

2011 Project Abstract

For the Period Ending June 30, 2014

PROJECT TITLE: Emerald Ash Borer Biocontrol Research and Implementation

PROJECT MANAGER: Monika Chandler

AFFILIATION: Minnesota Department of Agriculture

MAILING ADDRESS: 625 Robert Street North

CITY/STATE/ZIP: St. Paul, MN 55155

PHONE: 651-201-6537

E-MAIL: Monika.Chandler@state.mn.us

WEBSITE: www.mda.state.mn.us/plants/pestmanagement/eab/eabbiocontrol.aspx

FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 06b

APPROPRIATION AMOUNT: \$ 500,000

Overall Project Outcome and Results

We made great progress with the biological control for emerald ash borer (EAB) in Phase 1 of this project. We simultaneously released wasps that parasitize EAB while we studied them. EAB can kill ash trees quickly (within 6 years). We have responded rapidly to EAB finds so that we might avoid large numbers of EAB over extensive areas, a situation that would be difficult to manage effectively. At the same time, we studied the parasitoid wasps to understand their cold tolerance and dispersal capability. Our studies improved our implementation strategies.

Over 127,000 parasitoid wasps were released at 21 sites in the Twin Cities and southeastern Minnesota. Recovery of immature parasitoids in the field demonstrated that these agents are dispersing then finding and parasitizing EAB. We will continue releases in Phase 2. Research efforts demonstrated that the egg parasitoid, *Oobius agrili*, is the most cold tolerant and the larval parasitoid, *Tetrastichus planipennisi*, is the least cold tolerant. Therefore, we began releasing *T. planipennisi* earlier in the season to allow multiple generations to build a population sufficient to withstand anticipated cold induced mortality losses. We learned that *T. planipennisi* is capable of dispersing almost 5 miles within 24 hours but that most will fly $\frac{3}{4}$ miles in 24 hours. Therefore, we began releasing *T. planipennisi* over a large area at a release site rather than at a central cluster to enable faster *T. planipennisi* dispersal. Research efforts trained a total of six graduate students, five undergraduate students, and three technicians in whole or in part on these projects.

We will continue a study of ash health, EAB, and parasitoid wasps in the Twin Cities area where EAB was first found in 2009. To date, ash mortality within the study area has been substantially lower than anticipated.

Project Results Use and Dissemination

Information about this project has been and will continue to be disseminated to the public, land managers and researchers. Media releases (3) and social media were utilized to inform the public of major developments. There were 15 scientific presentations to researchers and land managers. Additional training presentations (24) were given to the public, professional land managers, and tree care professionals at many venues. Outreach at public events (20) helped us to connect with people about our activities. Two research papers on parasitoid cold tolerance were published. An additional two papers on parasitoid dispersal are anticipated. In addition, we participate in the EAB Forum, a multi-agency/organization venue for discussing EAB management. We maintain a website www.mda.state.mn.us/plants/pestmanagement/eab/eabbiocontrol.aspx with project information.



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2011 Work Plan

Date of Final Report: 08/15/2014

Date of Work Plan Approval: 6/23/2011

Project Completion Date: 6/30/2014

Is this an amendment request? Yes

Project Title: Emerald Ash Borer Biocontrol Research and Implementation

Project Manager: Monika Chandler

Affiliation: Minnesota Department of Agriculture

Address: 625 Robert St N

City: St Paul **State:** MN **Zipcode:** 55155

Telephone Number: (651) 201-6537

Email Address: Monika.Chandler@state.mn.us

Web Address: www.mda.state.mn.us/en/plants/pestmanagement/eab/eabbiocontrol.aspx

Location:

Counties Impacted: Statewide

Ecological Section Impacted: Lake Agassiz Aspen Parklands (223N), Minnesota and Northeast Iowa Morainal (222M), North Central Glaciated Plains (251B), Northern Minnesota and Ontario Peatlands (212M), Northern Minnesota Drift and lake Plains (212N), Northern Superior Uplands (212L), Paleozoic Plateau (222L), Red River Valley (251A), Southern Superior Uplands (212J), Western Superior Uplands (212K)

Total ENRTF Project Budget:	ENRTF Appropriation \$:	500,000
	Amount Spent \$:	500,000
	Balance \$:	0

Legal Citation: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 06b

Appropriation Language:

\$250,000 the first year and \$250,000 the second year are from the trust fund to the commissioner of agriculture to assess a biocontrol method for suppressing emerald ash borers by testing bioagent winter survival potential, developing release and monitoring methods, and piloting implementation of emerald ash borer biocontrol. This appropriation is available until June 30, 2014, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Emerald Ash Borer Biocontrol Research and Implementation

II. PROJECT SUMMARY: Biological control is currently the only promising long-term management strategy for emerald ash borer (EAB), a beetle that is native to Asia. It was first detected in North America near Detroit in 2002 and has killed millions of ash trees. In May 2009, Minnesota's first EAB was detected in St. Paul and has since been found in Minneapolis and in a natural ash stand on the Mississippi River in southeastern Minnesota. The loss of Minnesota's nearly 1 billion ash trees, more ash on forestland than any other state, would be catastrophic. Ash-dominated sites are essential to many native plants and wildlife.

Biological control is the only potential tool to save ash that can be implemented at a forest scale. Biological control reunites the target pest with the insects or diseases that control the pest in its native range. In this case, parasitoids that control EAB in Asia would be released to control EAB in Minnesota.

The project goal is to establish biological control agent populations that suppress EAB and minimize EAB damage. Our objectives are to assess biological control agent winter survival potential, and assess establishment and spread of biological control agents after release in order to successfully implement EAB biocontrol in Minnesota.

III. PROJECT STATUS UPDATES:

Project Status as of May 31, 2012:

Biological control of EAB is making good progress. There is a synergistic collaboration among project partners. Since we started our project in July of 2011, we have made strong progress on testing biological control agent cold-hardiness (Activity 1), developed methods for laboratory studies to measure biological control agent dispersal (Activity 2), and field-released thousands of biological control agents (Activity 3). We jointly initiated an ash health, EAB, and EAB biological control agent study in the Twin Cities.

Outreach to date has included the web, media coverage, events, and presentations.

To accomplish the above:

- Students and staff were hired.
- The capital equipment purchase was made of precision controlled freezers that are in use.
- A contract was written between the University of Minnesota (U of M) and the Minnesota Department of Agriculture (MDA) to transfer ENRTF project funds from MDA to the U of M.
- Federal 526 permits to receive and research or release biological control agents were obtained.

ENRTF dollars were leveraged to secure additional USDA APHIS CPHST funding at \$70,162 to provide quantities of the exact lifecycle stages of biological control agents needed for Activities 1 and 2.

Project Status as of November 30, 2012:

The biological control of EAB project is proceeding well. Data collection for testing biological control agent cold-hardiness (Activity 1) is near completion and ready for analysis. Data collection is well underway for studying parasitoid dispersal with the construction of a flight mill (Activity 2). We continue to field release biological control agents and collect data (Activity 3). Data collection is continuing for the ash health, EAB, and EAB biological control agent study in the Twin Cities.

Project Status as of May 31, 2013:

Project progress continued smoothly. All biological control agent cold-tolerance experiments are finished and a preliminary analysis completed (Activity 1). The impact of mating status, gender, size, age and feeding on parasitoid flight were measured and additional studies on impacts on temperature and humidity planned (Activity 2). Field monitoring of EAB and parasitoids continued over the winter

and plans made for the upcoming field season (Activity 3). Data collection continues for the ash health, EAB, and EAB biological control agent study in the Twin Cities.

Project Status as of November 29, 2013:

Data collection is nearly completed for research activities of parasitoid cold tolerance (Activity 1) and dispersal capacity (Activity 2). Data analysis will continue in preparation for our final report. Continuation of our ash health, EAB and EAB bioagent monitoring study was recommended for funding for an additional 3 years. Over 50,000 parasitoids were released during the 2013 field season at sites in Twin Cities and southeastern Minnesota. We are beginning to recover parasitoids in the field and received a definitive species confirmation. Continuation of EAB biocontrol implementation was recommended for funding for an additional 3 years.

Amendment Request November 29, 2013

Amendment request from 11/29/13 was approved

Activity 1 budgetary changes: This part of the project is on track to finish on budget. We are requesting permission for three variances between categories:

1. Move \$3,000 from Supplies to Salaries. The original proposal included funds to raise the parasitoids in our laboratory. This effort proved to be difficult and inefficient. USDA APHIS provided all parasitoids needed for this portion of the project and created a project savings for this line item.
2. Move \$2,500 from Travel to Salaries. Travel has been lower than originally estimated because most field work has been accomplished without the need for overnight stays.
3. Move \$1,640 from Equipment to Salaries. The original equipment estimates did not account for discounts that the University receives from some suppliers.

We are requesting the additional funding for graduate-student, undergraduate-student, and technician salaries for the processing of samples from the in-field overwintering study, data analysis, and write-up of the final report.

Please note that technician salary was reported in the Activity 1 column of the May 2013 status report but should have been in the Activity 2 column as it appears in this Nov 2013 report.

Activity 2 budgetary changes: This part of the project is on track to finish on budget. We are requesting permission for two variances between categories:

1. Move \$2,000 from Supplies to a new category, Services. Services include repair of environmental growth chamber (condenser breakdown during temperature flight trials) (\$1900) and scientific poster printing for research dissemination at local meetings (\$100).
2. Move combined \$8,000 from Travel to Salaries due to increasing benefit rates over past three years. Travel has been lower than expected due to a) travel scholarships of students on project, b) purchasing a vehicle with non-project funds as cost-savings vs. annual leasing, and c) proximate work in metro area.

Activity 3 budgetary changes: We are requesting permission for the following variances between categories.

1. Move \$4,500 from Travel meals and lodging to Travel vehicle/mileage (\$1,000), Supplies (\$500) and Salaries (\$3,000).
2. Move \$300 from Equipment to Salaries.

We anticipated that EAB would spread quickly throughout the state and much overnight travel would be needed. Fortunately, EAB has not been found throughout the state so we request to spend the funds on salary for monitoring/branch sampling.

Retroactive Amendment Request: September 17, 2014

Activity 1 budgetary changes: We are requesting permission for the following changes to reflect exact amounts spent in each category.

1. Increase personnel from \$134,740 to \$136,102.

2. Decrease capital equipment from \$12,360 to \$12,131.
3. Decrease supplies from \$3,000 to \$1,994.
4. Decrease travel meals and lodging from \$500 to \$373.

Activity 2 budgetary changes: We are requesting permission for the following changes to reflect exact amounts spent in each category.

1. Decrease personnel from \$153,100 to \$152,937.
2. Decrease services (equipment repair) from \$2,000 to \$1,917.
3. Increase supplies from \$4,000 to \$4,068.
4. Decrease travel vehicle/mileage from \$900 to \$650.
5. Increase travel meals and lodging from \$2,000 to \$2,429.

Activity 3 budgetary changes: We are requesting permission for the following changes to reflect exact amounts spent in each category.

1. Increase personnel from \$173,300 to \$175,685
2. Decrease supplies from \$2,000 to \$1,574.
3. Decrease travel vehicle/mileage from \$9,100 to \$7,858.
4. Increase travel meals and lodging from \$3,000 to \$2,282.

Amendment Approved: 10/07/14

Final Report Summary:

Overall Project Outcome and Results

We made great progress with the biological control for emerald ash borer (EAB) in Phase 1 of this project. We simultaneously released wasps that parasitize EAB while we studied them. EAB can kill ash trees quickly (within 6 years). We have responded rapidly to EAB finds so that we might avoid large numbers of EAB over extensive areas, a situation that would be difficult to manage effectively. At the same time, we studied the parasitoid wasps to understand their cold tolerance and dispersal capability. Our studies improved our implementation strategies.

