for generation of diversity and seed dispersal. We completed the general herbicide screen, providing key leads for additional chemistries that may offer additional control options. We developed a protocol to screen for potential differences in tolerance to herbicides for the three knotweed types. Field trials on the St. Paul campus provided insight into the biology and phenology of the three invasive knotweeds in the initial phases of invasion.

Status as of July 31, 2021:
The collection of genetic data from the Minnesota knotweed complex is completed for 868 samples. This collection is complemented with specimens from the western US, United Kingdom, Poland, Japan and Belgium, 312 additional samples putting the Minnesota populations into a broader context of world-wide knotweed populations. A preliminary analysis shows high genetic diversity among knotweeds in Minnesota. The degree of diversity among Japanese knotweeds was particularly surprising. Toward the goal of measuring important characteristics that may contribute to knotweed's invasiveness, germination was measured for seed collected in 2020. Twelve populations produced seed that did not germinate whereas eight other genotypes had 50.1 to 74.5% germination. Seed germination was observed in three Minnesota field locations providing evidence that seed dispersal and its associated variation is an important consideration in the ecology and management of knotweeds. A cold treatment of -75°C did not significantly reduce germination showing extreme cold tolerance of seed. Measurement of biomass production, competency for adventitious rooting, and male and female fertility continue for a second year.

Two herbicide trials for giant, Japanese, and Bohemian knotweed are underway and will be completed this summer, building on 2 greenhouse experiments that determined appropriate rates and methodology. We used visual ratings, biomass sampling, canopy determination with Canopeo, and the possibility of fluorescence to differentiate response to herbicides. Visual ratings and biomass sampling best described the effects of herbicide application. Monthly carbohydrate sampling continues of Bohemian and Japanese populations, with lab analysis and carbohydrate methodology / protocol development continuing.

Status as of January 31, 2022:
Analysis of knotweeds focused on genetic relatedness to identify species, assess clonal relationships and predict sexual and asexual dispersal mechanisms. Giant knotweed are genetically distinct from Bohemian and Japanese, whereas the Bohemian and Japanese individuals form a continuum similar to diverse individuals. This may result from continuous introgression events among hybrids and Japanese. The software poppr indicated there could be as few as 60 clonal genotypes among Minnesota knotweeds. However, we continue to refine these analyses to account for the technical variation that occurs in DarTseq genome sequencing data.

The key invasive phenotypes of rhizome cold tolerance, seed production and germination and pollen viability were measured. Rhizomes had a lethal temperature of 50% equal to -10°C, however significant inhibition of growth was seen at -8°C. Of the 42 female genotypes we grew in field conditions: 34 set seed indicating a high

level of female fertility. Preliminary results indicate the 2021 seeds are viable. Male fertility varied but was high among genotypes, with >50% viable pollen staining. Taken together, these findings predict dispersal of genetically diverse seed, which is a new dimension to knotweed invasion.

Samples for the seasonal carbohydrate monitoring and pre-dormancy break lateral distribution have been collected for two years, and are being prepped for analysis. For herbicide I50 trials, preliminary data shows giant knotweed is more sensitive to aminopyralid relative to Bohemian and Japanese knotweeds, with similar, but less distinct differences in response to imazapyr.

Status as of July 31, 2022:
Analysis of knotweeds is focused on genetic structure analysis, measurement of key invasive traits and optimization of herbicide management. Genetic analysis has definitively identified the knotweed taxa occurring in Minnesota including the previously unreported Fallopia compacta. Genetic analysis also clearly defined the complexity and level of hybridization occurring among F. japonica, F. sachalinensis, and F. compacta. Although dispersal occurs predominantly through asexual mechanisms, seed spread does occur and will impact management by increasing genetic diversity and development of resistance.

Experiments to optimize herbicide management of knotweeds are testing chemistries (Arsenal, Garlon, Habitat and Milestone) with a range of concentrations. Herbicide control of knotweed will be made more effective by coordinating applications with the movement of photosynthate from source leaves to rhizomes. The seasonal movement of photosynthate is being measured by sampling rhizomes each month of the growing season and determining their carbohydrate composition. These data will facilitate optimization of herbicide control.

Status as of January 31, 2023:
Analysis of knotweeds is focused on genetic structure analysis, measurement of key invasive traits and optimization of herbicide management. Genetic analysis has definitively identified the knotweed taxa occurring in Minnesota including the previously unreported Fallopia compacta. Genetic analysis also clearly defined the complexity and level of hybridization occurring among *F. japonica*, *F. sachalinensis*, and *F. compacta*. Although dispersal occurs predominantly through asexual mechanisms, seed spread does occur and will impact management by increasing genetic diversity and development of resistance.

A third general herbicide screen was conducted in 2022 (Table 1). The second and third run of experiments were similar, with the third run showing increased variability within treatments (Figures 1-4). Imazapyr and the auxin mimics flipped "best in show" where imazapyr provided the best control in the 3rd trial, and good but less consistent control was obtained with the auxin mimics triclopyr, picloram, and aminopyralid, about the opposite was observed in the 2nd trial. Glyphosate provided similar, differential responses between the two trials with proliferation of tiny leaf buds leaving questions as to long-term control, possibly requiring additional sequentials compared to other top-performing herbicides. Tebuthiuron provided a soil-applied option for control, performed extremely well in the second trial, but had a few escapes in the third trial requiring follow-up treatment. The campus "curiosity plots" were monitored weekly and phenology notes taken. Information will be combined with 2021 observations and reported in the final report. As in 2021, early senescence and low frost tolerance were observed.

Status as of June 30, 2023:
The project experienced some significant, unexpected setbacks. A Master's student who was funded by the project suddenly withdrew from graduate school. We were unable to recruit a replacement. A senior scientist was hospitalized or homebound for most of the past six months. These issues slowed the pace of progress and resulted in significant funding being unspent. Nevertheless, important progress was made. Experiments were complete with the exception of determining the segregation ratios for male vs female flowering. The remaining plants are from 15 open-pollinated maternal sources and should flower in approximately one month. Effort

during this reporting period was primarily directed at organizing and analyzing data and producing figures for publication. Nine manuscripts are in preparation. Their provisional titles are: 1) Cold Hardiness of Invasive Knotweed (*Fallopia* spp.) Rhizomes; 2) Genetic Diversity and Structure of Knotweeds with a focus on the Midwestern Knotweeds; 3) Ploidy Diversity in Four Taxa of Midwestern Knotweeds; 4) Sexual Reproduction: Production and Viability of Seeds and Pollen among Midwestern Knotweeds; 5) Asexual Reproduction: Adventitious Rooting Competency among Midwestern Knotweeds; 6) Growth Potential and Biomass Production a Key Invasive Trait of Midwestern Knotweeds; 7) Herbicide Sensitivity and Control Trials for Knotweed Management; 8) Seasonal Carbohydrate Distribution for Herbicide Optimization of Knotweed Taxa; and 9) Association of Key Invasive Traits of with Genotypes in Knotweeds. Genetic analysis has definitively identified the knotweed taxa occurring in Minnesota including the previously unreported *Fallopia compacta*. Genetic analysis also clearly defined the complexity and level of hybridization occurring among *F. japonica*, *F. sachalinensis*, and *F. compacta*. Although dispersal occurs predominantly through asexual mechanisms, seed spread does occur and will impact management by increasing genetic diversity and development of resistance.

Final Report Summary:
Three distinct species of knotweeds and their hybrids were confirmed present from a sampling that included Minnesota and beyond including the previously unreported species *Fallopia compacta.* Cold tolerance measures indicate all knotweeds have the potential to grow throughout Minnesota and into colder climates.

Seed production is inconsistent among populations and dispersal appears to be predominantly asexual and human facilitated. However, inconsistent viable seed, sometimes heavy, was documented and segregates 1:1 male and female plants. These data predict seed dispersal will become more prevalent increasing diversity and the probability of resistance to herbicides and other management practices.

We make two recommendations for knotweed management from the genetic and reproduction analyses. Remove all landscape plantings of the knotweed cultivar 'Compacta' that may set seed resulting in the production of the more vigorous *F. xbohemica* that is difficult to control and can have a higher environmental impact. *Fallopia xbohemica* has the highest rate of growth and therefore these hybrid populations should be prioritized for control to reduce environmental impacts and dispersal.

Carbohydrate storage and utilization indicates herbicide applications that rely on translocation to underground roots and rhizomes would be most effectively applied in August through mid-September, assuming adequate soil moisture supports plant growth and development. Mechanical or hand removal of above ground biomass would be most effective in late June through July, when starch and carbohydrate levels are at their lowest in below ground tissues, with at least one additional removal around September 1.

The best performing herbicides for knotweed control in order from highest to lowest environmental load in kg/ha were triclopyr, tebuthiuron, picloram, and imazapyr. Glyphosate was more inconsistent and likely would require more sequential follow-up treatments. Aminopyralid offers selective nontarget monocot tolerance compared to tebuthiuron, glyphosate, and higher rates of imazapyr; would reduce the environmental load in kg/ha of herbicide applied compared to all except imazapyr; and offers reduced water quality concerns compared to tebuthiuron, picloram and imazapyr. Aminopyralid may be less consistent however, occasionally needing more follow-up sequential treatment.

Fallopia sachalinensis was more susceptible to aminopyralid and imazapyr compared to *F. xbohemica* and *F. japonica.* These results need to be verified on established stands in the field, but the degree to which *F. sachalinensis* was much more responsive, especially to aminopyralid, indicates that these differences will also express more broadly on the landscape.

MITPPC Sub-project 7: Improved Detection and Future Management of Leafy Spurge and Common Tansy using Remote Sensing, Mechanistic Species Distribution Models, and Landscape Genomics
Sub-project managers: David Moller and Ryan Briscoe Runquist
Description: Predicting future range of invasive species is critical to management and control efforts. Species Distribution Models (SDMs) are developed to predict which geographic areas are under current risk of invasion and how distributions will expand or contract under climate change. Traditional SDMs are constructed only from environmental data and often underperform because they fail to account for how population demography and functional traits vary with environmental variables across geographic ranges. Here, we propose to develop mechanistic and process-based SDMs in order to provide fine-scale predictions of current and future distributions of two invasive species (leafy spurge and common tansy) that are widespread across much of the northern tier of the United States but considerably less common to the south. We will take a novel approach where we use remote sensing to gather demographic information on each species across MN. Publicly-available multi- and hyper-spectral satellite images will be analyzed to quantify abundance and population growth over the last 18 years. Those data will be used to develop process-based SDMs. We will also use manipulative controlled environmental experiments to assess population differentiation in ecologically-important traits across the region and determine critical thresholds that limit performance. Those data will be used to construct mechanistic SDMs. Finally, we will use landscape genomics to assess fine-scale population structure and patterns of dispersal across the region using low-cost, high resolution sequence data. Together, these integrative datasets will provide detailed predictions of habitat under current and future climates and inform near- and long-term management strategies.

This project is co-funded with ML 2015, Ch. 76, Art. 2, Sec. 6a, with $70,812 from ML 2015 and the balance of $351,888 from ML 2016, Ch. 186, Art. 2, Sec. 6a.

Summary Budget Information	ENRTF Budget (ML 2016, Ch. 186, Art. 2, Sec. 6a)	ENRTF Budget (ML 2015, Ch. 76, Art. 2, Sec. 6a):	Total
	$351,188	**$70,812**	**$422,000**

Outcomes, Activity 1 Remote sensing and machine learning to gather environmental, population, and demographic data	
1. Gather remotely sensed environmental data from publicly-available sources	12/13/2019
2. Develop classifier using DL and remotely-sensed data to detect leafy spurge	12/31/2020
3. Gather demographic data on leafy spurge populations identified by classifier	12/30/2021
4. Develop classifier using DL and remotely-sensed data to detect common tansy	12/31/2021
5. Gather demographic data on common tansy populations identified by classifier	6/30/2022
6. Validate classification model and demographic data through field surveys	6/30/2022
Outcomes, Activity 2: Common garden experiments to assess niche thresholds and trait differentiation among populations	
1. Collect seeds for leafy spurge and common tansy from across range	12/31/2019
2. Conduct growth chamber experiments on leafy spurge seeds at varying temperatures to assess germination niche	12/31/2021
3. Conduct growth chamber experiments on leafy spurge juveniles at varying temperatures to assess first year emergence	12/31/2021
4. Conduct growth chamber experiments on common tansy seeds at varying temperatures to assess germination niche	12/31/2021
5. Conduct growth chamber experiments on common tansy juveniles at varying temperatures to assess first year emergence	11/30/2022

Outcomes, Activity 1 Remote sensing and machine learning to gather environmental, population, and demographic data	
6. Conduct growth chamber experiments on common tansy juveniles and adults at varying temperatures to assess relative growth rate, reproductive allocation, and biomass allocation (above- and below-ground)	11/30/2022
Outcomes, Activity 3 Build traditional mechanistic and process based distribution models	
1. Build traditional SDMs (Maxent and Boosted Regression Trees) of leafy spurge	12/31/2020
2. Build traditional SDMs (Maxent and Boosted Regression Trees) of common tansy	12/31/2020
3. Build process-based SDMs of leafy spurge	6/30/2022
4. Build process-based SDMs of common tansy	6/30/2022
5. Build mechanistic SDMs of leafy spurge	6/30/2022
6. Build mechanistic SDMs of common tansy	4/1/2023
Outcomes, Activity 4 Using landscape genomics to infer major dispersal pathways and sources of new infestations	
1. Tissue collection and extraction	12/31/2020
2. Enzyme optimization for sequencing	6/30/2021
3. Sequencing and analysis of data within a spatial framework for leafy spurge	12/31/2021
4. Sequencing and analysis of data within a spatial framework for common tansy	4/1/2023
5. Final integrated report that summarizes current and future potential distributions of common tansy and leafy spurge complete	4/1/2023

Status as of July 31, 2019:
This sub-project was approved on April 2, 2019 and is in the early phases of organization. No update is required per LCCMR instruction.