Over 127,000 parasitoid wasps were released at 21 sites in the Twin Cities and southeastern Minnesota. Recovery of immature parasitoids in the field demonstrated that these agents are dispersing then finding and parasitizing EAB. We will continue releases in Phase 2. Research efforts demonstrated that the egg parasitoid, *Oobius agrili*, is the most cold tolerant and the larval parasitoid, *Tetrastichus planipennisi*, is the least cold tolerant. Therefore, we began releasing *T. planipennisi* earlier in the season to allow multiple generations to build a population sufficient to withstand anticipated cold induced mortality losses. We learned that *T. planipennisi* is capable of dispersing almost 5 miles within 24 hours but that most will fly $\frac{3}{4}$ miles in 24 hours. Therefore, we began releasing *T. planipennisi* over a large area at a release site rather than at a central cluster to enable faster *T. planipennisi* dispersal. Research efforts trained a total of six graduate students, five undergraduate students, and three technicians in whole or in part on these projects.

We will continue a study of ash health, EAB, and parasitoid wasps in the Twin Cities area where EAB was first found in 2009. To date, ash mortality within the study area has been substantially lower than anticipated.

Project Results Use and Dissemination

Information about this project has been and will continue to be disseminated to the public, land managers and researchers. Media releases (3) and social media were utilized to inform the public of major developments. There were 15 scientific presentations to researchers and land managers. Additional training presentations (24) were given to the public, professional land managers, and tree care professionals at many venues. Outreach at public events (20) helped us to connect with people about our activities. Two research papers on parasitoid cold tolerance were published. An additional two papers on parasitoid dispersal are anticipated. In addition, we participate in the EAB Forum, a multi-agency/organization venue for discussing EAB management. We maintain a website www.mda.state.mn.us/plants/pestmanagement/eab/eabbiocontrol.aspx with project information.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Assess biological control agent winter survival potential

Description: Cold hardiness of *S. agrili* and *T. planipennisi* will be assessed using established laboratory methods to measure the insect supercooling point, lower lethal temperature, and lower lethal times and field studies to measure actual agent survival. Temperatures experienced by the bioagents will be measured with thermocouples beneath the bark on various parts of the tree. This research will be completed by a graduate student, Anthony Hanson, and one undergraduate student advised by Dr. Robert Venette with the Forest Service and the University of Minnesota. This study complements Dr. Venette’s research on EAB larval cold weather survival potential.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 150,600
Amount Spent: \$ 156,600
Balance: \$ 0

Activity Completion Date: 06/30/2014

Outcome	Completion Date	Budget
1. Measure bioagent cold hardiness for two species	06/30/2014	\$108,100
2. Develop predictive model and map of expected bioagent survivorship	06/30/2014	\$42,500

Activity Status as of May 31, 2012:

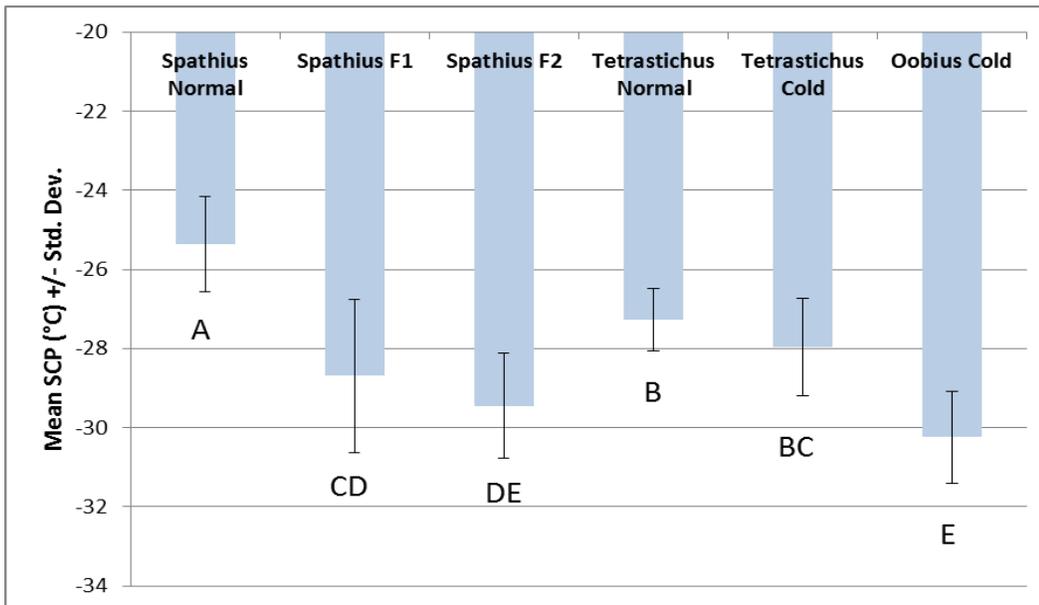
Parasitoid cold tolerance testing is progressing well. To determine cold tolerance, the following tests are almost completed.

1. Supercooling Point = the temperature at which an insect freezes
2. Lower Lethal Temperature = the lowest temperature that the insect can survive
3. Lower Lethal Time = the duration that an insect can survive at a specific temperature

For each species, comparisons are made between parasitoid larvae that were acclimated to cold before testing vs. parasitoid larvae that were not acclimated. Additional tests were performed on *S. agrili* to determine whether cold tolerance varies as individuals are put into diapause. A combination of these factors will used to determine cold tolerance.

The methodology is working well and the tests are approximately 75% completed. The developmental biology of the biological control agents slows progress. For example, it takes 16 weeks after cold exposure to break diapause. Data will be analyzed after test completion.

Figure 1. Preliminary average supercooling points (\pm standard deviation) of emerald ash borer parasitoids that were acclimated (cold) or not (normal) to cold temperatures before testing. Bars with the same letter are not significantly different (Tukey’s HSD at $\alpha=0.05$).



The supercooling point of three parasitoid species (*Spathius agrili*, *Tetrastichus planipennisi*, and *Oobius agrili*) is one factor that will be used to determine cold tolerance.

We are in the process of analyzing temperature records from the winter of 2011-2012 beneath the bark of cut logs and trees.

Budget note: Salary funds for the graduate and undergraduate students working on Activity 1 were covered to date from another funding source. Use of ENRTF funds for their salaries will begin in the fall of 2012.

Activity Status as of November 30, 2012:

Parasitoid cold tolerance testing is completed for both larval parasitoid species and lifecycle stages with the exception of *S. agrili* emergence from their 16 week diapause (see Lower Lethal Temperature). Results of these studies indicate *S. agrili* may be more cold tolerant than *T. planipennisi*.

Supercooling Point: Acclimation to shorter days and cooler temperatures decreases the likelihood that *S. agrili* will freeze at very cold temperatures (50% freezing at -29°C and 100% freezing at -34°C for the sampled individuals). In contrast, *T. planipennisi* did not show a difference after exposure to shorter days and cooler temperatures (50% freezing at -28°C and 100% freezing at -29°C for the sampled individuals).

Lower Lethal Temperature: Initial determinations of non-survival after exposure to cold temperatures were made by visually inspecting the larvae or pre-pupae (overwintering stage for each species) for discoloration or lack of movement. Final determinations of survival will also include adult emergence after exposure to warm temperatures for a sufficient period. The measure of adult emergence for *S. agrili* is ongoing until December, 2012. For *S. agrili*, survival of pre-pupae was increased by cold acclimation (50% mortality at -27.5°C and >99% mortality at -32.5°C). The close correlation of supercooling point and lower lethal temperature for *S. agrili* indicates that unlike some insect species, *S. agrili* is not freeze tolerant. It was difficult to cold acclimate *T. planipennisi* larvae as evidenced by high control mortality. For this reason, cold acclimated larvae were not used for these tests, but it is possible that cold acclimation could increase cold tolerance. The adult emergence measure was completed for this test and incorporated into results (50% mortality at -24.5°C and >99% mortality at -27°C). High mortality levels at temperatures above the supercooling (freeze) point indicate that *T. planipennisi* is chill-intolerant.

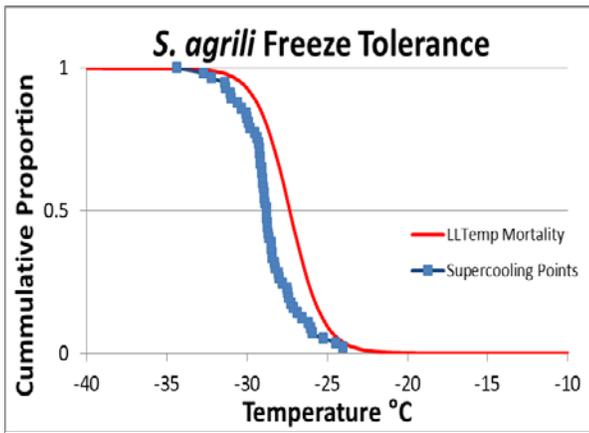


Figure 2. *S. agrili* lower lethal temperature 3 days after cold exposure and supercooling point distribution of pre-pupae reared under 1 generation diapause conditions.

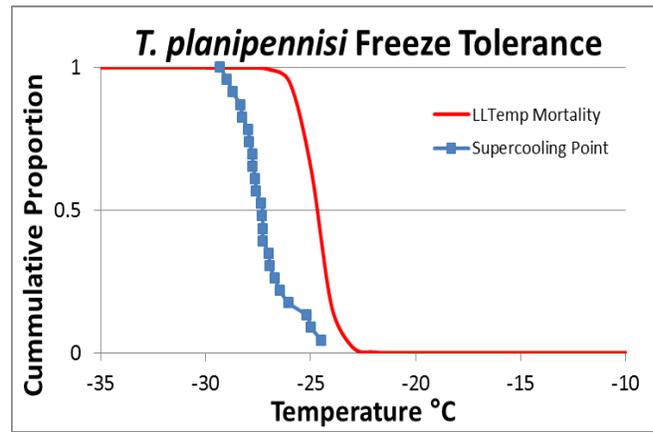


Figure 3. Lower lethal temperature based on adult emergence and supercooling point distribution of *T. planipennisi* reared under warm/long conditions.

Lower Lethal Time: Larvae and pre-pupae (overwintering stage for each species) were held at 0, -5, -10, or -15°C for 3, 14, 28, 56, or 84 days.

Table 1: Mortality induced by prolonged exposure to cold temperatures as measured by the number of days at specific temperatures.

Temperature (°C)	<i>Spathius agrili</i>		<i>Tetrastichus planipennisi</i>	
	25% Mortality	50% Mortality	25% Mortality	50% Mortality
0	83 days	>84 days	79 days	>84 days
-5	83 days	>84 days	76 days	>84 days
-10	83 days	>84 days	41 days	61 days
-15	61 days	84 days	23 days	32 days

The next step is to analyze these results in the context of long term climate data and recorded temperatures beneath bark.

Activity Status as of May 31, 2013:

Both species of larval parasitoids appear to be sufficiently cold tolerant for southern Minnesota but may be less suitable to northern Minnesota. In all parts of the state there is likely to be mortality during cold winters.

The impact of winter climate on population dynamics is related to population growth rate. Although *S. agrili* appears to be more cold tolerant than *T. planipennisi*, *S. agrili* has only 1-2 generations per year and produces 1-18 progeny per EAB larva. The population growth rate is slow. In contrast, *T. planipennisi* has 3-4 generations per year and produces 4-172 progeny per EAB larva. The higher population growth rate of *T. planipennisi* may allow it to withstand winter mortality losses better than *S. agrili*.

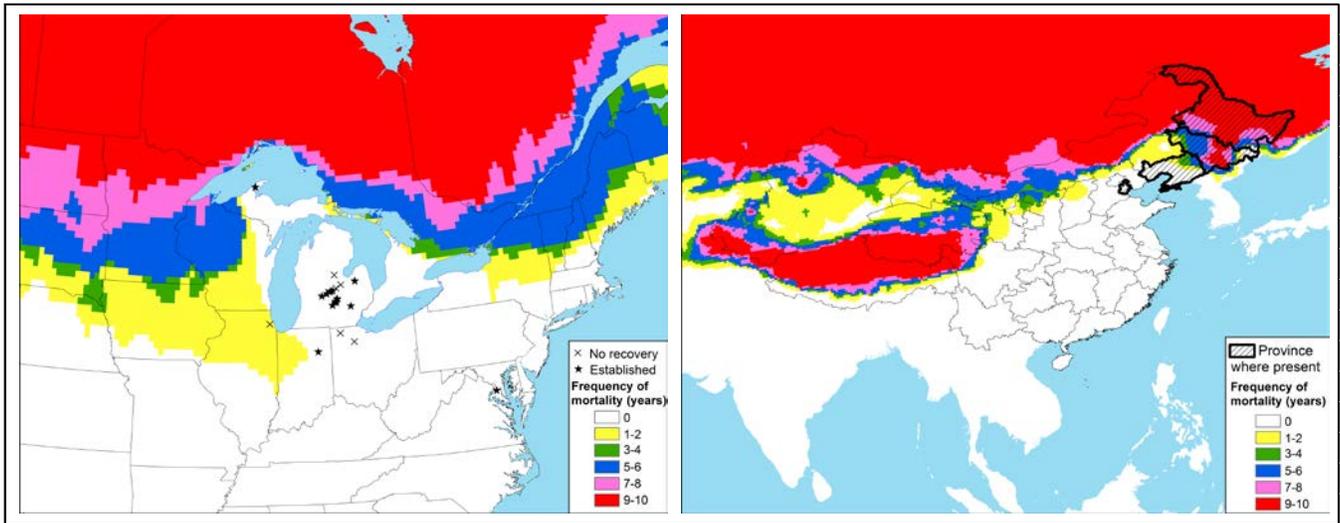


Figure 4. Frequency of North American (left) and Chinese (right) winters from 2002-2012 cold enough to cause 90% mortality of overwintering *Tetrastichus planipennisi* larvae. The native range of *T. planipennisi* in China is marked in hatch.

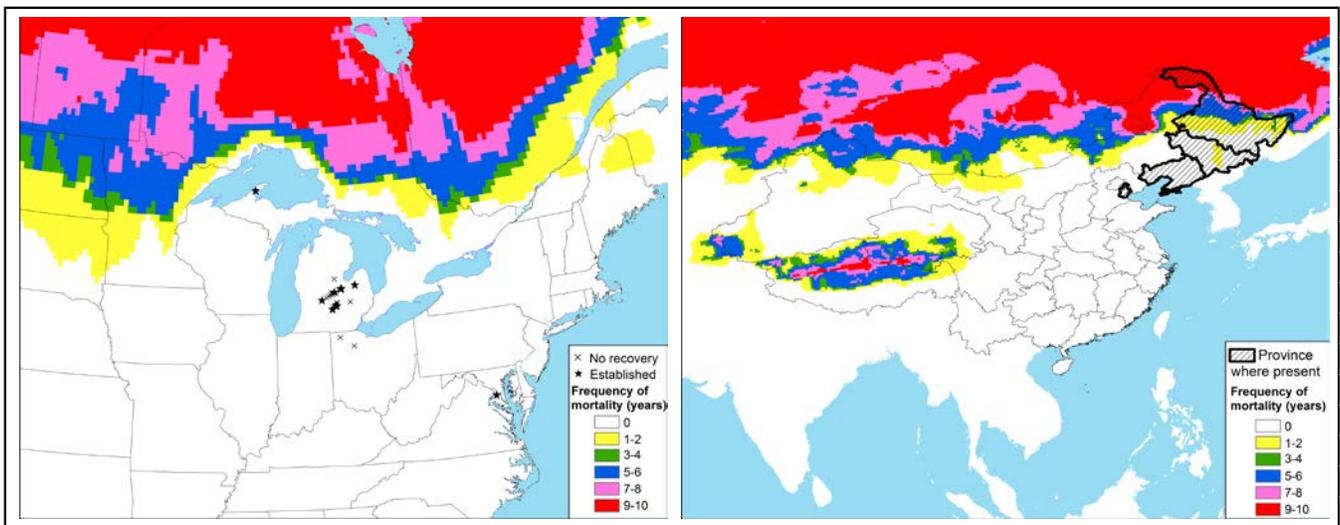


Figure 5. Frequency of North American (left) and Chinese (right) winters from 2002-2012 cold enough to cause 90% mortality of overwintering *Spathius agrili* pre-pupae. The native range of *S. agrili* in China is marked in hatch.

An important management recommendation is to prioritize early season releases of biological control agents to allow as many generations as possible to reproduce and the population to grow. Winter mortality of the overwintering generation will not have as large of an impact on the overall population.

Upcoming activities include studying cold tolerance of the egg parasitoid, *Oobius agrili*, and enclosing parasitoids on EAB infested branches to research their overwintering in the field.

Activity Status as of November 29, 2013:

Unfortunately, there was not a sufficient population of the egg parasitoid, *Oobius agrili*, available for cold tolerance research. This research is above and beyond our work plan commitments, but we had hoped to complete this work and may be able to at a future date.

The larval parasitoid, *Tetrastichus planipennisi*, was enclosed with EAB larvae in mesh covered cages on ash trees at Great River Bluffs State Park to study overwintering. Twenty-four cages were set up. Samples were collected from 12 cages this fall to determine the condition of the parasitoids going into winter. Data will be collected from these samples over the winter. Samples will be collected from the remaining 12 cages in spring 2014 to assess parasitoid condition after winter.

Final Report Summary:

Major conclusions

- Of the three parasitoid species that have been approved to control emerald ash borer, the egg parasitoid *Oobius agrili* appears to be the most cold-tolerant, while the larval parasitoid *Tetrastichus planipennis* is the least cold-tolerant. *Oobius agrili* had lower supercooling points than any other species tested (i.e., colder temperatures were required before this insect began to freeze). *Tetrastichus planipennis* did not appear to acclimate to colder temperatures or shortened day-lengths. The third parasitoid species, *Spathius agrili*, did acclimate to cold temperatures as it went into diapause in response to shortened day-lengths and cooler temperatures. Both *T. planipennis* and *S. agrili* were classified as chill intolerant (i.e., individuals may succumb to cold before they freeze). These results appear in the publication: Hanson, A.A., R.C. Venette, and J.P. Lelito. 2013. Cold tolerance of Chinese emerald ash borer parasitoids: *Spathius agrili* Yang (Hymenoptera: Braconidae), *Tetrastichus planipennis* Yang (Hymenoptera: Eulophidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae). *Biological Control* 67: 516-529.
- New tools were needed to measure the temperatures of tiny insects during cold tolerance testing. A new thermocouple design was developed and tested during the course of this project. The new thermocouple design, known as a 'cradle', proved to be more sensitive than traditional thermocouples. Thus, we are more confident in our assessments of the cold tolerance of emerald-ash-borer parasitoids, particularly measurements of supercooling points, than we otherwise would have been. The design and testing of the new thermocouple are described in the publication: Hanson, A.A., and R.C. Venette. 2013. Thermocouple design for measuring temperatures of small insects. *Cryoletters* 34: 261-267.

Management implications

- Our results suggest that *O. agrili* is least likely to freeze at temperatures that are common during Minnesota winters. This species might be best suited to overwinter in Minnesota and might be preferred for future releases (but see 'Future Research').
- *Tetrastichus planipennis* is vulnerable to winter cold. As such, for this species to survive the winter, releases should be conducted in spring. This strategy will allow populations of the parasitoid to build to large numbers. If large populations are present in the fall, the probability that at least some individuals will survive the winter improves.
- Although *S. agrili* is more cold-tolerant than *T. planipennis*, the Animal and Plant Health Inspection Service of the US Department of Agriculture is no longer rearing *S. agrili* for release at northern latitudes.

Future research

- At the start of this project, individual *O. agrili* were scarce, primarily because laboratory rearing techniques were still rudimentary. As a result, we were only able to focus on one dimension of insect cold hardiness. During the course of this project, rearing of *O. agrili* has improved. We would like to evaluate the lower lethal time of these insects (i.e., the effects of brief exposure to sub-freezing temperatures on survival) and lower lethal temperature (i.e., the extent of mortality at different periods of time when held at constant low temperatures). These two measures would substantially improve our ability to forecast the winter survival of this insect and would provide stronger support for the conclusion that this species is, indeed, the most cold tolerant.
- An initial attempt to measure winter survival of *T. planipennis* in the field failed. The main stems of 24 green ash trees in Great River Bluffs State Park were artificially infested with emerald-ash-borer eggs. Cages were placed on the trees and *T. planipennis* were released into the cages. That fall, half of the trees were harvested to confirm establishment of the parasitoids. The plan was to harvest the remaining trees in the spring and to evaluate the extent of parasitoid winter mortality. However, we recovered no parasitoids from the fall samples. Insect predators (especially ants and earwigs) were common on the trees and may have impacted the eggs that were put into trees or the parasitoids that were put in the cages. A recent report also suggests that *T. planipennis* is unable to parasitize larvae in trees that are greater than 3 inches in diameter because the bark becomes too thick. Our pilot taught us how to refine the study for future trials. We would like to repeat this study, but on smaller-diameter

branches or younger trees, with barriers to exclude the insect predators. Ultimately, we would like to elucidate the true overwintering capacity of *T. planipennisi* and subsequent emergence patterns of this insect in the subsequent spring.

- USDA-APHIS continues foreign explorations and testing of new parasitoid species to control emerald ash borer. Species currently being tested, such as *Spathius galinae* from Russia or an unidentified *Oobius* species from South Korea, may be more cold tolerant than the three species from China. It would be useful to evaluate the cold tolerance of these new species. Our laboratory is the only facility to have provided pre-release cold tolerance testing of emerald ash borer parasitoids.

ACTIVITY 2: Examining parasitoid establishment and dispersal

Note: Activity 2 was revised from the submitted proposal based upon peer review comments to the research addendum.

Description: The goal is to determine movement and potential establishment of parasitoids of EAB from the release site. Biological control agent traps will be placed at incremental distances of up to 1km from the sites where biological control agents have been or are being released (e.g., 0, 100, 200, 500, 1000m in four cardinal directions, or at points along an arc). These traps will serve to monitor EAB parasitoids emerging from the release site over time. This research will be conducted by a graduate student and one undergraduate student advised by Dr. Brian Aukema at the University of Minnesota. This study complements Dr. Aukema’s existing work on landscape ecology, movement patterns, and spatiotemporal modeling of insects undergoing range expansion events.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 162,000
Amount Spent: \$ 162,000
Balance: \$ 0

Activity Completion Date: 06/30/2014

Outcome	Completion Date	Budget
1. Identify monitoring points within/around release sites	10/01/2013	\$ 63,800
2. Determine dispersal gradient of parasitoids	06/30/2014	\$ 98,200

Activity Status as of May 31, 2012:

A graduate student, Samuel Fahrner, has been recruited to work on this project. In June 2011 after consultation with Drs. Julie Gould and Leah Bauer (USDA Forest Service), an array of yellow pan traps at increasing distances around two local release sites were deployed to detect dispersal of biological control agents. It is thought that yellow pan traps can be more efficient at sampling larval parasitoids than sentinel logs. A month of monitoring yielded negative results. This was disappointing but not unexpected. Biological control agents frequently require months to establish and reproduce, and we know little about their density-dependent dispersal. Over the winter we developed laboratory studies to understand reproduction of different densities of biological control agents on different densities of immature EAB. These results, integrated with better understandings of dispersal capacities and lower lethal temperature limits, will ultimately inform site selection and release strategies.