Status as of January 31, 2020:
The main objective during the first phase of our project has been to identify populations of leafy spurge and common tansy that span the range of environmental conditions and land uses across the state of MN. Systematically identifying and collecting from these populations allows us to characterize how plant traits vary across the state. In future experiments, we will assess if there are particular traits that confer an advantage in different environments, such as in warmer or cooler temperatures. We will also be able to characterize how plant genomic DNA varies across the state and use this information to determine important pathways of dispersal and invasion. In total, we collected from 185 leafy spurge populations, of which 69% were new records and two were new county records, and 187 common tansy populations, of which 49% were new records and one was a new county record. Our intensive effort has put us ahead of schedule for our main collection effort. During this period, we also exhaustively surveyed a twelve square-mile area of Dakota Co. for new leafy spurge populations. In this area, we identified 1,840 leafy spurge sites, most of which were previously undiscovered. This dataset will be used to develop a computer algorithm that detect populations of invasive species using satellite images.

Status as of July 31, 2020:
In the past six months, the team has been focused on two objectives: 1) developing a computer algorithm using cutting-edge deep-learning neural networks to detect populations of leafy spurge in Minnesota, and 2) preparing our plant collections of leafy spurge and common tansy from around the state for genomic analysis. In order to develop the deep-learning classifier, we generated a database of geo-referenced, digitized leafy spurge occurrence records to train and test our classification models. We have begun preliminary training and testing a number of different model designs. To produce more robust models, we have begun additional field work this summer. Additionally, we have been extracting DNA from collected plant specimens for genomic analysis. Currently, we have extracted high-quality genomic DNA for all 187 populations that we previously found and

surveyed across the state. High-quality DNA extraction of common tansy was more challenging and required troubleshooting. However, we have now developed a protocol to extract high-quality DNA from this species and have extracted DNA from 50 of the 185 populations collected from across the state. Along with these major objectives, we have also been running preliminary trials for our growth chamber and field experiments that we will use to quantify the differences in niche thresholds and functional trait diversity for both species from population across MN. Additionally, we have finished the traditional SDMs for both species and they are included in a manuscript that is currently in revision at the open-access journal *Diversity and Distributions*.

Status as of January 31, 2021:
Our team has made progress on all of our project activities over the last six months. We built multiple promising computer algorithms to detect Leafy Spurge populations from satellite imagery, and we are currently evaluating our models. We continued to improve our remote sensing dataset by performing additional ground-truthing and conducting population size and density surveys in order to gather demographic data. We were limited by COVID from conducting large experiments, however, we were able to pilot methodologies to help ensure a successful 2021 season. Last, we completed sample DNA extractions for both species and submitted samples to the UMN Genomic Center. We will use these data to evaluate the genetic diversity of the species across MN and determine patterns of dispersal and gene flow.

We published an article in Diversity and Distributions that evaluated commonly-used methods for building and evaluating species distribution models, particularly those aimed at predicting range expansion. This paper included traditional species distribution models for Leafy Spurge and Common Tansy and provided a baseline prediction of invasion risk. We also have a manuscript in revision at the Journal of Biogeography that presents a new method for predicting range expansion for invasive species using information on the environmental affinities of co-occurring native species. This new method also leveraged a large MN DNR dataset of >10,000 plant communities across MN. R. Briscoe Runquist and T. Lake presented the results of both manuscripts at the UMISC conference in November. We wrote a small grant and were awarded $8000 of in-kind support of very high-resolution satellite imagery from the European Space Agency and T. Lake received two graduate student grants from the Bell Museum ($2500) and the Botanical Society of America ($1500) in support of our experiment to test for genetic differentiation and responses to climate change in Common Tansy.

Status as of July 31, 2021:
Our team has made progress on all project activities over the last six months. We built multiple promising approaches to detect Leafy Spurge populations from satellite imagery, and we are currently evaluating those methods. We continued to improve our remote sensing dataset by performing additional ground-truthing and conducting population size and density surveys in order to gather demographic data. We were limited by COVID from conducting large experiments, however, we were able to pilot methodologies to help ensure a successful 2021 season. Last, we extracted DNA from multiple specimens of both species and submitted samples to the UMN Genomic Center. We will use these data to evaluate the genetic diversity of the species across MN and determine patterns of dispersal and gene flow.

We published an article in Diversity and Distributions that evaluated commonly used methods for building and evaluating species distribution models, particularly those aimed at predicting range expansion. This paper included traditional species distribution models for Leafy Spurge and Common Tansy and provided a baseline prediction of invasion risk. We also have a manuscript in revision at the Journal of Biogeography that presents a new method for predicting range expansion for invasive species using information on the environmental affinities of co-occurring native species. This new method also leveraged a large MN DNR dataset of >10,000 plant communities across MN. R. Briscoe Runquist and T. Lake presented the results of both manuscripts at the UMISC conference in November.

Status as of January 31, 2022:
In the past six months, we have focused on three main objectives: 1) validating our deep learning models for detecting leafy spurge from satellite imagery and preparing a manuscript with the models and results, 2) setting up and conducting a large common garden experiment on genetic differentiation in common tansy in MN, and 3) conducting bioinformatics and preliminary landscape and population genetic analysis of sequenced individuals. Our deep learning models leverage the use of publicly available satellite imagery to accurately predict areas of leafy spurge infestation. The most exciting development from our work has been the result that we can use a time series of satellite images over the growing season of leafy spurge to predict populations as accurately as models that use the more expensive and less widely available very high resolution satellite imagery. These results are currently under review at Remote Sensing of Ecology and Conservation. We have completed our first field season of our common garden, that will be used to determine niche thresholds and trait differentiation among MN populations of common tansy and leafy spurge. This data will be used to determine physiological limits and potential for future adaptation to climate in both species. Last, preliminary bioinformatics and genomic analysis are ongoing. In leafy spurge, we are working to confidently call genotypes for its polyploid genome. In common tansy, preliminary population genetic analysis indicates that there is structured genetic variation in Northern MN, which reveals genetic differentiation and potential differences in geneflow in the landscape.

Status as of July 31, 2022:
Our team continues to make progress towards completing the goals for our outlined activities. During the last six months we have made progress on all four activities. For Activity 1, we published the paper, "Deep learning detects invasive plant species across complex landscapes using Worldview-2 and Planetscope satellite imagery" in the journal Remote Sensing in Ecology and Conservation. Our models had an >96% accuracy rate at detecting leafy spurge populations from publicly-available satellite images. We are developing large-scale temporal models for leafy spurge to detect demographic changes from remotely sensed data. For Activity 2, we have successfully overwintered and re-established our common tansy field experiment at the St. Paul Experimental Agricultural Station. Approximately 95% of the plants re-emerged this spring; we are currently imposing drought and heat treatments and gathering data on phenology and growth. For Activity 3, we have started to synthesize our multiple species distribution models on common tansy and leafy spurge. We previously built and published traditional SDMs and have now built mechanistic species distribution models based for both species using on data from growth chamber and field experiments conducted last summer. We are working towards demographic models based on the data gathered from our deep learning models for leafy spurge. For Activity 4, we have finished all of the bioinformatics and genetic analysis for common tansy and leafy spurge. We are currently finishing our final data analyses and are in the process of preparing two manuscripts on our landscape and population genetic results.

Status as of January 31, 2023:
In the past six months, our team has continued to work towards the completion of all of our project activity goals as we approach the conclusion of the project. We presented work at the meeting of the joint Ecological Society of America and Canadian Society for Ecology and Evolution this summer and the Upper Midwest Invasive Species Conference this fall (travel expenses were paid with other funds, not this appropriation). For Activity 1, we are building deep learning models that can use the 37 years of archival imagery from Landsat to detect leafy spurge demographic data. Once we have collected all of the data, we will use it to build process-based SDMs of leafy spurge. For Activity 2, we have successfully finished our large field experiment assessing adaptation to climate change in common tansy. This was a large and complicated experiment. It required a lot of dedicated time to harvest and deconstruct without inadvertently introducing common tansy to the study site. We are currently processing all of the data and conducting preliminary analyses for manuscript preparation. For Activity 3, we have completed many of the SDMs and are awaiting results from Activities 1 & 2 to complete the remaining SDMs. For Activity 4, we have completed all of the genetic sequencing work and are preparing two manuscripts on the landscape and population genetics of leafy spurge and common tansy. We plan to submit

both manuscripts during winter 2022/23. Tom Lake received a Doctoral Dissertation Fellowship from the University of Minnesota to fund his salary and provide additional time to publish project results without any greater cost to the project.

Status as of June 30, 2023:
Our team has achieved the majority of the stated goals and outcomes from our initial proposal using cutting-edge species distribution models and genetics to improve predictions of invasive range expansion in leafy spurge and common tansy. In both species, we found substantial genetic and phenotypic evolution that may impact their invasive risk and change future strategic decision making. In the leafy spurge system, we built highly accurate detection models that use publicly-available satellite imagery and deep learning algorithms. These models allow for rapid field detection and monitoring as well as better predictive capacity because they 1) generate less biased occurrence records and 2) identify environmental factors that promote leafy spurge invasion. Population genomics and trait variation analysis of leafy spurge revealed that, despite relatively similar genetic variation across the expanding range, there was putatively adaptive evolution in germination dormancy in response to invasive expansion. In the common tansy system, we focused on understanding potentially adaptive genetic and trait variation. We conducted a large common garden experiment with climate manipulations. Data reveal important trait evolution that has occurred during range expansion that may impact their response to climate change. Landscape genomic analysis of common tansy revealed that there are two distinct genetic clusters in Minnesota that are distinguished by changes in soil features and land usage. For the project, we have produced five major peer-reviewed publications. We are currently working on three additional publications on leafy spurge population genomics, common tansy landscape genomics, and trait evolution in common tansy across Minnesota.

Final Report Summary:
In our project, we used cutting-edge deep learning computer models, large scale field experiments, and genomic analyses to improve predictions of invasive range expansion for the two problematic weeds: leafy spurge and common tansy. In both species, we found substantial genetic and phenotypic evolution that may impact their invasive risk and change future strategic decision making. In the leafy spurge system, we built highly accurate detection models that leveraged publicly available satellite imagery, which allowed us to predict occurrences across large landscapes. Predictions of this magnitude allow for rapid field detection and monitoring as well as the identification of environmental factors that promote leafy spurge invasion. Population genomics and trait analysis of leafy spurge further revealed that, despite relatively similar genetic variation across the expanding range, populations from different regions of the state may have evolved differences in germination niche. In the common tansy system, we focused on understanding potentially adaptive genetic and trait variation using growth chamber experiments and a large common garden experiment with climate manipulations. Data reveal important trait evolution occurred during range expansion which has the potential to impact further invasion and that may influence the response to climate change. Landscape genomic analysis of common tansy revealed that there are two distinct genetic clusters in Minnesota that are distinguished by changes in soil features and land usage.

MITPPC Sub-project 8: Using Plants to Control Buckthorn: an Expanded Approach
Sub-project manager: Peter Reich
Description: European buckthorn (*Rhamnus cathartica*) is an invader of woodlands throughout eastern North America. Current control methods are costly and offer little-to-no long-term benefit due to rapid buckthorn recolonization after removal. Preliminary results from ongoing experiments around Minneapolis/Saint Paul established under the MITPPC-funded Cover It Up! Project suggest that densely revegetating woodlands can reduce buckthorn recolonization by blocking at least 96% of incoming light. Within the two year duration of those experiments, this level of shading was achieved using dense, costly plantings of native shrubs. It remains unclear whether other planting treatments or lower-cost seeding treatments can achieve this level of shading and associated buckthorn suppression, and under which contexts revegetation will most effectively suppress

buckthorn. Here, we propose to leverage and expand on Cover It Up! To discover how revegetation can synergize with other management practices to help suppress buckthorn over longer time frames, at lower cost, and more widely through the state. Activity 1 extends the aforementioned experiments to evaluate the ability of slow-growing native perennial plants (still immature after two years) to suppress buckthorn and examine how herbicide, deer and fire management may interact with revegetation to strengthen buckthorn suppression. Activity 2 investigates new methods to cost-effectively establish dense, suppressive shrub cover from seed, in comparison with traditional low-density bare-root plantings. Activity 3 expands the experimental network to citizen scientists, to evaluate the performance of revegetation seed mixtures in diverse environments across Minnesota. If effective, the revegetation treatments designed and tested here can serve as a template for managers throughout Minnesota, potentially resulting in significant cost and labor savings and improving the health of woodlands by excluding invaders, reducing herbicide applications, and increasing forest understory plant diversity.