To further address dispersal of biological control agents, project partners initiated a field study of ash health, EAB, and EAB biological control agents in the Twin Cities. This is a large, collaborative project with multiple partners including USDA Forest Service, the Department of Natural Resources, and the cities of Falcon Heights, Minneapolis, Lauderdale, Roseville, and St. Paul. Three hundred ash trees were selected in the late summer of 2011. Each tree will be monitored for three years. We collect data on tree size and health. We are using branch sampling methodology to subsample ash trees for evidence of EAB and EAB biological control agents. Two branches per year are removed from each tree for three years. A length is cut from each branch then peeled. Detailed information is collected on each EAB gallery, EAB larvae, biological control agent parasitoids, and native parasitoids. Data collection for this study is coordinated by Jonathan Osthus with MDA (Activity 3), and will be analyzed

by Drs. Aukema (Activity 2) and Venette (Activity 1). The first year of branch sampling was completed in mid-April. We learned that this method, though labor-intensive, can detect EAB in asymptomatic trees and improve data resolution in areas where EAB are known to occur. We did not find any surprise infestations outside of the known infested area to date.

Activity Status as of November 30, 2012:

Parasitoid flight capability will inform decisions about where to release and possibly recover parasitoids and increase the accuracy of estimates of population spread in the field. We are studying the dispersal potential of *Tetrastichus planipennisi* using 24 custom, computer-monitored flight mills. This sophisticated system collects robust data on a large number of individuals. We learned that *T. planipennisi* can fly long distances – up to 7 km (4.3 miles) within a 24 hour period with a mean flight speed of 0.8 km per hour. *Tetrastichus planipennisi* has the potential to move very far over its adult lifespan (mean adult lifespan of 45 days in the lab) and multiple generations per year. We will continue flight studies to determine the effects of age, feeding, mating status, sex, size, and temperature on the flight patterns. We plan to correlate these data with field release and recovery data.

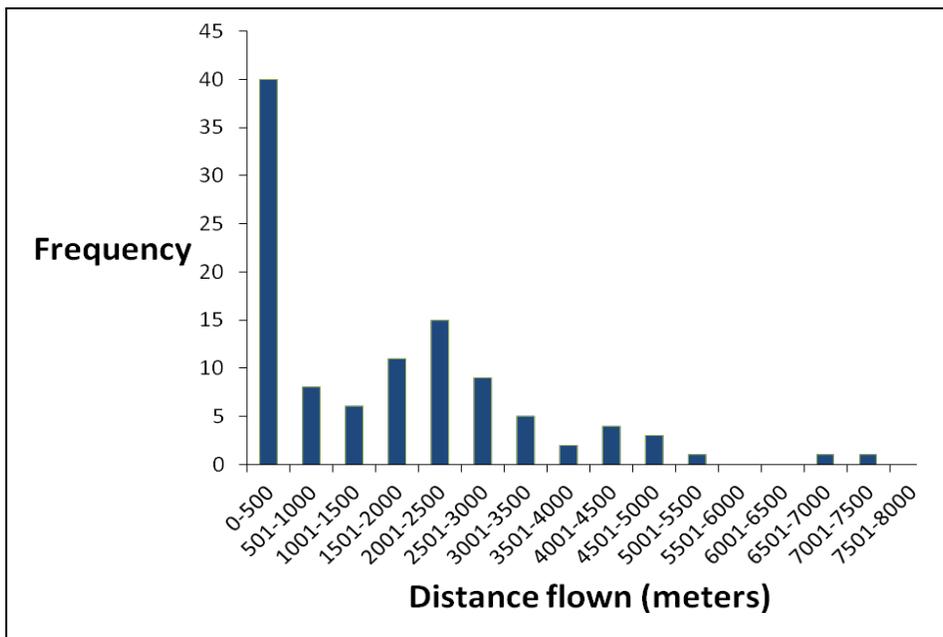


Figure 1. Measured distance frequency that *Tetrastichus planipennisi* flew in a 24 hour period on a flight mill.

Unlike the other EAB biocontrol parasitoid species, *T. planipennisi* is well-suited to flight mill studies. Although a strong flier in the field, *Spathius agrili* will not fly when attached to a flight mill. *Oobius agrili* is simply too small to attach to a flight mill.

In the previous status report we stated that we planned an EAB larval density dependence study on the flight pattern of larval parasitoids. We decided to study flight capability instead after learning that other researchers had studied parasitoid flight response to EAB larval density. We will use their findings in addition to our flight studies to better understand parasitoid dispersal.

Budget note: In lieu of an undergraduate student worker, a recent graduate with applicable experience was hired on this project. The total salary and fringe budget amounts are not changed.

Project partners continue to work jointly on the ash health, EAB, and EAB bioagent monitoring study. Ash health data were collected last summer. Approximately 1/4 of the branches have been cut, peeled and data collection for the second year of sampling. Of the 300 tree initially selected, 298 were sampled in the winter of 2011/2012 (2 trees were removed before sampling began). 260 trees will be sampled again in the winter of 2012/2013 (39 trees were removed since initial study tree selection). 20 infested trees were identified through branch sampling in 2011/2012.

Activity Status as of May 31, 2013:

The studies of flight behavior of *T. planipennis* were expanded to study the effects of several factors on dispersal capacity (e.g., sex, size, age, feeding status). Our goal is to understand what conditions will result in the best coverage upon release. On average, females flew farther than males. Further studies revealed this is likely because females are larger and larger *T. planipennis* fly farther. Age did not affect flight distance until the parasitoids were very old – they appear to be viable into their second month post hatching from their pupal cases. Mating status also did not affect flight distance. Feeding the parasitoids sugar enabled them to fly much farther than starved parasitoids. The starved parasitoids barely flew. This highlights the importance of having a nectar source for the adults to feed on. The parasitoids that we receive and release are fed honey before release. Our work confirms that this pre-release step is critical and suggests that the parasitoids may perform well in field settings where there are available nectar sources.

Future studies on the effect of temperature and humidity on flight will be conducted.

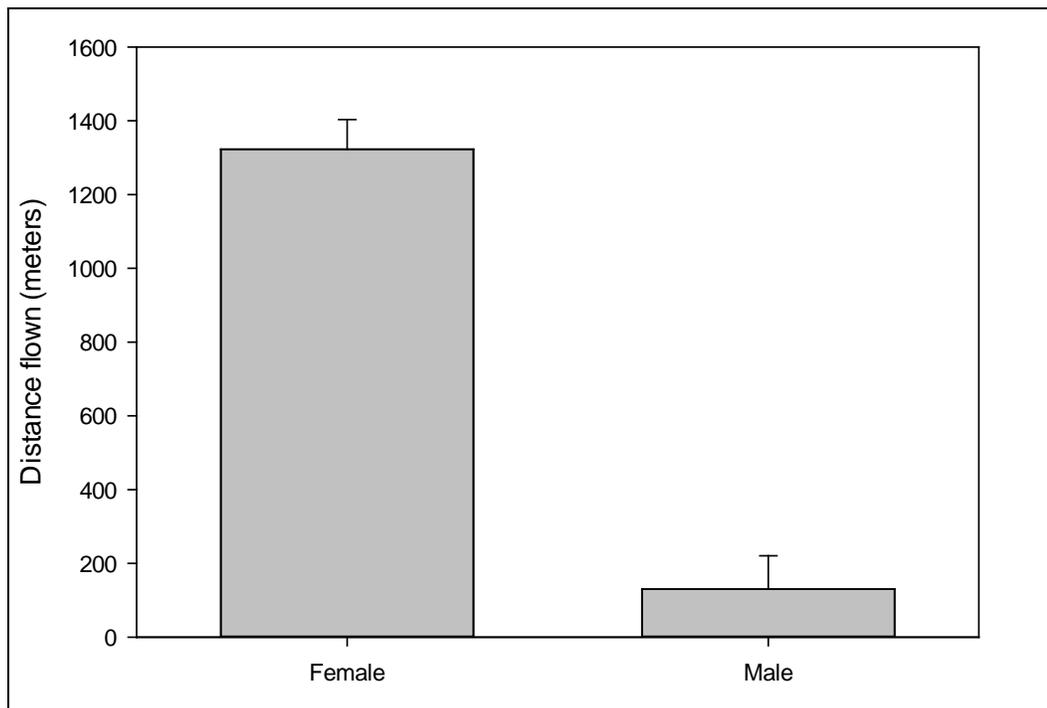


Figure 2. Distance flown by female and male parasitoids. Each parasitoid used in this study was flown once for a 24-hour period. Flight distances were measured using 24 custom-built computer-monitored flight mills. Parasitoids were fed a dilute honey solution and water prior to flight.

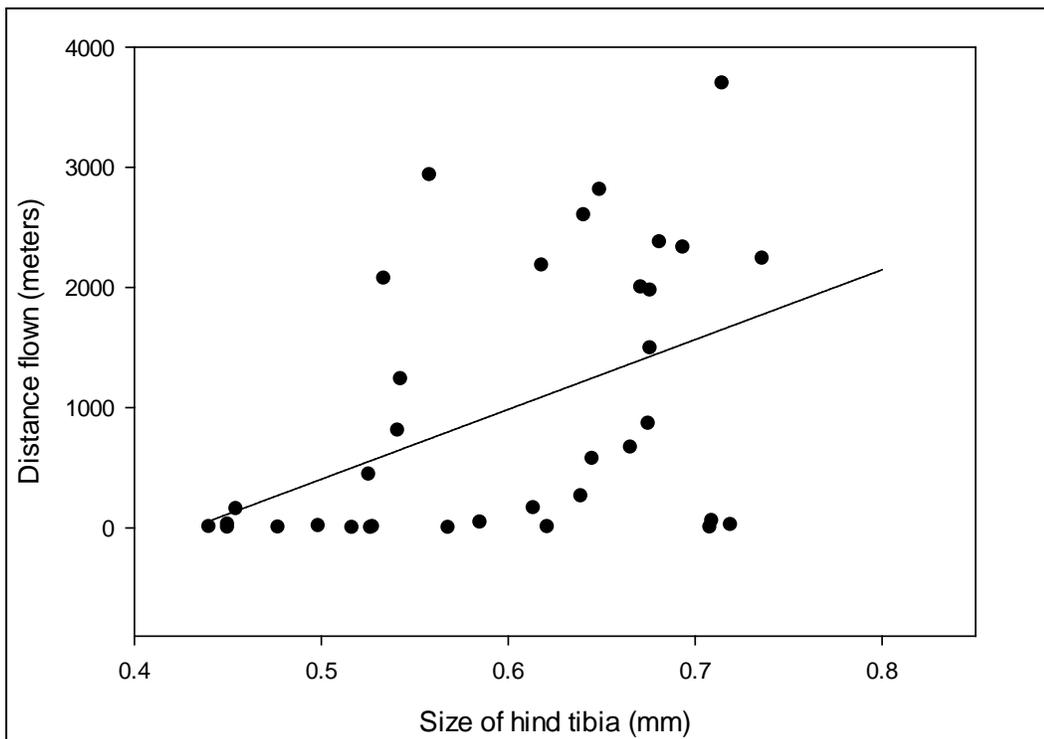
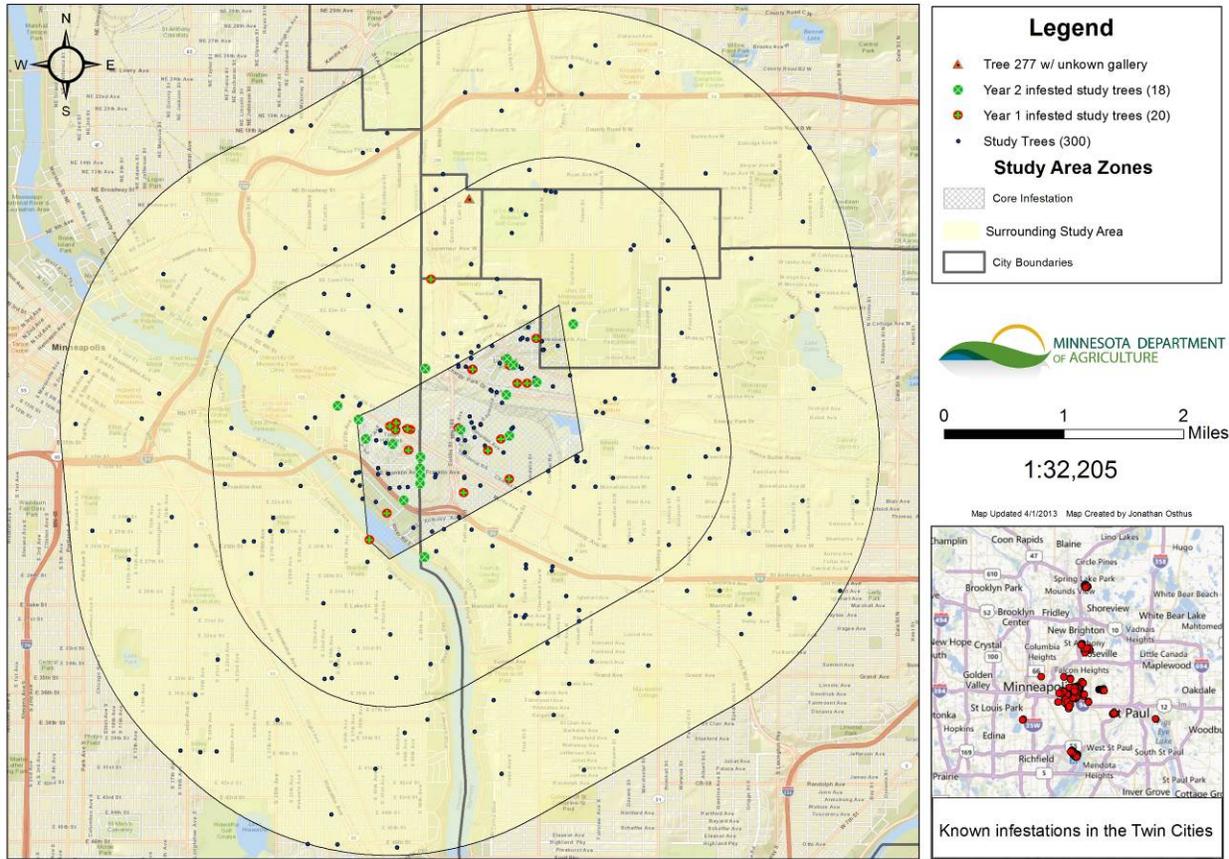


Figure 3. Parasitoid size (as measured by the size of a hind leg segment) as an indicator of flight distance potential. Each parasitoid used in this study was flown once for a 24-hour period. Flight distances were measured using 24 custom-built computer-monitored flight mills. Parasitoids were fed a dilute honey solution and water prior to flight. Parasitoid size was determined by measuring the mean of the hind tibias.

Project partners continue to work jointly on the ash health, EAB, and EAB bioagent monitoring study. Branch sampling has been completed for the year with overall positive results. 18 infested trees were identified or about 7% of the study trees sampled. Also, EAB gallery densities remained quite low in the majority of the branch samples. The results highlight the impact that management is having in the core infestation area where much higher rates of infestation were expected with how long EAB has been established.

Ash Health, EAB, and EAB Bioagent Monitoring Study



Activity Status as of November 29, 2013:

Studies of EAB and the larval parasitoid, *Tetrastichus planipennis*, flight continue and will be completed in early 2014. Mean flight speed increases with temperature for EAB. EAB appears to have higher mortality rates after flying at high speed. We will study mortality as it relates to temperature and flight speed.

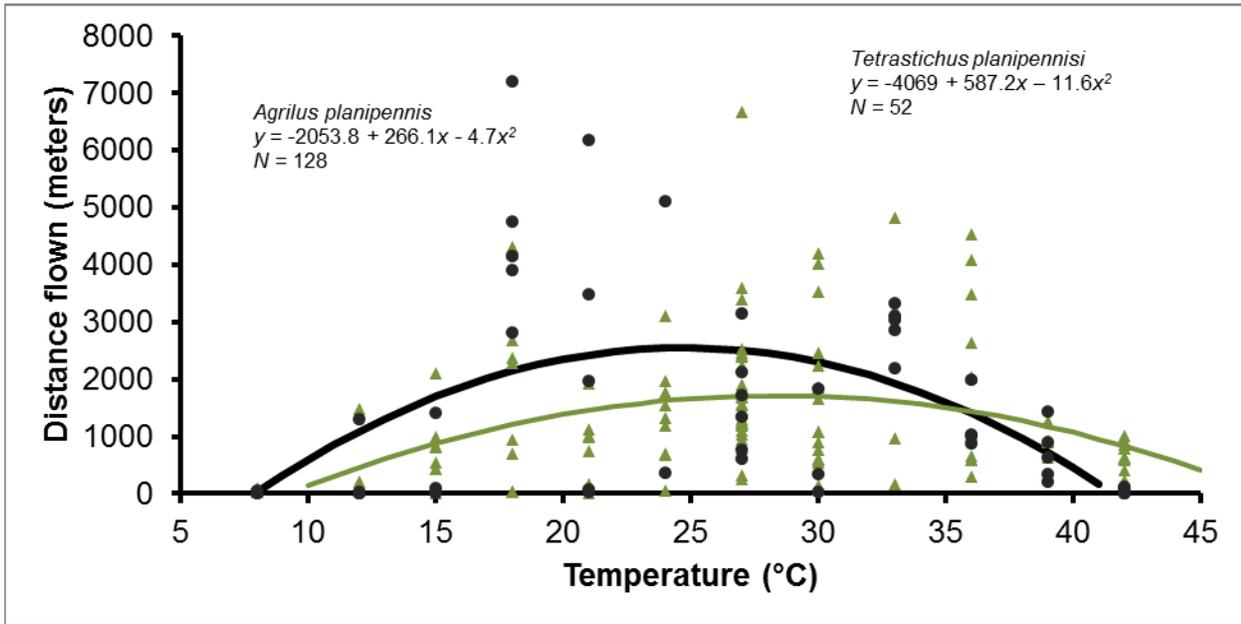


Figure 4. Dispersal capacity of emerald ash borer, *Agrilus planipennis*, and the larval parasitoid, *Tetrastichus planipennisi*. Insects were flown at increments of temperature between 8 and 42° C. All insects were tethered to computer-monitored flight mills and were flown continuously for one 24-hour period. Flight mills were placed inside of environmental chambers and insects were not provided water or nutrition during flight trials.

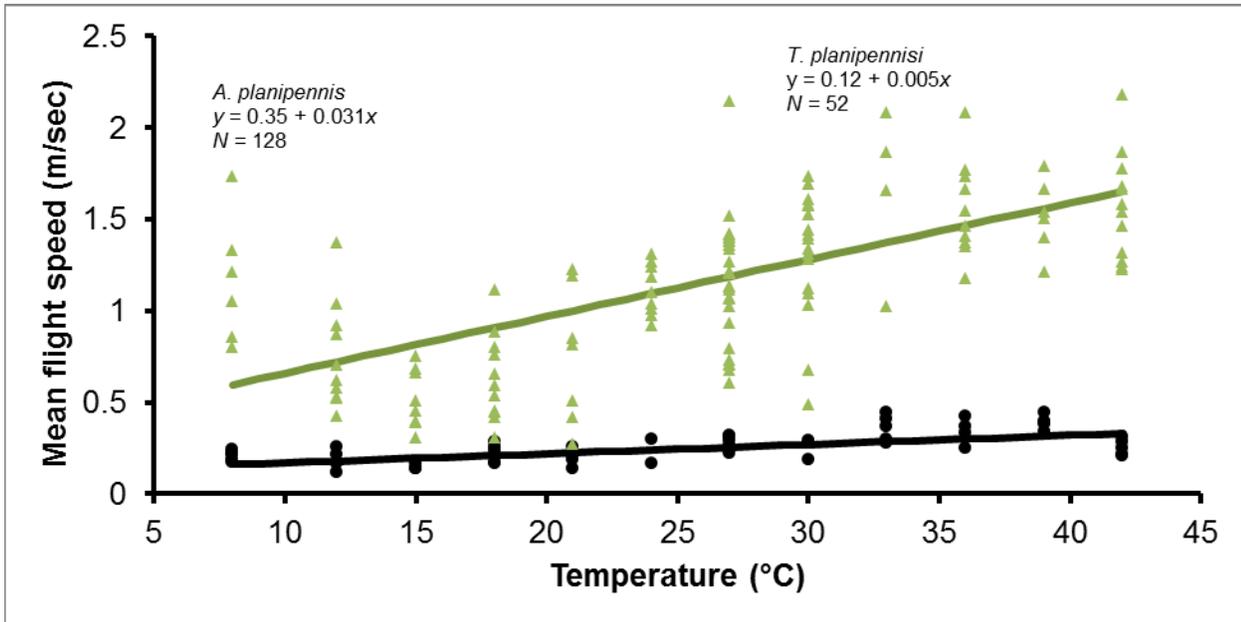


Figure 5. Flight speed of emerald ash borer, *Agrilus planipennis*, and the larval parasitoid *Tetrastichus planipennisi*. Insects were flown at increments of temperature between 8 and 42° C. All insects were tethered to computer-monitored flight mills and were flown continuously for one 24-hour period. Flight mills were placed inside of environmental chambers and insects were not provided water or nutrition during flight trials.

Ash health data were collected from all ash health, EAB and EAB bioagent monitoring study trees. Branch sampling has started and will continue over the winter.

Final Report Summary:

We have learned a great deal about dispersal capacity of *Tetrastichus planipennisi* (aka “Tets”) during the last three years of opportunity provided by ENRTF funds. Below we provide an overview of challenges in field studies with Tets and subsequent adjustments to study flight capacity across a range of environmental conditions on a state-of-the-art laboratory flight mill.

When we commenced this project, initial studies of dispersal of biological control agents on the urban and natural landscape provided little information. Despite several lines of traps and recapture attempts in as little as six hours post-release, we were not able to recapture any biological control agents using standard yellow pan traps. Indeed, to date in Minnesota, very few Tets have been recovered post-release in comparison to other states (see Activity 3). This is likely indicative of low pest densities to date in Minnesota. For example, the state detected EAB very early in the Twin Cities urban environment (2009), and densities of the pest have not yet built to tree-killing levels where biological control agents would typically cluster. We suspect there may be an advantage to our strategies of “carpet-bombing” these stingless wasps into low density infestations, from whence they spread (see Activity 3).