Summary Budget Information	ENRTF Budget:	$ 560,000

Outcomes, Activity 1 Revegetating after buckthorn removal as a component of integrated management strategies to suppress re-invasion over the long term	
1. Characterize and relate fuel loads, fire behavior, and buckthorn performance	8/30/2022
2. Herbicide applications	9/1/2021
3. Annual censuses of performance of buckthorn, planted species, and other native plants	10/1/2022
4. Statistical analyses and publication-ready manuscripts	6/30/2023
Outcomes, Activity 2. Scaling up shrub revegetation using cost-effective direct seeding and low-density planting.	
1. Sites identified and pre-seeding and seeding treatments imposed	12/1/2019
2. Census performance of buckthorn, seeded species, and other native plants	10/1/2022
3. Statistical analyses and publication-ready manuscripts	6/30/2023
Outcome, Activity 3 Exploring revegetation efficacy in diverse Minnesota environments	
1. Develop web portal for volunteer data submission and associated website	6/1/2020
2. Participants are registered and have received training and experimental supplies	6/1/2020
3. Participant submission of data complete	10/1/2022
4. Statistical analyses and publication ready manuscript completed	6/30/2023

Status as of July 31, 2019:
This sub-project was approved on April 2, 2019 and is in the early phases of organization. No update is required per LCCMR instruction.

Status as of January 31, 2020:
Over the past six months, significant progress has been made in all three activities and all goals are progressing according to schedule. For Activity 1, we have worked to transition existing experimental units into a state conducive to continued monitoring and manipulation. This includes continuing established measurement protocols, but also introducing new experimental tests. For Activity 2, we have established plots at two sites around the Twin Cities to test the efficacy of various seeding densities and methods to create buckthorn-suppressing shrub cover. For Activity 3, we have further developed the infrastructure, protocols, and materials needed for the citizen science component of the project. We have also recruited a sufficient number of participants throughout the state.

Status as of July 31, 2020:
Field experimental work for Activities 1 and 2 is proceeding as planned, with minor reductions to accommodate COVID-19 constraints. In particular, COVID-19 necessitates separate travel by all team members, so to avoid exceeding our travel budget we have reduced some field measurements for this year in a strategic way that will not compromise final outcomes. Activity 1 experiments are already completely set up; we are continuing measurements. For Activity 2, we completed experimental setup in May by planting the outstanding treatment (bare-root elder shrubs, as a baseline for comparison with the shrub and tree seeds that are the focus of the Activity). We made major progress on Activity 3, our citizen science project. We completed the first two outcomes as scheduled: 123 citizen scientists are now running 110 distinct experiments (following a consistent protocol) and submitting their data via a web portal. We supplied these participants with materials, training, and support to establish these experiments. Given COVID-19 restrictions on lab use and student assistance, we needed to redirect substantial amounts of effort from other tasks such as publications to ensure that the citizen science project launched as planned. We are writing several manuscripts based (at least in part) on data obtained through this grant; those nearest submission are "Leaf phenology of native shrubs relative to invasive European buckthorn: implications for revegetation" (90% complete), "High density revegetation of shrubs and trees suppresses buckthorn regeneration" (60% complete), and "Effects of native herb seeding and foliar herbicide on buckthorn regeneration" (60% complete).

Status as of January 31, 2021:
Field experimental work proceeded as planned this summer & fall, with some reductions to accommodate COVID-19. We maintained and monitored our long-term revegetation experiments around the Twin Cities (Activities 1 & 2) and analysed results that we presented at the Upper Midwest Invasive Species Conference (2 talks), Ecological Society of America meeting, and the MN Noxious Weed Advisory Committee. We found that seeding native grasses and wildflowers after removing buckthorn can reduce buckthorn regeneration. This suggests that revegetating using grasses and wildflowers can reduce the need for further follow-up control of buckthorn using herbicide or other methods, though it cannot eliminate this need: follow-up control must be combined with seeding for successful restoration. We made major progress on our citizen science project (Activity 3): we created training materials for participants to measure revegetation efficacy and buckthorn performance in their experiments and supported them in making their measurements and submitting them via the internet. We held 11 virtual meetings via Zoom with participants to provide training, Q&A, discuss results from other parts of the project, and thank them for their efforts. We are writing several manuscripts based in part on data obtained through this grant. "Phenological niche overlap between invasive buckthorn (*Rhamnus cathartica*) and native woody species" has been submitted for publication. Other papers in progress include "High density revegetation of shrubs and trees suppresses buckthorn regeneration" (70% complete) and "Effects of native herb seeding and foliar herbicide on buckthorn regeneration" (70% complete).

Status as of July 31, 2021:
Field experimental work proceeded as planned this summer & fall, with some adjustments to accommodate COVID-19. We maintained and monitored our long-term revegetation experiments around the Twin Cities (Activities 1 & 2) and analyzed results that we presented at the Upper Midwest Invasive Species Conference (2 talks), Ecological Society of America meeting, and the MN Noxious Weed Advisory Committee. We found that seeding native grasses and wildflowers after removing buckthorn can reduce buckthorn regeneration. This suggests that revegetating using grasses and wildflowers can reduce the need for further follow-up control of buckthorn using herbicide or other methods, though it cannot eliminate this need: follow-up control must be combined with seeding for successful restoration. We made major progress on our citizen science project (Activity 3): we created training materials for participants to measure revegetation efficacy and buckthorn performance in their experiments and supported them in making their measurements and submitting them via the internet. We held 11 virtual meetings via Zoom with participants to provide training, Q&A, discuss results from other parts of the project, and thank them for their efforts. We are writing several manuscripts based in part on data obtained through this grant. "Phenological niche overlap between invasive buckthorn (*Rhamnus*

cathartica) and native woody species" has been submitted for publication. Other papers in progress include "High density revegetation of shrubs and trees suppresses buckthorn regeneration" (70% complete) and "Effects of native herb seeding and foliar herbicide on buckthorn regeneration" (70% complete).

Status as of January 31, 2022:
Field experimental work proceeded as planned this summer and fall. We maintained and monitored our long-term revegetation experiments around the Twin Cities (Activities 1 & 2), including completing the herbicide application milestone for Activity 1 in September 2021 as scheduled, and continued to analyze and write up results (Activity 1). Work in Activity 3 focused on processing photos from 2020 and 2021, the field season, and growing a sense of community among participants. Our paper "Phenological niche overlap between invasive buckthorn (*Rhamnus cathartica*) and native woody species" was published in Forest Ecology & Management in August 2021. Our manuscript "Using plants to control buckthorn (*Rhamnus cathartica*): improved biotic resistance of forests through revegetation" has been submitted to Ecological Applications. We are writing additional manuscripts based wholly or in part on data obtained through this grant, including "Effects of native herb seeding and foliar herbicide on buckthorn regeneration" (80% complete), "Revegetating herbs enables controlled burning for buckthorn control" (60% complete), and "Common buckthorn (*Rhamnus cathartica*) does not have an extended seedbank" (25% complete).

Status as of July 31, 2022:
Field experimental work proceeded as planned this spring and early summer. We maintained and monitored our long-term revegetation experiments around the Twin Cities (Activities 1 & 2) and continued to analyze and summarize results (Activity 1). Work in Activity 3 focused on processing photos and data from 2020 and 2021 citizen science volunteers, preparing for the 2022 field season, and supporting the community of volunteers. We revised a paper "Using plants to control buckthorn (*Rhamnus cathartica*): improved biotic resistance of forests through revegetation" in response to peer review and it has now been accepted for publication in Ecological Engineering. We are writing additional manuscripts based wholly or in part on information obtained through this grant, including "Effects of native herb seeding and foliar herbicide on plant cover and buckthorn regeneration" (80% complete), "Revegetating following invasive buckthorn removal increases native plant cover and diversity and long-term resistance to re-invasion" (40% complete), "Herbaceous revegetation facilitates prescribed burns in buckthorn-invaded woodlands" (60% complete), "No evidence for an extended longevity of common buckthorn (*Rhamnus cathartica*) in soil seedbanks" (30% complete), and "Direct seeding native shrubs and trees results in strong seedling establishment for revegetation" (20% complete).

Status as of January 31, 2023:
Field experimental work proceeded as planned this summer and fall. We maintained and monitored our long-term revegetation experiments around the Twin Cities, and continued to analyze and write up results (Activities 1 & 2). Work in Activity 3 similarly focused on collecting the final field season of data, and processing these data including photos for analysis, as well as guiding participants who are concluding their participation in dismantling their experiments. Thus, we completed our penultimate milestones for all three activities; the remaining milestone for all three activities is completing analyses and manuscripts. We have prepared two manuscripts for submission to academic journals: "Understory revegetation enhances efficacy of prescribed burning after common buckthorn (*Rhamnus cathartica*) management " and "No evidence for a long-lived seedbank in common buckthorn, *Rhamnus cathartica L.*". We are writing additional manuscripts based wholly or in part on data obtained through this grant, including "Effects of native herb seeding and foliar herbicide on plant cover and buckthorn regeneration" (85% complete), "Revegetating following invasive buckthorn removal increases native plant cover and diversity and long-term resistance to re-invasion" (50% complete), and "Direct seeding native shrubs and trees results in strong seedling establishment for revegetation" (50% complete). We presented a synthesis of results from several of these manuscripts at the Upper Midwest Invasive Species Conference in October 2022 (virtual attendance, no travel involved).

Status as of June 30, 2023:
Effort associated with this project focused on analyses and dissemination in early 2023 in accordance with the project timeline. We successfully published one manuscript, "No evidence of a long-lived seedbank in common buckthorn (*Rhamnus cathartica* L.) within Minnesota deciduous forests" in Biological Invasions. Another manuscript "Understory revegetation enhances efficacy of prescribed burning after common buckthorn (*Rhamnus cathartica*) management" remains in review at Forest Ecology and Management. Two additional manuscripts, "Effects of native herb seeding and foliar herbicide on plant cover and buckthorn regeneration" and "Revegetating following invasive buckthorn removal increases native plant cover and diversity and long-term resistance to re-invasion," were also advanced during this time period (roughly 90% complete) and will be submitted for peer review within the year. We also decommissioned existing experimental sites that will not continue to be monitored as part of new and ongoing MITPPC-funded research. Virtually all of the sites utilized in Activities 1 and 2, and many of the sites used in Activity 3 will be maintained for future monitoring to better contextualize their findings and amplify the lessons learned from them for Minnesota land managers. The combined needs of decommissioning discontinued sites and maintaining ongoing sites required us to dedicate significant time to personnel management during this update window. Several key personnel, including Wragg, Anderson, and Granstrom-Arndt left the project prior to its conclusion, necessitating the hiring of additional postdoctoral and civil service staff and delaying publications associated with the lost personnel. Findings from this project were covered in popular news media.

Final Report Summary:
Buckthorn is an invasive shrub that outcompetes native plants and degrades Minnesota forests. Removal of buckthorn is a common management activity but often only provides short-lived benefits since buckthorn rapidly re-establishes. In earlier MITPPC-funded research, we characterized the limits of buckthorn shade tolerance and illustrated that buckthorn seedlings could be suppressed by dense plantings of shrubs and trees within a three-year period. This project built upon those earlier findings by examining the efficacy of slower-establishing seed mixtures of herbaceous and woody species and evaluating how native plant seeding functions in the context of other forest management activities.

We continued monitoring the field experiments we established in 2017 to test how densely establishing native plant cover affects buckthorn seedlings. We found that densely planting native shrubs, particularly *Sambucus racemosa* and *Sambucus canadensis* led to greatly reduced light availability and frequent exclusion of buckthorn seedlings (most plots contained no buckthorn after 4 years). Plantings of *Abies balsamea* and *Acer saccharum* had similar results (Schuster et al. 2022). These results were due to a combination of high rates of growth and overlapping leaf phenology between native plants and buckthorn (Schuster et al. 2021). Herbaceous seeding (primarily of native *Elymus* grasses) was less effective at reducing buckthorn abundance over 4 years compared to shrub planting (Schuster et al. 2022). However, a parallel experiment demonstrated that these effects grow over time, especially in sites with more than 10% canopy openness (Schuster et al. in prep). These findings suggest that planting and seeding can significantly reduce the amount of buckthorn returning from seed and consequently limit the amount of follow-up management effort required.

MITPPC Sub-project 9: Genetic Control of Invasive Insect Species: Phase II
Sub-project manager: Michael Smanski
Description: Our phase II project has revealed that temperature impacts the performance of engineered genetic incompatibility (EGI), a form of genetic biocontrol, in flies in unpredictable ways. To de-risk translation of the genetic biocontrol approach into Spotted Wing Drosophila applications, we propose to create additional EGI variants that (i) target different developmental morphogen genes (genes that govern the size and appearance of the insect; when expressed at the wrong time, these genes will cause an insect to die), (ii) use alternative programmable transcription activator gene expression cassettes (varying the strength and tissue specificity of gene expression) and (iii) vary in the chromosomal location of the transgenes.

Summary Budget Information	ENRTF Budget:	$55,100

Outcomes, Activity 1 Creation of additional EGI variants	
1. Plasmid construction and fly injection.	12/31/2022
2. Husbandry, genetic validation, and behavioral/molecular characterization.	12/31/2022

Status as of July 31, 2022:

Overall, we have made substantial progress on this project by performing experiments in our model system built in D. melanogaster. Highlights from the past six months include new results from our simulation models of spotted wind drosophila (SWD) biocontrol that show interesting effects when the genetic biocontrol is applied in populations that have a low frequency of resistant individuals. We prepared and published an approach to compare the effectiveness of alternative genetic biocontrol approaches for SWD populations. Results were published as part of an eLife paper in early 2022. We then used this simulation model to examine several strategies by which the Sex-Sorting Incompatible Male System (SSIMS) could be applied. Specifically, we tested whether targeting two genes with a single biocontrol agent was better than targeting two genes with two separately engineered biocontrol agents, either released in an alternating sequence or at the same time, in terms of overcoming the evolution of genetic resistance. Surprisingly, we found that all approaches resulted in a switch from general population suppression to population replacement in the first season. In a surprising twist, this unexpected outcome from the first season actually made it easier to completely suppress the population in the second year, and the emerging resistant population after year one was more homogenous than the original (year 0) starting wild population. This result, as well as the population genetics study, is currently being written for submission to a peer-reviewed journal soon. Progress on completing the first proof of concept in SWD has been slow, but with continued effort and with help from colleagues in other universities we are making progress.

Final Report Summary:

Overall, we accomplished each of the goals of this funding period, but not necessarily via the route we described in the original proposal. Our goal for Activity 1 was to determine the robustness of hybrid lethality (arising from mating of a biocontrol agent and wild type) in diverse environments. Every strain of biocontrol agent that we studied had a temperature-dependent activity that could be readily observed. Our goal for Activity 2 was to confirm the molecular mechanism hybrid lethality. We confirmed that it is lethal ectopic (wrong tissue) expression of our target developmental genes, or lethal overexpression of our target developmental genes, depending on the specific genetic design. Our goal for Activity 3 was to develop a simulation model to predict how the performance of genetic biocontrol was impacted by the presence of resistance genes in the target population. We developed nuanced strategies to mitigate this effect based on the modeling results. Our results from Activities 2 and 3 have been published in peer-reviewed journals, and our results from Activity 1 will lead to two new manuscripts submitted in the next 6 months.

MITPPC Sub-project 10: Novel Diagnostic Tools for Rapid and Early Detection of Oak Wilt

Sub-project manager: Abdennour Abbas

Description: The purpose of this proposal is to develop a new assay technology for rapid and early detection of the invasive fungus *Bretziella fagacearum* (*B. fagacearum*), causal organism of Oak Wilt. In Phase I (2016-2018), a new technology named Nanoparticles-Enhanced Chemiluminescence (NEC) Assay was developed for early and rapid detection of *B. fagacearum*. In Phase II (ended in December 2019), the NEC technology was tested for field detection of oak wilt. Phase III of the project began by applying the NEC technology for the detection of real oak wood samples infected by the fungal pathogen. However, the data obtained suggested that the assay suffered

from interferences in the optical signal and repeatability issues for in field testing. To overcome this problem, a new technology based on loop mediated isothermal amplification (LAMP) will be designed for the rapid detection of oak wilt in the field. If successful, this will be extended to the detection of other invasive forest pathogens including *Ophiostoma novo-ulmi* that causes Dutch Elm Disease, *Heterobasidion irregulare* that causes *Heterobasidion* root rot, *Tubakia iowensis* that causes bur oak blight and *Geosmithia morbida* that causes thousand cankers disease in walnut species and wingnut.

Summary Budget Information	ENRTF Budget:	~~$190,740~~ $170,637

Outcomes, Activity 1	
1. Identification of 4-6 primers specific to *Bretziella fagacearum*	07/30/2022
2. Proof of concept of the new detection technology using these primers	09/30/2022
Outcomes, Activity 2	
1. Demonstration of the detection of oak wilt on real samples using the new assay	12/31/2022
2. Characterization of the analytical parameters of the new assay	03/31/2023
3. Optimization of commercial prototypes of the assay technology	06/30/2023

Status as of January 31, 2023:
The purpose of this proposal is to develop a new assay based on loop-mediated isothermal amplification (LAMP) for rapid and early detection of the invasive fungus *Bretziella fagacearum*, the causal organism of oak wilt. Specifically, the project aims to:
1- Specific Aim 1: Develop DNA primers and probes specific to the fungus *Bretziella fagacearum*
Completion: 100%-Successful Outcome
LAMP is an isothermal nucleic acid amplification method that requires 4 to 6 primers with at least two primers in the form of a loop. The assay developed in this study uses 6 primers that were successfully designed based on DNA sequences specific to *Bretziella fagacearum*. DNA extractions of given samples were used as the template for the assay. The amplification reaction was conducted at 65°C for 30 minutes at the end of which the amplicons were detected using fluorescence.
To check the specificity of the designed primers to *Bretziella fagacearum*, LAMP assays were conducted for five other fungal species that are similar to the target pathogen. *Dicarpella sp., Fusarium sporotrichoides*, *Graphostroma sp., Querciphoma carteri*, and *Epicoccum nigrum*, were used for these tests. Based on the results obtained, the primer set labbeled P87 showed the highest specificity for the targeted detection of *Bretziella fagacearum* and was selected for the next phase of this study.

2- Specific Aim 2: Use the primers and probes with LAMP technology to detect oak wilt.
Completion: 90%- Successful outcome
Once specific primers have been developed and tested, experiments are performed to evaluate the analytical parameters: detection limit, specificity, and operational range of the detection assay.
To find the lowest concentration of the target DNA that can be detected using the assay, LOD tests were conducted. The LOD of the assay was found to be 30 fg/µl. This is lower than the concentration of DNA usually found in infected samples, which means the assay would be capable of detecting oak wilt in real samples.
To test the efficiency of the assay in identifying diseased oaks in real-world samples, tests were conducted on DNA samples extracted from known healthy and infected red oaks. Ten samples each of healthy and infected red oak wood shavings were used for DNA extraction and LAMP tests. Based on the preliminary assessments, the assay shows 100% sensitivity and 95-100% specificity in detecting infected oak samples.

Furthermore, to make the detection faster without the use of fluorescence, a rapid optical detection method was developed based on gold nanoparticles (AuNPs) that were coated with probes specific to *Bretziella fagacearum* DNA. These nanoparticles have been developed and optimized during Phase I and Phase II of this project and found their important use with the LAMP assay in this Phase III. Based on the new optical detection concept, tests were conducted on ten healthy and ten infected oak samples. The test was optimized to determine the ideal incubation time required for the differentiation. The results obtained show 100% specificity and sensitivity in detecting the target pathogen with the optical method.

Ongoing experiments aim to optimize the optical detection aspect of the assay on real-world samples. We expect to achieve all the goals of this project on time.

Status as of June 30, 2023:
The project resulted in two major advancements: the development of a rapid visualization method for DNA amplicons. We showed that amplified DNA and oligonucleotide-coated gold nanoparticles assemble into globular nanostructures, that form a distinct and visible red spot upon precipitation, enabling naked-eye detection. This new visualization method was applied for the development of a rapid assay based on loop mediated isothermal amplification (LAMP) for oak wilt pathogen identification. Six different primers were designed to specifically bind and amplify the pathogen's DNA, which was detected by fluorescence after 30 min reaction time. Furthermore, to simplify the assay for field applications, gold nanoparticles (AuNPs) were designed to bind to the amplified DNA (amplicon) obtained from the LAMP reaction. Upon inducing precipitation, the AuNP-amplicons settles as a red spot visible to the naked eye, indicative of an infection. Both infected and healthy red oak samples were tested using this visualization method and both the sensitivity and specificity of the assay were calculated to be 95-100%. Moreover, the developed assay was able to identify infections from crude DNA extracts of oak wood samples, which reduces the time taken for DNA purification steps. This shows promising applications of the developed technique in the early detection of infected oak trees directly in the field.

Products and Deliverables: The project resulted in the filing of one patent, submission of two manuscripts and two abstracts accepted for oral presentations at two major national conferences in 2023.

Final Report Summary:
The project led to a successful development of a new nucleic acid visualization technology that was successfully used to develop and test a new LAMP assay for field detection of oak wilt. This development led to initiating a collaboration with the State of Iowa Department of Natural Resources to work on a field test demonstration (ongoing), and to establishing a collaboration with an industry partner for potential product development. Furthermore, the research results were disseminated in 2 publications and two oral presentations in national conferences. In addition, an invention disclosure was submitted to the University of Minnesota Office of Technology Commercialization.

IV. DISSEMINATION:

Description:
Findings will be shared with agencies and citizen groups so that public information and decision making is based on the best available science. Updates on progress and research results will be disseminated through University of Minnesota, College of Food, Agricultural, and Natural Resource Sciences, and College of Biological Sciences via websites, social media, and publications. Media releases will also be used when warranted. Additionally, findings will be presented at local and national conferences and via peer-reviewed publication and student theses.

Status as of *January 31, 2017*:
There has been no activity under this appropriation to-date.

Status as of *July 31, 2017*:
There has been no activity under this appropriation to-date.

Status as of January 31, 2018:
For a description of dissemination by the MITPPC on the ML 2014, 2015, and 2016 appropriations may be accessed in the ML 2014 workplan.

Status as of *July 10, 2018*:
For a description of dissemination by the MITPPC on the ML 2014, 2015, and 2016 appropriations may be accessed in the ML 2014 workplan.

Status as of January 31, 2019:
The following presentations were made by sub-project research teams funded under this appropriation:
(Sub-project 1)
- Held, B.W., A.J. Cotton, K.E. Bushley and R.A. Blanchette. 2018. Fungi associated with the Emerald Ash Borer: the role of fungi during ash mortality and finding effective biocontrol agents. Proceedings of Upper Midwest Invasive Species Conference, October 15-18, Rochester, MN.

(Sub-project 2)
- Meet and greet with the goats at Indian Mounds park in St. Paul (June 16, 2018): Presented information about our project to members of the public during an event organized by St. Paul Parks and Recreation.
- University of Minnesota College of Veterinary Medicine Points of Pride presentation (Oct. 10, 2018): Presented a poster with preliminary results from our first field season.
- Tech Savvy at St. Cloud State (Oct. 27, 2018): Presented information about parasite transmission, including that of meningeal worm, to junior high girls using live outreach snails. The event included activities that simulated parasite transmission under different scenarios.
- Informational meeting at Dakota County Extension office for 4-H members (Nov. 13, 2018): Informed 4-H members about our project and recruited help with raising our poultry for the coming field season.

(Sub-project 3)
- "Three Healthtech Teams Win Big at Sixth Walleye Tank" Rochester Rising.
  http://rochesterrising.org/main/three-healthtech-teams-win-big-at-sixth-walleye-tank
- "U of M biologists 'hard-wiring' male sterility in mosquitoes to lower the risk of Zika" MinnPost.
  https://www.minnpost.com/twin-cities-business/2018/08/u-m-biologists-hard-wiring-male-sterility-mosquitoes-lower-risk-zika/

(Sub-project 4)
- Contact with all collaborators on the project to inform them of the successful project award (April 2018) and to begin reaching out about sites, surveys, and the collaboration network. Contact include
  o State: MN DNR & MLEP
  o Private Industrial: UPM Blandin & Molpus
  o County: Itasca, Koochiching, and St. Louis County
- Presentations

- o Skay, R., Windmuller-Campione, M., Anderson, B., & Russell, M. (2018). *Black Spruce Alternative Silviculture Methods and Dwarf Mistletoe In Minnesota.* SAF National Convention. October 5th, 2018. Portland, OR. ****** Funding to attend conference not through MITPPC**
  - o Skay, R. (2018). *Black Spruce Alternative Silviculture Methods and Dwarf Mistletoe In Minnesota.* Natural Resources Science and Management Graduate Student Seminar. October 24th, 2018. St. Paul, MN.
  - o Windmuller-Campione, M. (2018). *Status of Current Silvicultural Collaboration with MN DNR*. MN DNR Annual Winter Silviculture Meeting. December 12th, 2019. Cloquet, MN. (Invited presentation).
- ● Social Media Post
  - o One Instagram post on the Silva Lab Instagram page about sampling (https://www.instagram.com/p/Bq0apxAh1Kq/)

Status as of July 31, 2019:
(Sub-project 1)
- ● No activity during this reporting period.

(Sub-project 2)
Presentations:
- ● Dr. Marchetto presented on meningeal worm and to the UMN Vet School's small ruminant club. She also presented information about our study to the public at the Germanic American Institute's German Days on June 8. We worked with 4H families to rear our ducklings.
- ● Dr. Marchetto gave feedback for the Science of Agriculture Challenge to a group of students at the Dakota County Extension doing a project on backyard chickens.
Publications:
- ● A manuscript about the effects of goat digestion on common buckthorn seed recovery and viability has been submitted to the Natural Areas Journal.

(Sub-project 3)
Presentations:
- ● This work was presented as part of the Data Needs Assessment for Self-Limiting Genetic Biocontrol Workshop at the National Academy of Sciences in May 2019.
- ● It was also presented at the MN DNA Workshop on Genetic Biocontrol in June 2019.
- ●   A staff member (soon to be PhD student) Nate Feltman presented a poster on this research at the Genome Writer's Guild Conference in June 2019.

(Sub-project 4)
Presentations:
- ● [Workshop: Dwarf Mistletoe on Black Spruce: Practical Management Options.](#) Held on Wednesday May 15, 2019 in International Falls, MN. Sponsored by the Sustainable Forests Education Cooperative and Minnesota Logger Education Program. 39 attendees of loggers and natural resource professionals. Several members of this project team provided presentations, including F. Baker and M. Russell. Photos of the workshop [can be found here](#).
- ● Windmuller-Campione, M., *Skay, R.,* Russell, M., (2019). Influence of eastern spruce dwarf mistletoe on structure and composition of black spruce forests in northern Minnesota. Hearts of the Continent's Science Symposium. Duluth, MN. April, 4th 2019.
- ● Baker, F. (2019). Dwarf mistletoe fun facts. Project Fact Sheet.

(Sub-project 5)
Presentations:

- *Senait D. Senay presented the proposed BMSB dispersal platform on the IAA 4.0 GEMS meeting (https://agroinformatics.org/) on May 29, 2019.*

(Sub-project 6)
No activity during this reporting period.