We have concentrated our efforts on laboratory studies over the past three years. We contributed additional funds from a McKnight Land-Grant faculty award to construct a laboratory flight mill. This award, distinguishing Prof. Aukema as a top early-career faculty within the University of Minnesota system, allowed us to study flight energetics of the Tets and EAB across a range of situations.

Research findings have included:

- Flight distances of female Tets representative of populations released in the biological control program average 3/4 miles in 24 hours. Half of them do not fly farther than 450 yards, however. The farthest a female will fly is almost 5 miles.
- Larger females fly farther than smaller ones.
- Females who feed on a honey-water solution prior to flight fly 40X farther than those who do not fly. Tets that do not receive honey-water do not fly very far at all. Clean, uncontaminated nectar sources in the field may be critical to success of these biological control agents.
- Females fly very well for 10 weeks after they hatch. We do not know if their eggs are viable for that long, but these data strongly suggest that Tets are viable biological control agents for two months after emerging.
- Both Tets and EAB fly fastest at warmer temperatures (95-100F). However, on the flight mill, they tended to die quickly at these warmer temperatures. This may change in field situations where they can stop and feed on dew or rainwater or nectar.
- Overall, Tets and EAB fly the farthest at temperatures between 80-85F. They can engage in flight all the way down to 55F, however.
- In Minnesota, our temperature data suggest that Tets should be able to fly through early to mid-October. This estimate is based upon temperature equations developed in the laboratory and the past 10 years of meteorological data from the MSP airport.

These studies formed the basis of a MS thesis for Samuel Fahrner, who received the 2014 Peterson Award for MS thesis research in the Department of Entomology at the University of Minnesota. Both thesis chapters have been or are being disseminated to reputable scientific peer-reviewed journals. Final copies of these papers will be submitted when available.

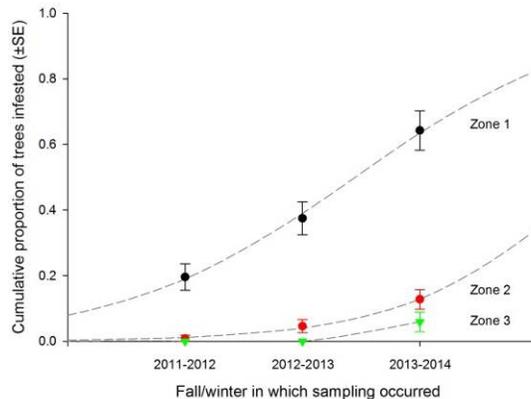
Fahrner, S.J., Lelito, J.P., Blaedow, K., Heimpel, G.E., and B.H. Aukema. (2015) Factors affecting the flight capacity of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a classical biological control agent of emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae). *Environmental Entomology*. Accepted pending minor revisions.

Fahrner, S.J., Lelito, J.P., and B.H. Aukema. (2015) The effect of temperature and humidity on the flight capacity of *Tetrastichus planipennis* (Hymenoptera: Eulophidae) and its host, *Agrilus planipennis* (Coleoptera: Buprestidae). To be submitted to *Biocontrol*.

Tracking the EAB infestation core

Major conclusions

- Mortality of ash in the study area has been substantially less than anticipated. Earlier research suggested that 100% of ash in Hennepin and Ramsey counties would be killed by emerald ash borer five years after the insect was first detected in 2009. Of the 300 trees in this study, none have been killed by emerald ash borer. A total of 68 trees were found to be infested, and of those 58 were removed or treated with insecticide. At the time of detection, often only one or two galleries were present in branch samples. This small number of galleries suggests that many infestations were found early.
- Approximately 64% of the ash trees in the core area (Zone 1) are infested, while only 13% are infested in Zone 2, and 6% are infested in Zone 3.
- The rate at which the proportion of infested trees is increasing is the same in the core area (Zone 1) and the adjacent land (Zone 2). Fewer trees were initially infested in Zone 2 than Zone 1. None of our study trees in Zone 3 were found to be infested until the 2013-2014 sampling was completed.
- No parasitoids were recovered from these trees. This result suggests that the parasitoids are either not established or population densities are still below detectable levels.



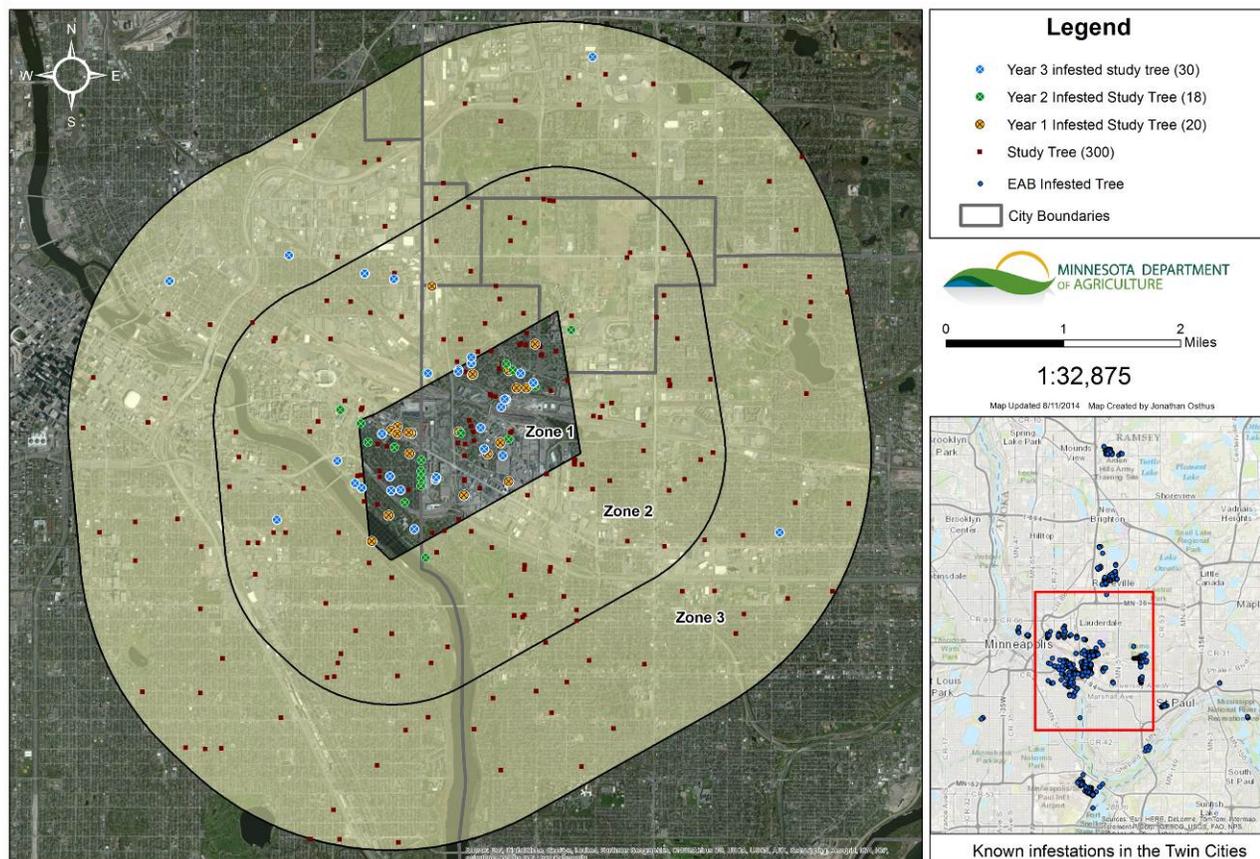
Management implications

- The similar rate of increase in Zone 1 and Zone 2 suggests that emerald-ash-borer management in the core area is not slowing the spread in adjacent areas. Cities may need to intensify emerald-ash-borer management.
- Releases of parasitoids in and around the Twin Cities continue to be justified.

Future research

- Observations of these 300 trees will continue under Phase II of the biological control implementation project. New trees will be added to replace those that were treated or removed to maintain the sensitivity of the monitoring network.
- This study provides one of the most rigorous in-field assessments of the efficacy of emerald-ash-borer management techniques in the nation. Our approach provides a reliable estimate of the proportion of ash trees that are infested in each zone. We are currently using volunteers to estimate the number of publically-owned ash trees in each zone. With that information, we can estimate the number of publically-owned ash trees that are likely to be infested in each zone. We can then compare this number with records of tree removals or insecticide treatments to determine the proportion of infested trees that were managed. These values provide a basis to intensify management activities as needed.

Tracking the EAB Infestation Core (300 Tree Study)



ACTIVITY 3: Coordinate Minnesota’s biological control implementation

Description: Strategic implementation of EAB biocontrol will require coordination, communication, and facilitation with other agencies, private landowners, and the general public. Potential release sites will be assessed and information related to field releases will be tracked. A new position will be created within the Plant Protection Division at MDA to coordinate implementation.

Summary Budget Information for Activity 3:

ENRTF Budget:	\$ 187,400
Amount Spent:	\$ 187,400
Balance:	\$ 0

Activity Completion Date: 06/30/2014

Outcome	Completion Date	Budget
1. Webpage developed for outreach	12/30/2011	\$ 500
2. Phase one implementation strategy for Minnesota developed	06/30/2012	\$ 50,000
3. Potential release sites delimited and assessed	04/30/2014	\$ 77,800
4. Field data collected and entered into database	06/30/2014	\$ 59,100

Activity Status as of May 31, 2012:

EAB biological control implementation scaled up in response to new EAB finds in 2011 and the hiring of Jonathan Osthus as the EAB Biocontrol Coordinator at MDA. Jon has years of experience working with EAB and was up to speed quickly. There were new EAB finds in Shoreview, at the Summit and Dale area of St. Paul, at several sites in Winona County, and a single trap catch in La Crescent. Biological

control agents were released at all of these sites in addition to existing sites in Houston County and the Twin Cities. The one exception is La Crescent. We received permission from the city to release but we have not found the EAB infestation pocket yet. This will determine where we release.

Bioagent releases were conducted throughout the 2011 field season for a total of 30,717 released.

Site Name	Biological Control Agent Species			
	Spathius agrili	Tetrastichus planipennis	Oobius agrili	All Species
E. River Pkwy 1	269	973	260	1,502
E. River Pkwy 2	745	2,531	660	3,936
Great River Bluffs State Park 1	521	1,103	0	1,624
Great River Bluffs State Park 2	899	2,663	0	3,562
Houston Release 1	138	842	200	1,180
Lamoille	234	282	0	516
Langford Park	809	2,398	394	3,601
Shoreview 1	888	1,536	338	2,762
Summit & Dale	736	1,983	0	2,719
Tower Hill Park	1,050	2,970	774	4,794
W. River Pkwy 1	1,307	2,199	1,015	4,521
Totals	7,596	19,480	3,641	30,717

Data were collected according to USDA APHIS guidelines and entered into a MDA database. Data were sent to the national database for EAB biocontrol. In addition, 10 release trees in Minneapolis were felled because they were confirmed infested. These trees were peeled, but no bioagents were recovered. This is in keeping with bioagent recoveries in other states at least two years after release. We are still at one year after release at our sites.

Releases have begun for the 2012 field season. We anticipate releasing bioagents at all sites and will consider new sites as additional EAB detections are reported.