(Sub-project 7)
- No activity during this reporting period.

(Sub-project 8)
- No activity during this reporting period.

Status as of January 30, 2020:
(Sub-project 1)
Presentations:
Held B, Simeto S, Rajtar N, Cotton A, Showalter DN, Bushley K, Blanchette R. 2019. Canker, decay, and entomopathogenic fungi associated with emerald ash borer galleries. 87th Annual Meeting of the Mycological Society of America. Minneapolis, MN. 10-14 August.

Faulkner A, Showalter DN, Bushley KE. 2019. Links between ash fungal endophytes and emerald ash borer gut communities. 87th Annual Meeting of the Mycological Society of America. Minneapolis, MN. 10-14 August.

Public relations:
Research story on MITPPC website,"Researchers, local park boards – and fungi – come together to fight the emerald ash borer in Minnesota." https://mitppc.umn.edu/news/fungal-friends-foes
Meet the researcher, Nick Rajtar, MITPPC website, https://mitppc.umn.edu/news/meet-researcher-nick-rajtar
Meet the researcher, Benjamin Held, MITPPC website, https://mitppc.umn.edu/news/meet-researcher-benjamin-held

(Sub-project 2):
Publications:
Article in the Invasive Plants Association of Wisconsin newsletter by Nelson. "Assessing the role of goats in restoration." Issue 57, Nov. 2019.
Article written about our project: "Ahead of the herd: how goat-grazing research is helping with buckthorn control" by Caro Silvola. July 17, 2019 on the MITPPC website.
Our manuscript, "Goat digestion leads to low survival and viability of common buckthorn (*Rhamnus cathartica*) seeds" is currently in revision with the *Natural Areas Journal*.

Presentations:
Interviewed by *Prairie Lawn and Garden* on PBS for Goats and Invasive Plants to air on April 9, 2020 with repeats on April 11 at 4 p.m. and April 13 at 1 p.m. (will also be posted to YouTube)
Interview with Minnesota Virtual Academy biology teacher, Beth Robelia, that will be used as a virtual field trip for her students and also posted to YouTube

Field day at Rosemount Research and Outreach Center for 4H student volunteers and their families. The students got to see the poultry and goats at work, and we discussed the experiment as a whole.

UMN Conservation Sciences seminar series talk, "The use of goat browsing to control invasive plants: determining when conservation goals are congruent or conflicting" by Marchetto. Nov. 1, 2019.

(Sub-project 3):
Presentation:
October 22, 2019. Presentation to the Invasive Species Community of Practice Workshop. Attendees included a general audience aimed to teach the key differences between existing genetic biocontrol strategies.

(Sub-project 4):
Presentations:
Raychel Skay successfully defended her thesis on November 13th, 2019. The defense included a public presentation. She is currently working on completing final edits, and her thesis will be available online in early 2020 through the University of Minnesota website.

(Sub-project 5):
No activity during this period.

(Sub-project 6):
Public relations:
To facilitate stakeholder input into our research the knotweed research team held an in person and Webex meeting with participation from Minnesota Departments of Agriculture, Transportation and Natural Resources and other stakeholders interested in knotweed management. A similar meeting will be held in 2020 to update stakeholders and facilitate their feedback on the research results and plans for 2020.

Although not a formal outreach effort, the knotweed team interacted with many private landowners during collection of samples for DNA analysis. Anecdotally, most landowners with knotweed infestations were aware of the problem, however some were not. Most landowners appreciated receiving the Minnesota Department of Agriculture pamphlet "KNOTWEEDS" and almost all were very interested in how to best manage their population.

Of exceptional note is the first identification of Giant knotweed in Minnesota by knotweed team member, Dallas Drazan. The identification was confirmed by the Minnesota Department of Agriculture and a voucher specimen deposited in the University of Minnesota Steere Herbarium.

(Sub-project 7):

Public relations:
D. A. Moeller was interviewed by the Pioneer Press as a part of the article on the MITPPC

R. D. Briscoe Runquist met with Chris Klatt from Dakota County Parks about leafy spurge and common tansy populations in Dakota County Parks and in the County

T. M. Lake gave a guest lecture about invasive species and distribution modeling for the UMN class EEB 3001: Ecology and Society

D. A. Moeller and T. M. Lake taught three lab sections about species distribution models for the UMN class PMB3007W: Plant, Algal, and Fungal Diversity and Adaptation

(Sub-project 8):
Presentations:

May 2019. Gathering Partners. Oral presentations by Wragg & Knosalla of project findings and plans to approximately 30 citizen scientists and practitioners.

November 2019. Duluth Cooperative Invasive Species Management Area. Oral presentations by Wragg & Knosalla to approximately 20 people.

November 2019. Duluth Invaders R^{2}ED Team. Oral presentation by Wragg & Knosalla to approximately 30 people.

November 2019. CitSciMN. Discussions with Knosalla & Schuster (approximately 30 interactions).

Public relations:
Pioneer Press. https://www.twincities.com/2019/12/02/u-of-m-center-battles-hungry-invasives/
KARE 11. https://www.kare11.com/article/life/home-garden/grow-with-kare/grow-with-kare-be-a-citizen-scientist-and-fight-buckthorn-university-minnesota-native-plants-woods/89-33f5cd4e-a5af-4d26-8e7e-c1655f482f84?utm_source=MITPPC+Master+List&utm_campaign=411ad7fc2d-EMAIL_CAMPAIGN_APRIL_COPY_01&utm_medium=email&utm_term=0_3667ffbf32-411ad7fc2d-189336331

Status as of July 31, 2020:
(Sub-project 1)

Rajtar, N. 2020. A Closer Look at Fungi Interacting with the Emerald Ash Borer. Presentation to the Minnesota Mycological Society. June 8, 2020.

(Sub-project 2)
Marchetto, K. M., Heuschele, D. J., Larkin, D. J., & Wolf, T. M. (2020). Goat Digestion Leads to Low Survival and Viability of Common Buckthorn (*Rhamnus cathartica*) Seeds. *Natural Areas Journal*, *40*(2), 150-154.

Prairie Lawn and Garden interview aired on PBS in April
(https://www.youtube.com/watch?v=Po3wAKsG_cQ&list=PLBbXJqSJahrm4FLsC6zMsZCFV1wMQUTdZ&index=5&t=0s)

Radio interview about the above paper with WTIP in Grand Marais, MN (https://soundcloud.com/wtip-community-radio/20200410-katherine-marchetto)

Print interview about the above paper with Tri-State Neighbor

A Natural Areas Association webinar on May 19, with 391 participants in attendance during live broadcast and 690 registered. Two hundred and thirty-one YouTube views as of June 23, 2020
(https://www.youtube.com/watch?v=oFBKZKBvmCU)

Dr. Marchetto was interviewed by Minnehaha Academy 7th grade students about local mollusks.

Dr. Marchetto participated in Skype a Scientist, a project to connect scientists with K-12 students, 4 times (3 with classrooms and 1 with families doing online learning)

Dr. Marchetto also wrote and posted two summaries of our research project for lower and upper elementary students on her blog, to be used as resources for Skype a Scientist teachers (www.greenworldhypothesis.com).

(Sub-project 3)

(Sub-project 4)
First paper submitted on results from Activity 1 currently under revision to Forest Ecology and Management

> Skay, R.*, Windmuller-Campione, M. A., Russell, M., & Reuling, L. F. Influence of Eastern Spruce
> Dwarf Mistletoe on Stand Structure and Composition in Northern Minnesota. *Forest Ecology and*
> *Management*. [Revising to Resubmit]

Two presentations and one poster on results from Activity 1 and Activity 2 including modeling, influence of
mistletoe on stand structure and composition and our ability to detect, and on loggers and forests perceptions

> Gray, E.*, Windmuller-Campione, M. A., Russell, M. B. "Is Infestation Predictable? Risk Factors
> Associated with Eastern Dwarf Mistletoe Infestation in Lowland Black Spruce Stands of
> Minnesota," MN SAF Winter Meeting MN SAF, Brainder, Minnesota, United States. (February 18,
> 2020). *Invited*.

> Windmuller-Campione, M. A., Blinn, C. R., Russell, M. B., Roth, S., Snyder, S., Skay, R.* "Dwarf
> Mistletoe & Forest Management: What, Who, and How?," Research Review SFEC, Cloquet,
> Minnesota, United States. (January 9, 2020).

> Gray, E.*, Windmuller-Campione, M. A., Russell, M. B. "Is Infestation Predictable? Risk Factors
> Associated with Eastern Dwarf Mistletoe Infestation in Lowland Black Spruce Stands of
> Minnesota.," SFEC Research Review, Cloquet, Minnesota, United States. (January 9, 2020).

(Sub-project 6)

Drazan, Dallas. 2020. Seminar: Management of Knotweeds in Minnesota. Applied Plant Science thesis proposal.
4 May 2020.

(Sub-project 7)
Ryan Briscoe Runquist, Scientist Spotlight on MITPPC website.
 Uploading occurrence data taken during population surveys via EddMaps and GLEDN

(Sub-project 8)

Papers from a prior phase of this project ("Cover It Up: Using Plants to Control Buckthorn") published this year:

Schuster MJ, Wragg PD, Williams LJ, Butler EE, Stefanski A, and Reich PB (2020) Phenology matters: Extended
spring and autumn canopy cover increases biotic resistance of forests to invasion by common buckthorn
(*Rhamnus cathartica*). Forest Ecology and Management 464:118067

Anfang C, Schuster MJ, Wragg PD, and Reich PB (2020) Increased light availability due to forestry mowing of
invasive European buckthorn promotes its regeneration. Restoration Ecology 28:475-482

Schuster MJ, Bockenstedt P, Wragg PD, and Reich PB (2020) Krenite (fosamine ammonium) impacts on targeted
invasive shrub *Rhamnus cathartica* and non-target herbs. Invasive Plant Science and Management:1-19

Media coverage:
"Forests can take cover to resist alien invaders" (May 2, 2020) Mark Kinver, BBC

Digital outreach associated with Activity 3 (Citizen Science):
Newsletter circulated to full mailing list (including citizen scientists, county agricultural inspectors, and other people who expressed interest in participating but were not able to do so): 917
Participants: 123
Experimental sites: 110

Status as of January 31, 2021

(Sub-project 1), no activity

(Sub-project 2):

Marchetto, KM, TM Wolf, and DJ Larkin. "The effectiveness of using targeted grazing for vegetation management: a meta-analysis." Presented at the Upper Midwest Invasive Species Conference on Nov. 3, 2020. The session was attended by over eighty people.

Marchetto, KM, TM Wolf, and DJ Larkin. "The effectiveness of using targeted grazing for vegetation management: a meta-analysis." In revision with Restoration Ecology.

Dr. Wolf gave an invited seminar for the UMN Natural Resources Extension Faculty and Staff Development program June 2, 2020.

(Sub-project 3):

Maselko M., Feltman N., Upadhyay A. et al., **Smanski MJ**. Engineering multiple species-like genetic incompatibilities in insects. Nat Commun 11, 4468 (2020). https://doi.org/10.1038/s41467-020-18348-1

Das SR, Maselko M, Upadhyay A, **Smanski MJ** (2020) Genetic engineering of sex chromosomes for batch cultivation of non-transgenic, sex-sorted males. PLoS Genet 16(11): e1009180. https://doi.org/10.1371/journal.pgen.1009180.

Casas-Mollano JA, Zinselmeier MH, Erickson SE, and **Smanski MJ**. The CRISPR Journal. Oct 2020.350-364. http://doi.org/10.1089/crispr.2020.0064

Presentations:
§ Upper Midwest Invasive Species Conference (webcast) "Genetic technologies to control invasive species", November 2020
§ MN DNR Invasive Species Working Group (webcast) "Genetic technologies to control invasive species", October 2020
§ FNIH Global GeneConvene Virtual Institute Talk (webcast) "Engineering Species-like Barriers to Sexual Reproduction" October 2020

(Sub-project 4):

Skay, R., Windmuller-Campione, M.A., Russell, M.B., Reuling, L. (2020). Influence of eastern spruce dwarf mistletoe on stand structure and composition in northern Minnesota. Forest Ecology and Management, 481: 118712

Gray, E., Windmuller-Campione, M.A., Russell, M.A. (2020). Minnesota's Dwarf Mistletoes: Investigating a Novel Invasive Through a Native System. Upper Midwest Invasive Species Conference. November 2, 2020.

Gray, E., Windmuller-Campione, M.A., Russell, M.A. (2020). Influence of Eastern Dwarf Mistletoe on Stand Structure in Lowland Black Spruce Forests of Minnesota. National Meeting of Society of American Foresters. October 31, 2020.

Snyder, S.A., Blinn, C.R., and Roth, S. 2020. Understanding Minnesota Foresters' Attitudes and Treatment Practices for Eastern Spruce Dwarf Mistletoe in Black Spruce Systems. North American Invasive Species Management Association Annual Conference. Virtual (10/6-10/8/20).

(Sub-project 5): no activity

(Sub-project 6): no activity

(Sub-project 7):

Publications

Lake, T. A., Briscoe Runquist, R. D., & Moeller, D. A. (2020). Predicting range expansion of invasive species: Pitfalls and best practices for obtaining biologically realistic projections. Diversity & Distributions, 26(12), 1767–1779.

Briscoe Runquist, R. D., Gorton, A. J., Yoder, J. B., Deacon, N. J., Grossman, J. J., Kothari, S., Lyons, M. P., Sheth, S. N., Tiffin, P., & Moeller, D. A. (2020). Context Dependence of Local Adaptation to Abiotic and Biotic Environments: A Quantitative and Qualitative Synthesis. American Naturalist, 195(3), 412–431.

Local Adaptation meta-analysis featured in Proceedings of the National Academy of Sciences: Front Matter Journal Club (http://blog.pnas.org/2020/03/a-combination-of-living-and-nonliving-selective-forces-drive-local-adaptation-across-species/)
        -
Manuscript in revision at Journal of Biogeography:
Briscoe Runquist, R. D., Lake, T. A, & Moeller, D. A., Improving predictions of range expansion for invasive species using joint species distribution models and surrogate co-occurring species

Oral presentation at UMISC: Briscoe Runquist, R. D., "Improving predictions of range expansion for invasive species using joint species distribution models and surrogate co-occurring species", 3 November 2020
        -        Poster presentation at UMISC: Lake, T. A. "Predicting range expansion of invasive species: Pitfalls and best practices for obtaining biologically realistic projections" 2-6 November 2020
        -        Dr. Briscoe Runquist was featured as an expert in an article written by Popular Science on the mysterious seed packets sent to people (https://www.popsci.com/story/environment/seeds-mail-advice/)

(Sub-project 8):

Wragg PD, Schuster MJ, Roth AM, Bockenstedt P, Frelich LE, and Reich PB (2020). Revegetation to slow buckthorn reinvasion: Strengths and limits of evaluating management techniques retrospectively. Restoration Ecology.

 Paper submitted for publication, currently in review:

Schuster MJ, Wragg PD, and Reich PB. Phenological niche overlap between invasive buckthorn (Rhamnus cathartica) and native woody species.

 Promotion:

Stantec, Inc. is promoting the above Wragg et al. paper (co-author Paul Bockenstedt works for them) through their social media channels including LinkedIn and twitter, in collaboration with Christine Lee of MITPPC.

This paper will be featured on the cover of the January issue of Restoration Ecology, using a photograph of a post-buckthorn restoration project taken by David Hansen and provided to us by MITPPC.

 Presentations:

Wragg PD, Schuster MJ, Frelich LE, Roth AM, Bockenstedt P, Schottler S, Goodnature M, Kortebein P and Reich PB (2020). Cover it Up! Four Years of Restoring Herbs to Control Common Buckthorn. Upper Midwest Invasive Species Conference, November 2020. 88 people in the virtual audience, video available after the conference for others to watch.

Schuster MJ, Wragg PD and Reich PB (2020). Some native shrubs can mitigate phenological advantages of invasive buckthorn (Rhamnus cathartica). Upper Midwest Invasive Species Conference, November 2020.

Schuster MJ (2020). Some native shrubs can mitigate phenological advantages of invasive buckthorn (Rhamnus cathartica). Ecological Society of America annual meeting, August 2020.

Schuster MJ, Wragg PD and Reich PB (2020). Cover It Up!: Using Plants to Control Buckthorn -- Findings for better management of Minnesota's woodlands. Minnesota Noxious Weed Advisory Committee, November 2020.

Status as of July 31, 2021

(Sub-project 1)

Publications:

Held, B. W., S. Simeto, N. N. Rajtar, A. J. Cotton, D. N. Showalter, K. E. Bushley and R. A. Blanchette. 2021. Fungi associated with galleries of the emerald ash borer. Fungal Biology 125: 551-559. https://doi.org/10.1016/j.funbio.2021.02.004

Rajtar, N. N., B. W. Held and R. A. Blanchette. 2021. Fungi from galleries of the emerald ash borer produce cankers in ash trees. Submitted for publication.

Guest column in the Cotton County Citizen: A possible silver lining in the Region's Ash Borer, April 19th, 2021.

Other reports:

U of M study: Fungi may stop the emerald ash borer Duluth News Tribune March 15 2021

University of Minnesota works on 'attack fungi' that could fight emerald ash borer Star Tribune March 10 2021

U of M scientists discover attacking fungi that show promise against emerald ash borer Minnesota News

U of M researchers find potential cure for emerald ash borer beetle, March 11, 2021 Axios

U of M researchers look at fungi to stop emerald ash borer, Rochester Post Bulletin, March 19, 2021

Our View: The tree pests are freezing, too — and dying, Duluth News Tribune, Feb 16, 2021

U of M Researchers Make Gains on Emerald Ash Borer Control, Mpls, St. Paul Magazine, March 12, 2021.

U Of M Scientists Discover Fungi That 'Attack' Emerald Ash Borer, WCCO-TV, March 10, 2021

Fungi shows promise against emerald ash borer, Wisconsin State Farmer, March 12, 2021

Interviews

U of M researchers hope fungus can help fight Emerald Ash Borer, KSTP, March 18th, 2021

Wisconsin Public Radio, The Morning Show interview: 'Fungi could beat the emerald ash borer', May 12, 2021.

Presentations:
Simeto, S., Bushley, E. K., Showalter, D. N., Held, B. W., Rajtar, N. N.; Blanchette, R. A. 2021. Screening of entomopathogenic fungi for virulence against Emerald Ash Borer eggs. International Congress on Invertebrate Pathology and Microbial Control & 53rd Annual Meeting of the Society for Invertebrate Pathology.

(Sub-project 2)

Marchetto, KM, TM Wolf, and DJ Larkin. 2021. The effectiveness of using targeted grazing for vegetation management: a meta-analysis. Restoration Ecology. DOI: 10.1111/rec.13422.

Marchetto, KM. "Feeling sluggish: Learning about Minnesota's terrestrial gastropods." Gathering Partners Conference on May 13, 2021. Live online session was attended by 38 people.

KM Marchetto provided consultation to the Prairie Island Indian Community about methods for assessing the impact of goat bark stripping on Siberian Elms (June 2, 2021).

(Sub-project 4)

We have presented at the joint MN SAF/SFEC research review in February. Planning is underway for the species workshops that will be held in Fall 2021 in collaboration with SFEC and the National Advanced Silviculture Program.

Ella Gray and Alex Gorman have submitted abstract to present at the national SAF convention in November

(Sub-project 8)

Pesky Plants website: 1.2K users since Jan 1, 2021. Average session: 4 minutes, 26 seconds.

Monthly newsletter (Mailchimp) to 269 subscribers, >40% open rate. Subscribers up from 164 in January 2021.

May 13-15, 2021: Virtual table/poster at Gathering Partners Exhibit Hall

May 5, 2021: Community Science with Noxious Weeds (During a Pandemic and an Uprising). Poster and panel discussion by Abbie Anderson for CitSciVirtual. 33 attendees.

Feb 25, 2021: Anoka Master Gardeners meeting, spot for Abbie Anderson. 89 attendees.

Feb 25, 2021: Wild Ones - Arrowhead chapter meeting, spot for Stephan Carlson. 6 attendees.

Feb 9, 2021: Presented at Washington Co CWMA meeting. 34 attendees.

Feb 2, 2021 KAXE Interview with host John Latimer. February 2, 2021.

Feb 2021: Local papers (e.g., Duluth News Tribune) announce volunteer opportunities with PPT

Feb 2021: Minnesota Township Insider, winter 2021 issue, pages 18-20. Cover article, readership of ~9,000.

Jan 15, 2021: Presented at Ramsey County CWMA meeting. 17 attendees.

Jan 13, 2021: Info Session for St. Louis County (co-hosted by Duluth CISMA, City of Duluth, St. Louis County Public Works). 26 attendees.

Status as of January 31, 2022

(Sub-project 1)
Rajtar N. N., B. W. Held and R. A. Blanchette. 2021. Fungi from galleries of the emerald ash borer produce cankers in ash trees. Forests 12(11):1509. https://doi.org/10.3390/f12111509

Wargo Nature Center Newsletter (October 6th) EAB field trials at Wargo Nature Center - Anoka County Parks

Simeto, S. Entomopathogenic Fungi associated with the Emerald Ash Borer. Minnesota Mycological Society Newsletter, July 2021

Presentations:
Simeto, S. Entomopathogenic Fungi associated with the Emerald Ash Borer. Minnesota Mycological Society meeting, May 24th 2021.

(Sub-project 2)

Project coverage by popular press:

•   Minnesota Ag Connection. How effective is targeted livestock grazing? (http://www.minnesotaagconnection.com/story-state.php?Id=738&yr=2021)

•   Mirage News. How effective is targeted livestock grazing? (https://www.miragenews.com/how-effective-is-targeted-livestock-grazing-592753/)

- Brownfield Ag News. Targeted livestock grazing to reduce soybean aphid habitat. (https://brownfieldagnews.com/news/targeted-livestock-grazing-to-reduce-soybean-aphid-habitat/)

- Pope County Tribune: Goats join Barsness buckthorn bridage. (https://pctribune.com/2021/10/goats-to-join-barsness-buckthorn-brigade/)

- Mankato Free Press. Grazing goats, popular buckthorn foes, come to Waseca county park. (https://www.mankatofreepress.com/news/local_news/grazing-goats-popular-buckthorn-foes-come-to-waseca-county-park/article_4875714c-32aa-11ec-b046-5707bb5991cb.html)

Presentation:
Marchetto, KM. Meningeal worm and your herd. Southwest Wisconsin Technical College, July 29, 2021.

Interviewed by Princeton University student for an undergraduate writing assignment

(Sub-project 3) No activity

(Sub-project 4)
Gray, E. R., Russell, M. B., & Windmuller-Campione, M. A. (2021). The Difficulty of Predicting Eastern Spruce Dwarf Mistletoe in Lowland Black Spruce: Model Benchmarking in Northern Minnesota, USA. Forests, 12(7), 843.

Windmuller-Campione, M.A. (2021). Pine Forests – A Brief Overview. SFEC Wet Forest Symposium. December 14, 2021. Total Attendance - 82

Windmuller-Campione, M.A. (2021). Wet Forests – A Brief Overview. SFEC Wet Forest Symposium. October 26, 2021. Total Attendance - 41

Windmuller-Campione, M.A. (2021). Eastern Spruce Dwarf Mistletoe Biology & Influence of Stand Structure and Composition. SFEC Wet Forest Symposium. October 26, 2021.

Windmuller-Campione, M.A. (2021). Podcast Guest on SilviCast – Wet Forests Conversation. https://www.uwsp.edu/cnr-ap/WFC/Pages/SilviCast.aspx

Windmuller-Campione, M., Blinn, C., Rusell, M.B., Snyder, S., Gray, E, Gorman, A., Roth, S., Skay, R. (2021) Eastern Spruce Dwarf Mistletoe Influence on Stand Dynamics and Forest Management Practices in Minnesota. International Boreal Forest Research Association (IBFRA) (Virtual), August 20, 2021.

Snyder, S. A., Blinn, C. R., & Peterson, R. R. (2021). Examining Loggers' Attitudes and Behaviors Toward Invasive Forest Plants: A Minnesota Case Study. Journal of Sustainable Forestry, 40(3), 300-318.

Snyder, S. A., Blinn, C. R., & Roth, S. (2021). Results of a Survey of Minnesota Foresters Regarding Knowledge of and Treatment Practices for Dwarf Mistletoe in Black Spruce Stands in Northern Minnesota. https://conservancy.umn.edu/handle/11299/218324

(Sub-project 5) No activity

(Sub-project 6)
Drazan, D. A.G. Smith, N.O. Anderson, R. Becker, and M. Clark. 2021. History of knotweed (Fallopia spp .) invasiveness. Weed Science. 1-21. 0.1017/wsc.2021.62.

(Sub-project 8)
Papers:

Schuster MJ, Wragg PD, and Reich PB (2021) Phenological niche overlap between invasive buckthorn (Rhamnus cathartica) and native woody species. Forest Ecology and Management 498:119568

Media coverage:
April 16, 2021, Star Tribune profile on Jim Nicholas, CIU Citizen Scientist (In retirement, Minneapolis man takes on stubborn foe: Buckthorn - StarTribune.com)

Winter 2020/2021, Tree Farming for Better Forests profile on Damon Campbell, CIU Citizen Scientist (https://coveritup.umn.edu/sites/coveritup.umn.edu/files/2021-08/Surgeon%20By%20Day%3B%20Buckthorn%20Warrior%20By%20Night.pdf)

Presentations:
July 19, 2021: Abbie presented at the "Annual Short Course" for MN Association of County Agricultural Inspectors, coordinated by Joe Enfield

November 4 2021: Mike Schuster presented findings as part of the "Improving Restorations" webinar series hosted by UMN Extension/DNR. There were 160 concurrent viewers.

October 2021: Rob Venette highlighted results from this project in a presentation at the Minnesota Society of Arboriculture's annual meeting.

Status as of July 31, 2022:

(Sub-project 1)
Simeto, S. Fungi associated with Emerald Ash Borer: Understanding their role and looking for their potential use in biocontrol. 59th Annual Minnesota Shade Tree Short Course, March 15th 2022.
Simeto S. Emerald Ash Borer: Identification, ecology and management. Emerald Ash Borer (EAB) training in Fort Worth Nature Center, March 13th 2022.
Simeto, S.,K. E. Bushley, D. N. Showalter, B. W. Held and R. A. Blanchette. 2022. Ovicidal effect of entomopathogenic fungi on emerald ash borer eggs. Mycological Society of America Annual Meeting, Gainesville, FL.

(Sub-project 7)
Publication: "Deep learning detects invasive plant species across complex landscapes using Worldview-2 and Planetscope satellite imagery" in the journal Remote Sensing in Ecology and Conservation

Google Cloud Research Credit program has granted us $1800 in research credits and another application has been elevated to consideration for their Climate Change Initiative Challenge program.