EAB detection and defining the leading edges of infestations are critical to site selection for bioagent releases. Therefore much effort is put into coordinating data collection for the ash health, EAB, and EAB bioagent monitoring study (details in Activity 2 section). Cutting and peeling 600 branches was a lengthy and labor intensive task completed over the winter. Visual surveys of infested areas were completed to better understand the extent and severity of infestations. In addition, we started a pilot study called EAB STUC. Male EAB beetles fly over ash leaves searching for female beetles then land nearby to investigate. We exploit this behavior by setting traps of dead female beetle decoys covered with sticky spray to catch the live males – they get STUC (Stick Traps Using Cadavers). Results of this preliminary study will determine whether this method is employed on a larger scale.

This study will be conducted during EAB's peak flight season. We selected 125 trap trees in Falcon Heights, Lauderdale, Minneapolis and St. Paul. Five dead beetles are placed on different areas of the tree and covered with Tanglefoot®, a sticky substance. STUC is modeled on field research by Penn State University conducted in Michigan. This will be the first implementation test of this methodology. This is a collaborative project with the University of Minnesota, USDA, and cities of Minneapolis and St. Paul.

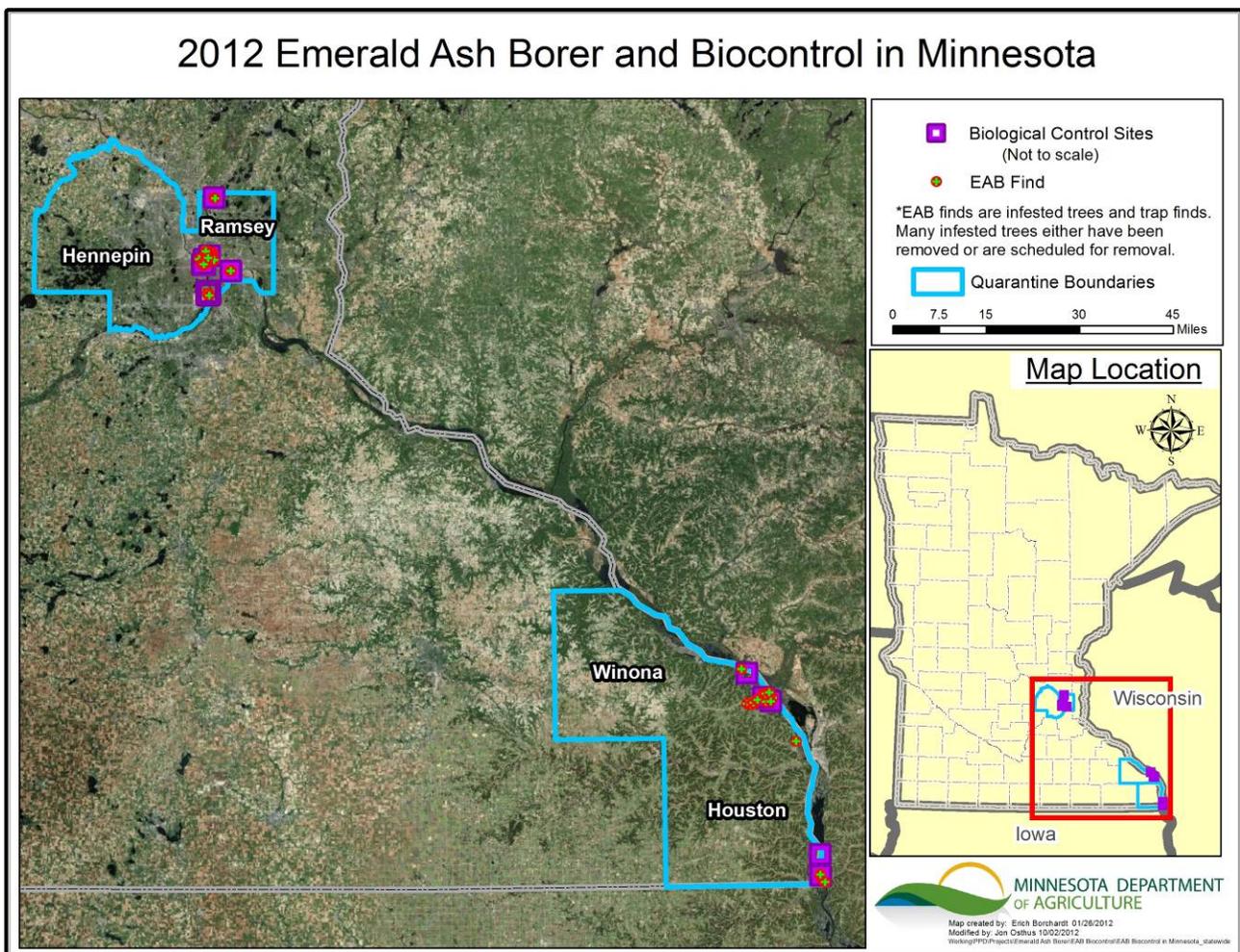
In March 2012, we hired an undergraduate student worker to help with all of the branch and log peeling and the EAB STUC pilot study. He worked part-time during the school year and started full-time in mid-May.

Activity Status as of November 30, 2012:

Biological control agent releases continued at all established sites and were initiated at the Fort Snelling golf course in response to a new EAB find.

Release Site	2010 Total Released	2011 Total Released	2012 Total Released	All Years Site Total
E. River Pkwy 1	0	1,502	2,532	4,034
E. River Pkwy 2	0	3,936	6,788	10,724
W. River Pkwy 1	0	4,521	5,861	10,382
Tower Hill Park	0	4,670	4,176	8,846
Summit & Dale	0	2,719	4,223	6,942
Langford Park	0	3,601	5,016	8,617
Shoreview 1	0	2,762	3,429	6,191
Fort Snelling	0	0	2,557	2,557
Houston Release 1	3,326	1,180	2,948	7,454
Lamoille	0	516	441	957
GRB SP 1	0	1,624	1,346	2,970
GRB SP 2	0	3,686	3,389	7,075
GRB SP 3	0	0	2,615	2,615
All Sites Total	3,326	30,717	45,321	79,364

GRB SP = Great River Bluffs State Park



Data were collected for biological control agent releases. Monitoring data collection will continue throughout the winter with intensive branch and tree sampling.

We completed our test of EAB STUC (Sticky Traps Using Cadavers) and determined that it was not an effective method for detecting and delimiting EAB populations at low levels. Neither the STUC nor adjacent purple traps caught a single EAB beetle. Therefore, we will not employ this method unless there is a significant increase in the EAB population.

Activity Status as of May 31, 2013:

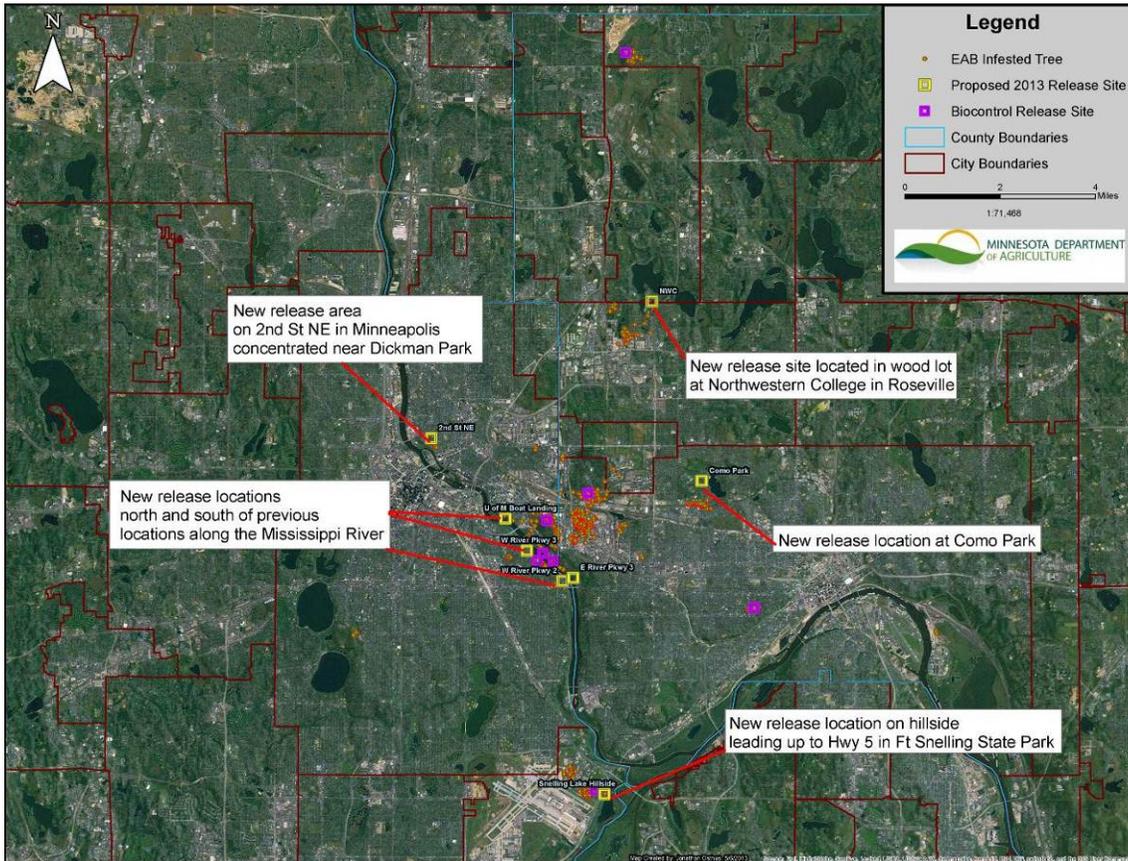
Sampling and Visual Survey: During the winter, 48 metro and 6 southern Minnesota trees from bioagent release areas were sub-sampled to gauge EAB density and look for EAB parasitoids. Visual survey was also used at all sites. EAB density was low in most trees with a few notable exceptions of medium to high density at Great River Bluffs State Park.

Logs from two sample trees (not release trees but near release trees) at Great River Bluffs State Park were brought back to MDA to be peeled carefully in the lab. Four parasitoid samples were recovered from EAB pupal chambers in one of the logs. Two different types of cocoons were observed and were sent to Dr. Juli Gould with USDA APHIS PPQ in Massachusetts and a parasitoid expert. One type appears to be a native parasitoid in the genus *Atanycolus*. *Atanycolus* has been documented attacking EAB in other states but has not been documented in Minnesota. The other type has not been found associated with EAB in other states. Multiple tiny wasps of an undetermined species emerged from one of these samples and a wasp in the family Crabronidae (species undetermined to date) emerged from a cocoon. These are solitary wasps that nest in wood cavities so are unlikely to have anything to do with EAB except use the galleries. No parasitoid samples of the species released have been recovered to date.