(Sub-project 8)
Papers:
● 	Michael J. Schuster, Peter D. Wragg, Alexander M. Roth, Paul Bockenstedt, Lee E. Frelich, Peter B. Reich. in press. Using plants to control buckthorn (*Rhamnus cathartica*): improved biotic resistance of forests through revegetation. Ecological Engineering
● 	White paper on revegetating to suppress buckthorn re-invasion - completed by MITPPC staff and project researchers.

(Sub-project 9)
-Invited talk on this project given at the Engineering Biology Research Consortium annual meeting
-Invited talk on this project given at the inaugural Gordon Research Conference on Genetic Biocontrol

-Hosted a stakeholder workshop on genetic biocontrol of aquatic invasive species at UMN (officially part of a MAISRC-funded project, but many of the stakeholders that were engaged (e.g. EPA, MN-DNR, tribal representatives from the Leech Lake Band and Fond du Lac Band of Anishinaabe) are equally relevant to the SWD project).
-Outreach to 4th grade class (1 of 4 planned) to teach about genetic engineering (officially part of a MnDRIVE-funded project).

Status as of January 31, 2023:

(Sub-project 6)
Three presentations were made at UMISC 2022, Green Bay WI.
Roger Becker, Alan Smith, Neil Anderson, Elizabeth Katovich, Ryan Mentz. 2022. Herbicidal Control of Invasive Knotweed and Possible Differential Response.

Alan Smith, Dallas Drazan, Neil Anderson, Roger Becker, Matthew Clark. 2022. Genetic Diversity and Reproduction of Invasive Knotweeds with a Focus on Minnesota.

Alan Smith, Dallas Drazan, Neil Anderson, Roger Becker, Matthew Clark. 2022. Cold Hardiness of Invasive Knotweed (Fallopia spp.) Rhizomes.

(Sub-project 7)
UMISC Presentations:
Landscape genetics of common tansy in Minnesota reveal two genetic clusters with distinct environmental tolerances (Ryan)
Deep learning detects invasive plant species across complex landscapes using Worldview-2 and Planetscope Satellite imagery (Tom)

Ecological Society of America - Montreal Presentation:
Deep learning detects invasive plant species across complex landscapes using Worldview-2 and Planetscope Satellite imagery (Tom)

UMN American Society of Photogrammetry and Remote Sensing Student Chapter Presentation:
Time series classification with convolutional neural networks (Tom)

(Sub-project 8)
Papers:
Michael J. Schuster, Peter D. Wragg, Peter B. Reich. in prep. Understory revegetation enhances efficacy of prescribed burning after common buckthorn (Rhamnus cathartica) management. Restoration Ecology.
Michael J. Schuster, Peter D. Wragg, Peter B. Reich. in review. No evidence of a long-lived seedbank in Common Buckthorn, Rhamnus cathartica L.. Biological Invasions.

Media coverage:
October 13, 2022. Mike Schuster appeared on FOX 9 to discuss buckthorn management.
https://www.fox9.com/video/1129883

Presentations:
Upper Midwest Invasive Species Conference. October 26, 2022. Remote presentation: Cover It Up! Seven years of restoring herbs to control common buckthorn.

(Sub-project 9)

We brought (dead) RFP-expressing fruit flies and live GFP-expressing aquarium fish to 5 elementary school classrooms in 2022 (one in Falcon Heights, MN and 4 in a URM-serving school in Cloquet, MN). In each classroom, we taught a 90 min STEM less related to genes, genetics, and genetic engineering.

June 30, 2023:
(Sub-project 6)
Data and power point slides from this project were used by Maggie Barnick, Biological Technician (Plants), U.S. Forest Service (formerly with the MDA) at the Minnesota Shade Tree Short Course on March 14 & 15, 2023, estimated total number of attendees was 70.

(Sub-project 7)
New Collaborators:
- Lex Flagel, Gencove; Leafy spurge genomics
- Debalin Sarangi & Datta Chiruvelli, CFANS; SDMs
- Theresa Chen, Knowledge Computing Lab, CSE; Remote sensing of invasive species

- UMN Research and Outreach Centers (ROC):
  - St. Paul Experimental Station, St. Paul, MN
  - Southwest ROC, Lamberton, MN
- Cloquet Forestry Center in collaboration with the Fond Du Lac Band
- Minnesota State University, Moorhead, Science Outreach Center, Glyndon, MN

(Sub-project 10)
The team has been invited and accepted to present oral presentations at two major scientific conferences:
1- 2023 American Institute of Chemical Engineers (AIChE) Annual Meeting, Orlando, FL, November 6, 2023.
2- American Chemical Society Fall Meeting, San Francisco, CA, August 13-17, 2023.

The team is also building connections with plant diagnostic laboratories in different states for potential field testing.

Final Report Summary:
Research teams were very active in disseminating their research results to broad audiences. Eight research teams published 30 peer reviewed articles in esteemed journals such as Restoration Ecology, PLoS Genetics, and Biological Invasions. Research teams made over 70 presentations to professional conferences, educational institutions, and land management groups. Regional and statewide media, ranging from the Duluth News Tribune to KAXE radio in Grand Rapids, to the Mankato Free Press to WCCO, carried stories about our research and extended the reach of our results. Whenever possible, the ENRTF was appropriately recognized.

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: 0
Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 10.5 FTE
Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0

B. Other Funds:

SOURCE OF AND USE OF OTHER FUNDS	Amount Proposed	Amount Spent	Status and Timeframe
Other Non-State $ To Be Applied To Project During Project Period:			
	$	$	
Other State $ To Be Applied To Project During Project Period:			
UMN Indirect Recovery Rate @52%	$ 1,950,000	$	
ML 2014 Chapter 312, Article 12, Section 8 (general fund)	$3,400,000		6/30/2022
Past and Current ENRTF Appropriation:			
M.L. 2014, Ch. 312, Art. 12, Section 8 (ENRTF)	$1,460,000		6/30/2022
M.L. 2015, Ch. 76, Sec. 2, Subd. 6a (ENRTF)	$5,000,000		6/30/2023
Other Funding History:			
N/A	$	$	

VI. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role

VII. PROJECT STRATEGY:
A. Project Partners:

Project Partners (not receiving funds):
- USDA Forest Service Northern Research Station
- Minnesota Department of Agriculture
- Minnesota Department of Natural Resources
- Minnesota Forest Resource Council
- Agencies and organizations involved in invasive species outreach programs so public information is based on the best available science.
- Networks of citizen scientists could be an important part of implementing early detection programs and monitoring the effectiveness of control efforts.

This will be updated in more detail once the priorities for research are established.

B.   Project Impact and Long-term Strategy:

The Center's ultimate goal is to eliminate, reduce, mitigate or prevent the introduction, expansion or damage done by terrestrial invasive species in Minnesota. Metrics of success include: threat awareness, response efficiency, control effectiveness, non-target species protection, and mitigation strategies. Ancillary goals include: workforce development, citizen engagement, focused research strategies, improved response time to emerging threats, and improved coordination of efforts.

Success will depend on the ability to marshal multi-disciplinary teams in timely and prioritized ways to deliver results. Funding provided will be used to support additional multi-disciplinary research teams. With adequate funding, the Center's efforts are expected to result in numerous, effective prevention and control methods within an eight year time frame for a significant portion of the species upon which we will focus.

IX. VISUAL COMPONENT or MAP(S): See attached

X. RESEARCH ADDENDUM: A research addendum was developed after a panel of researchers from the University of Minnesota provided competitive reviews of the pre-proposals under this appropriation. The addendum was distributed to relevant experts outside the University of Minnesota and reviewed for scientific novelty, appropriateness of methods, qualifications of the research team, and potential impact on invasive species management. The research addendums were modified as necessary in response to comments received during the peer-review process. The final documents provide a technically detailed description of the research to be completed under this sub-project work plan. The research addendums are on file with the Minnesota Invasive Terrestrial Plant and Pest Center**.**

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 31 and July 31 each year (every six months). A final report and associated products will be submitted between June 30 and August 15, 2023.

Environment and Natural Resources Trust Fund
Minnesota Invasive Terrestrial Pests and Plants Center-- Sub Project List
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a **FINAL**
Project Manager: Robert Venette
Project Title: Minnesota Invasive Terrestrial Plants and Pests Center – Phase III
Organization: Regents of the University of Minnesota
College/Department/Division: College of Food, Agricultural, and Natural Resources Sciences
Project Budget: $3,750,000
Project Length and Completion Date: 7 years; June 30, 2023
Current Date: August 18, 2023

Sub Project #	Sub-Project Title	Species	Sub-Project Manager	LCCMR Approval Date	Revised final budget [8-18-2023]	Amount Spent	Balance	Status (select from dropdown menu)
reserve					$145,458		$145,458	
1	Sub-project 1 Fungi in ash trees: towards protecting trees from emerald ash borer and new diseases	Emerald ash borer & challara disease of ash	Robert Blanchette	2/1/2018	$500,000	$500,000	$0	Complete
2	Sub-project 2 Understanding the benefits and limitations of using goats for invasive plant control	Buckthorn	Tiffany Wolf	2/19/2018	$410,267	$410,267	$0	Complete
3	Sub-project 3 Genetic control of invasive insect species: Phase I	Spotted wing drosophila	Michael Smanski	2/28/2018	$295,717	$295,717	$0	Complete
4	Sub-project 4 Dwarf mistletoe detection and management in Minnesota	Dwarf mistletoe	Marcella Windmuller-Campione	4/19/2018	$455,606	$455,606	$0	Complete
5	Sub-project 5 Developing spatially explicit bio-economic dispersal model to aid with the management of brown marmorated stink bug	Brown marmorated stink bug	Senait Senay & Terrance Hurley	8/6/2018	$329,304	$329,304	$0	Complete
6	Sub-project 6 Management of invasive knotweeds	Japanese, giant, and bohemian knotweeds	Alan Smith	4/2/2019	$476,723	$476,723	$0	Complete
7	Sub-project 7 Building mechanistic and process based species distribution models for common tansy and leafy spurge: from landscapes to genomes*	Common tansy and leafy spurge	David Moeller & Ryan Briscoe Runquist	4/2/2019	$351,188	$351,188	$0	Complete
8	Sub-project 8: Using plants to control buckthorn: an expanded approach	Common buckthorn	Peter Reich	4/2/2019	$560,000	$560,000	$0	Complete
9	Sub-project 9: Genetic control of invasive insects, Phase 2	Spotted wing drosophila	Mike Smanski	2/18/2022	$55,100	$55,100	$0	Complete
10	Sub-project 10: Novel Diagnostic Tools for Rapid and Early Detection of Oak Wilt	Oak wilt	Abdennour Abbas	6/14/2022	$170,637	$170,637	$0	Complete
Notes:	*This project is co-funded with ML 2015, Ch. 76, Art. 2, Sec. 6a, with $70,812 from ML 2015 and the balance of $351,888 from ML 2016, Ch. 186, Art. 2, Sec. 6a (total project allocation = $422,000). This table only summarizes expenditures made through M.L. 2016, Ch. 186, Sec. 2, Subd. 6a.				$3,750,000	$3,604,542	$145,458	

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial Pests and Plants Center

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Sub-Project Title: Sub-project 1 Fungi in Ash Trees: towards protecting trees from emerald ash borer and new diseases
Sub-Project Manager: Robert Blanchette
Organization: University of Minnesota
M.L. 2016 ENRTF Appropriation: $500,000
Project Length and Completion Date: 4 Years, June 30, 2022
Date of Report: Revised August 18, 2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Budget [8-18-2023]	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$451,417	$451,417	$0
PhD graduate student: $42,518 (56% salary, 8% benefits; 36% tuition); 0.5 FTE per year for 3.5 years			
Research Scientist: $24,202 12 weeks salary for each year (75% salary, 25% benefits) 0.17 FTE per year for 4 years			
Postdoctoral Research Associate, $57,636 (82% salary, 12% benefits) 1 FTE for 3.5 years			
Undergraduate Students, $8,000 (100% salary) .23 FTE for 4 years			
Professional Services			
Molecular sequencing and greenhouse space fees	$14,480	$14,480	$0
Equipment/Tools/Supplies			
Culture media for growing microorganism, such as molecular primers, probes and reagents, petri dishes.Materials for laboratory and field biocontrol studies, such as containers for EAB infected logs, screen, funnel traps, biocontrol beetle contamination chambers, biocontrol tree bands, etc.	$30,997	$30,997	$0
Travel expenses in Minnesota			
$2,400 per year for vehicle travel costs to collect samples from EAB infected areas in Minnesota	$3,106	$3,106	$0
Other			
COLUMN TOTAL	**$500,000**	**$500,000**	**$0**

Returned to reserve (negative, taken)

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial
Pests and Plants Center

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Sub-Project Title: Sub-project 2 Understanding the benefits and limitations of using
Sub-Project Manager: Tiffany Wolf
Organization: University of Minnesota
M.L. 2016 ENRTF Budget: $410,267
Sub-Project Length and Completion Date: 4 Years, February 28, 2022
Date of Report: April 26, 2022 (Completed)

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	TOTAL BUDGET	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$392,388	$392,388	$0
Assistant Professor Larkin, Faculty investigator: $25,100 (75% salary, 25% benefits) summer salary; 3.8% FTE each year for 4 years			
Post-doc: $236,771 (82% salary, 18% benefits); 100% FTE each year for 4 years			
Graduate Research Assistant: $25,000 (58% salary, 42% benefits); 25% FTE each year for 2 years			
Graduate Research Assistant: $85,597 (55% salary, 45% benefits); 50% FTE each year for 2 years.			
Laboratory technician: $10,020 (79% salary, 21% benefits); 30% FTE for 1 year.			
Undergraduate research assistants: $22,400 (100% salary); 560 hours @ $10/hour per year for four years. 27% FTE per year for 4 years.			
Professional/Technical/Service Contracts			
Rental of 56 goats to graze a site over four field seasons on UMN land, such as the Rosemount Research and Outreach Center, in order to have the greatest long term access to the field site. Other sites will be located on land of Goat Dispatch customers at no cost.	$5,770	$5,770	$0
Research software for data management and analysis (Fulcrum Custom Subscription)	$1,038	$1,038	$0
Equipment/Tools/Supplies			
Experimental supplies - goat fencing, field stakes, flagging, yard sticks, gloves, and related items & Sample collection consumables - vials, research grade ethanol, vinyl gloves, forceps, and related items	$2,595	$2,595	$0
Laboratory testing- -This work will now be undertaken by students and accounts for the increase in personnel to accommodate.	$2,370	$2,370	$0
Travel expenses in Minnesota			
Vehicle rental/gas/mileage, trailer rental for goose transportation between field sites. These expenses are necessary for project investigators, staff, and students to be able to make multiple trips each year from the U of M's St. Paul campus to field sites throughout southern Minnesota. Travel expenses will comply with U of M travel policies.	$3,960	$3,960	$0
Publications	$2,146	$2,146	$0
COLUMN TOTAL	**$410,267**	**$410,267**	**$0**

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial Pests and Plants Center

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Sub-Project Title: Sub-project 3 Genetic Control of Invasive Insect Species: Phase I
Sub-Project Manager: Michael Smanski
Organization: University of Minnesota
M.L. 2016 ENRTF Budget: $295,717
Sub-Project Length and Completion Date: 3.1 years, February 28, 2021
Date of Report: Revised August 18, 2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	REVISED BUDGET [8-18-2023]	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$234,324	$234,324	$0
Maciej Maselko, postdoc: $150,588 (75% salary, 25% benefits); 100% FTE each year for 2 years			
Graduate Student, $85,067 (60% salary, 40% benefits); 50% FTE each year for 2 years			
Professional/Technical/Service Contracts			
Core facilities (DNA sequencing costs)	$21,196	$21,196	$0
Equipment/Tools/Supplies			
Lab/Field Supplies, such as plasmid extraction kits, PCR strib tubes, parafilm, RNA extraction kits, DNA oligos, eppendorf tubes	$40,197	$40,197	$0
Printing			
Publication costs	$0	$0	$0
COLUMN TOTAL	**$295,717**	**$295,717**	**$0**

Returned to reserve

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial Pests and Plants Center

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Sub-Project Title: Sub-project 4 Dwarf Mistletoe Detection and Management in Minnesota
Sub-Project Manager: Marcella Windmuller-Campione
Organization: University of Minnesota
M.L. 2016 ENRTF Budget: $455,606
Sub-Project Length and Completion Date: 3.5 years: November 30, 2022
Date of Report: April 26, 2022 (Completed)

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Total Budget	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$398,484	$398,484	$0
M. Windmuller-Campione, project manager: $23,766 (75% salary, 25% benefits) summer salary; 4% FTE each year for 4 years.			
M. Russell, Project investigator: $24,452 (75% salary, 25% benefits) summer salary, 4% FTE each year for 4 years			
3 graduate research assistants: $268,104 (52% salary, 48% benefits during academic year, 85% salary and 15% benefits during summer); Each positon 50% FTE each year for 2 years (total FTEs 3.0)			
3 undergraduate research assistants: $51,424 (100% salary, 0% benefits summer, 92% salary 8% benefit acedmic year), Each position 20% FTE for 3 years (total FTE 0.6)			
Equipment/Tools/Supplies			
Mail Survey	$0	$0	$0
Field equipment	$786	$786	$0
Computers for PhD Student and MS student - computers are a necessary expense for the development of sampling locations and design on GIS software and the analysis of data collected in the field for Objective 1 and Objective 2. Computers will be used solely for project-related work. Computers will be cleaned of data and programs and returned to MITPPC after the completion of the project.	$1,399	$1,399	$0
Travel expenses in Minnesota			
Travel to the multiple research sites multiple times each year to collect data related for Activity 1 and 2. We estimate over 5000 miles traveled each year due to remote site location, long distances between sites, and many of the project team members being based in the Twin Cities with sites located in northern Minnesota.Travel expenses includes lodging and meal allowance for graduate students, research associates, and field technicians	$54,937	$54,937	$0
Rental time in airplane for sampling of dwarf mistletoe	$0	$0	$0
In state conference travel and registration	$0	$0	$0
Unmanned aircraft work	$0	$0	$0
COLUMN TOTAL	**$455,606**	**$455,606**	**$0**

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial Pests and Plants Center

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Project Managers: Senait Senay & Terrance Hurley
Sub-Project Title: Sub-project 5 Developing Spatially Explicit Bio-economic Dispersal Model to Aid with
Sub-Project Budget: $329,304
Sub-Project Length and Completion Date: 3 4 years, July 18, 2021
Date of Report: Revised August 18, 2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised final budget [8-	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$326,579	$326,579	$0
Terrance Hurley, Co-Project Manager: $57,684 (75% salary; 25% benefits) 5.77% FTE (partial summer salary) each year for 4 years			
Post-doc in Applied Economics: $66,750 (75% salary; 25% fringe); 100% FTE for 1 year.			
Graduate Research Assistant in Entomology: $80,000 (54% salary; 25% fringe); 100% FTE each year for 2 years			
Researcher: $70,361 (75% slarary; 25% fringe); 15% FTE per year for 4 years			
Post doc with International AgroInformatics Alliance: $135,169 (75% salary; 25% fringe); 100% FTE each year for 2 years			
Programmer with International AgroInformatics Alliance: $132,569 (75% salary; 25% fringe); 65% FTE each year for 2 years			
Senior Software Engineer with International AgroInformatics Alliance: $5,095 (75% salary; 25% fringe); 2% FTE each year over 2 yrs			
Equipment/Tools/Supplies			
$3,500 for ERDAS IMAGINE image analysis software, $4,000 for 2 laptops with monitors and docking station (2 @ $2000 each). This research project is computer intensive and requires capabilities beyond normal office desktops. Software and computers will be used solely for this project and will be transferred to MITPPC upon project completion for future use.	$2,725	$2,725	$0
Printing			
Publication costs for four original research articles		$0	$0
Travel expenses in Minnesota			
Travel to share results and receive feedback from stakeholder groups within the state. Travel is for up to three trips (estimated @ $290 / trip) and may include presentations to technical experts.		$0	$0
Other			
Travel: $1,350 for one traveller to present results and receive project feedback at an out-of-state technical conference		$0	$0
COLUMN TOTAL	**$329,304**	**$329,304**	**$0**

Returned to reserve

personnel (Wages and Benefits)

M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial Pests
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Project Managers: Alan Smith
Sub-Project Title: Sub-project 6 Management of Invasive Knotweeds
Sub-Project Budget: $579,670
Sub-Project Length and Completion Date: 4 years, June 30, 2023
Date of Report: August 18, 2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised final budget [8-18-2023]	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$375,141	$375,141	$0
Graduate students $ 25,498 (47.5% salary, 17.7% fringe; 35% tuition) .5 FTE for 2.5 yrs			
Undergraduate student: $33,000 (100% salary) .25 FTE during school year; 1 FTE summer for 4 years			
Civil service: $44,219 (72.8% salary, 27.2% fringe) .5 FTE for 4 years			
Professional Services			
Contract with FSN for CHO analysis	$0	$0	$0
Equipment/Tools/Supplies			
GPS device, field and greenhouse supplies, microscopy supplies	$21,197	$21,197	$0
Printing			
Publication costs for four research articles	$1,524	$1,524	$0
Travel expenses in Minnesota			
Travel to collect knotweed samples	$4,311	$4,311	$0
Other			
Greenhouse fees, field space, soil testing	$74,550	$74,550	$0
COLUMN TOTAL	**$476,723**	**$476,723**	**$0**

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial Pests and Plants
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Project Managers: David Moeller and Ryan Briscoe Runquist
Sub-Project Title: Sub-project 7: Building mechanistic and process based species distribution models for
Sub-Project Budget: $351,188
Sub-Project Length and Completion Date: 3.8 years April 1, 2023
Date of Report: February 13, 2023

Funding for this sub-project is split with
ML 2015 Ch. 76, Art. 2, Sec. 6a

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	TOTAL BUDGET	AMOUNT SPENT	TOTAL BALANCE	TOTAL BUDGET	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM						
Personnel (Wages and Benefits)	$313,695	$313,695	$0	$70,812	$70,812	$0
David Moeller, Project Manager: $39,835 (74% salary, 26% fringe) .03% FTE (partial summer salary) for 3 yrs						
Ryan Briscoe Runquist, Researcher: $248,879 (65.8% salary, 34.2% fringe) 1 FTE for 4 yrs.						
Graduate student: $58,669 (68.3% salary, 17.7% benefits, 14% tuition) 0.5 FTE for four semesters (2 academic years)						
Undergraduates: $27,322 (40 hrs/wk for 13 wks @ $13.13-$13.43/hr; 0% fringe) for 4 years						
Professional services						
Lab services for DNA sequencing/growth chamber fees	$22,053	$22,053	$0			
Equipment/Tools/Supplies 6295						
Supplies for seed collection, growth chamber experiments, plant processing from experiments	$5,062	$5,062	$0			
computer software for remotely-sensed data & GIS analysis; genomic analysis	$4,008	$4,008	$0			
Printing						
publications' costs for journal articles	$0	$0	$0			
Travel						
In-state travel ($3,000/yr)	$6,370	$6,370	$0			
COLUMN TOTAL	$351,188	$351,188	$0	$70,812	$70,812	$0

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota Invasive Terrestrial Pests
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Project Managers: Peter Reich
Sub-Project Title: Sub-project 8 Using Plants to Control Buckthorn: an Expanded
Sub-Project Budget: $560,000
Sub-Project Length and Completion Date: 4 years, June 30, 2023
Date of Report: August 18, 2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revisied final budget [8-	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$500,676	$500,676	$0
2 Post-doctoral associates: $321,103 (78.6%salary, 21.4% fringe) 2 FTE, 1 for 9 mos, 1 for 6 month/yr for 4 yrs.			
2 undergraduates: $60,224 (100% salary) @12/hr * 1,254 hrs/yr for 4 yrs			
1 civil service technician: $106, 432 (78% salary, 22% fringe) 0.75 FTE for 4 yrs.			
1 temp casual: $16,221 (92.3% salary; 7.7% fringe) 0.5 FTE for 1 yr			
Contracts			
Contractor to supply and apply bud inhibitor herbicide at research plots	$27,500	$27,500	$0
Postage and mailing			
Mailing costs associated with citizen science activities	$4,078	$4,078	$0
Equipment/Tools/Supplies			
Field supplies, fencing, tools, seeds, plants, equipment to measure light availability, maintenance of equipment to measure light availability	$12,614	$12,614	$0
Travel expenses in Minnesota			
In-state travel to field and training sites and UMISC conference, including mileage on personal vehicles	$15,132	$15,132	$0
COLUMN TOTAL	**$560,000**	**$560,000**	**$0**

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota
Invasive Terrestrial Pests and Plants Center

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Project Managers: Michael Smanski
Sub-Project Title: Sub-project 9 Genetic control of
Sub-Project Budget: $60,000
Sub-Project Length and Completion Date: 6 months December 31, 2022
Date of Report: February 13, 2023 (completed)

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	TOTAL BUDGET	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$55,100	$55,100	$0
1 Post-doctoral associate: $33,000 (78.6% salary, 21.4% fringe) 1 FTE, 1 for 6 mos			
1 graduate student: $27,000 (56% salary; 30%; 13 % benefits) 1 FTE for 6 mos			
COLUMN TOTAL	**$55,100**	**$55,100**	**$0**

Returned to reserve | $4,900 |

Environment and Natural Resources Trust Fund
M.L. 2016 Budget Spreadsheet- Minnesota
Invasive Terrestrial Pests and Plants Center

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06a
Project Managers: Abdennour Abbas
Sub-Project Title: Sub-project 10, Novel Diagnostics for Rapid and Early Detection of Oak Wilt
Sub-Project Budget: $170,637
Sub-Project Length and Completion Date: 12 months, June 30, 2023
Date of Report: August 18, 2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised final budget [8-18-2023]	AMOUNT SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$127,060	$127,060	$0
1 faculty: $13,350 (66.5% salary; 33.5% benefits) 1 FTE for one month			
1 Post-doctoral associate: $72,540 (79.1% salary, 20.9.% fringe) 1 FTE, for one year			
1 technician: $64,350 (71.3% salary; 30%; 28.7 % benefits) 1 FTE for one year			
Equipment/Tools/Supplies	$42,104	$42,104	$0
General lab supplies, cleaning consumables, reagents, PCR kits			
Professional Services	$1,096	$1,096	$0
User fees at UMN core facilities, PCR maintenance			
Travel	$377	$377	$0
Domestic travel for consultation and presentation			
COLUMN TOTAL	**$170,637**	**$170,637**	**$0**

Returned to reserve