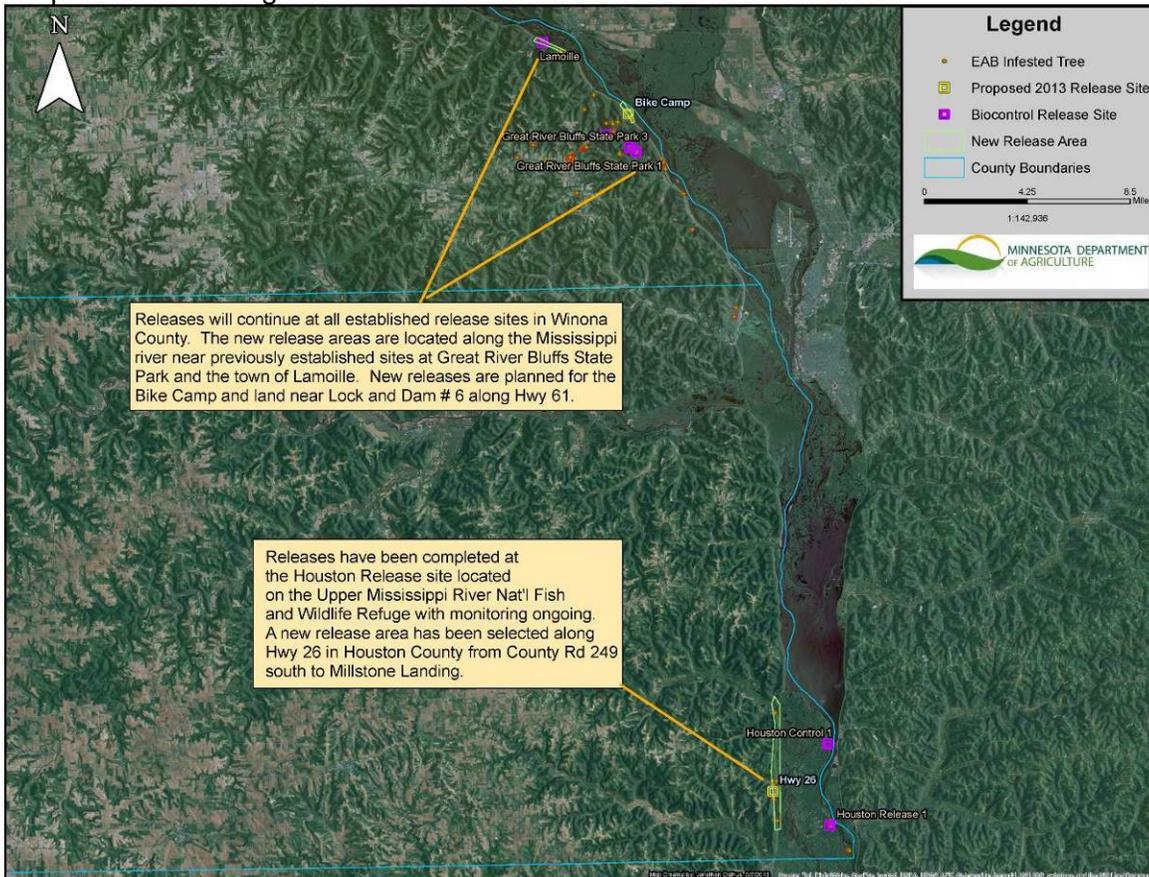
Planning: Meetings were held with individual project partners to discuss progress, sampling results and plan for the upcoming field season. This included planning for infestations that were detected over the winter. The EAB infestation density was greater at new finds in the metro than at known sites where the infestation had been detected at an earlier stage and managed.

- **New Sites:** Five metro and three southeastern Minnesota areas were selected to initiate releases in 2013. Bioagent availability for releases may be a limiting factor.
- **Continuation Sites:** Releases at three metro (Shoreview, Summit and Dale and Fort Snelling) and four southeastern (Lamoille and 3 sites at Great River Bluffs State Park) will continue in 2013.
- **Completed Release Sites:** Federal guidelines for releases are to release for two years, wait an additional year then monitor for parasitoid recovery. The E. River Pkwy 1 & 2, W. River Pkwy 1, Tower Hill Park, Langford Park and Houston 1 sites will not receive additional releases but will be monitored.

We are working with federal and state partners to increase releases at sites over broad areas rather than in tight clusters of ash trees.



Proposed new biological control release sites in the Twin Cities for 2013



Proposed new biological control release sites in southeastern Minnesota for 2013

Activity Status as of November 29, 2013:

Over 51,000 parasitoids were released at 14 sites over the field season. Releases were made on a weekly basis alternating between southeast Minnesota and the Twin Cities. Ash health data were collected from both new and existing release sites (20 sites total). Data were entered into state and federal databases.

Region	2013	2012	2011	2010	All Years
Twin Cities	16,703	34,582	23,711	0	74,996
SE MN	34,473	10,739	7,006	3,326	55,544
Total	51,176	45,321	30,717	3,326	130,540

We receive excellent support from USDA APHIS and MN DNR for our parasitoid release efforts. 40,000 (30% of statewide total) wasps were released at Great River Bluffs State Park from 2011-2013. This is one of the few sites selected nationwide for “carpet bombing” the site with high release numbers. The site was selected because of its location in the heart of a large EAB infestation in SE MN and its appeal to EAB (sunny and open with ash trees stressed from prescribed fire). The site is strategically located with EAB movement corridors of the Mississippi River to the east and Interstate 90 to the south.

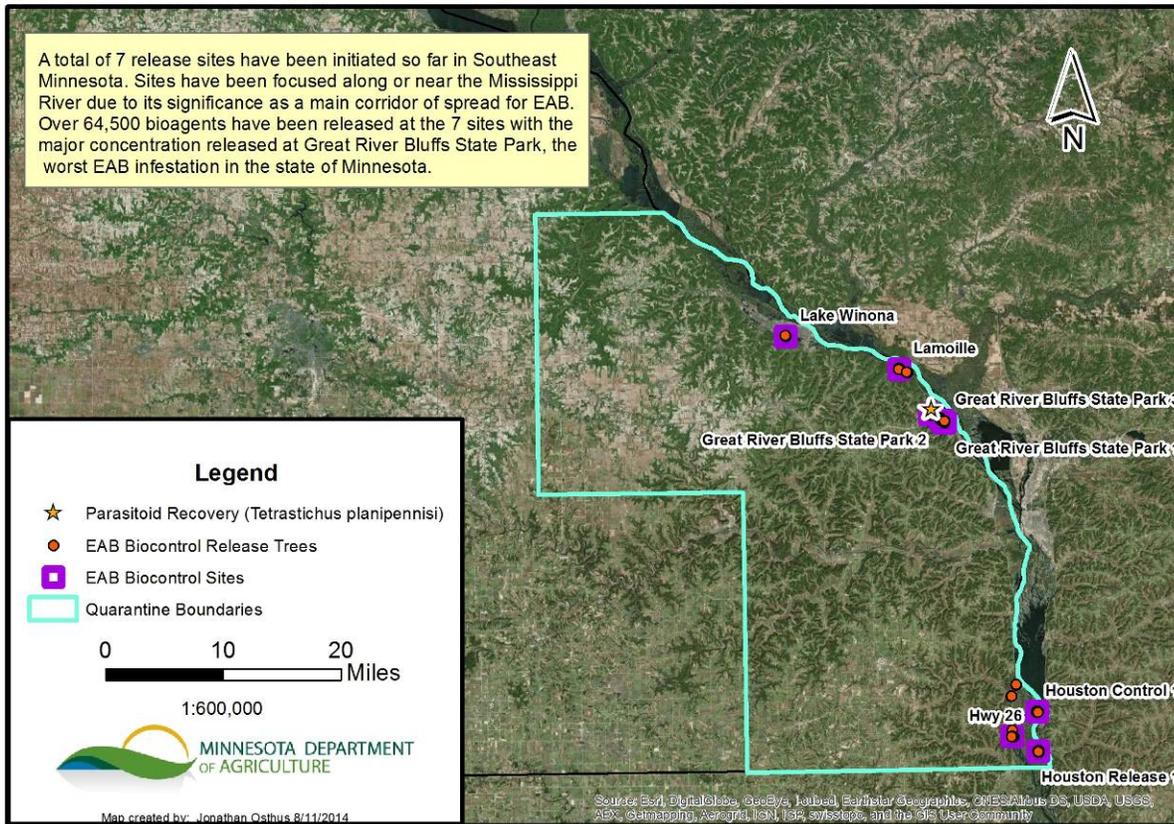
We are beginning to recover parasitoids. MDA found *Tetrastichus planipennis* larvae that had consumed most of an EAB carcass in an EAB gallery (pictured below) on 10/23/13 at Great River Bluffs State Park. A second clutch of parasitoid larvae were found on 11/01/13. MDA emerged several adults and received a definitive species confirmation from USDA APHIS PPQ. These parasitoid finds confirm that *T. planipennis* is attacking EAB and reproducing in the field. Also, we would know that the parasitoids are dispersing well. These larvae were found approximately 0.5 miles from the nearest release site. Releases of *T. planipennis* were initiated in fall 2011 at the park.



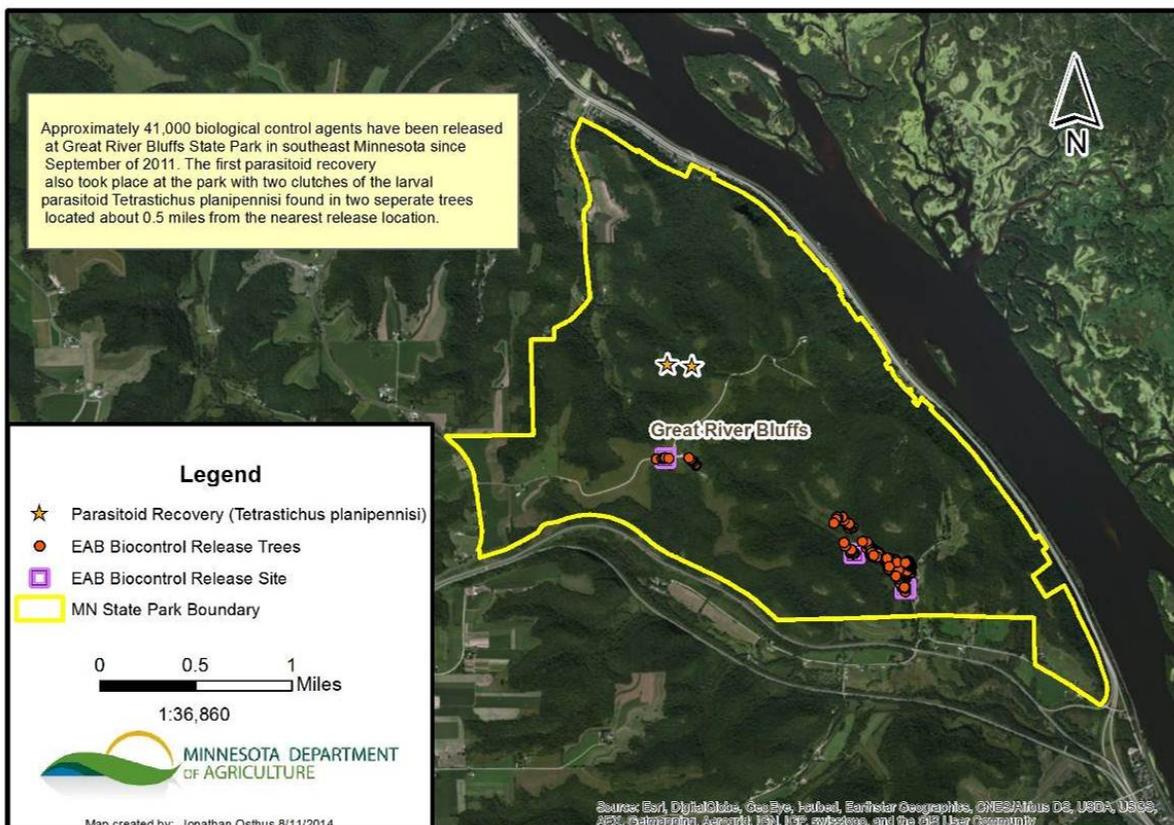
EAB carcass and the developing parasitoid larvae that consumed it found within an EAB gallery.

Upcoming plans include continued ash health monitoring. This will include the Duluth area due to the August 2013 EAB find in nearby Superior, WI.

Emerald Ash Borer Biocontrol in Southeast Minnesota



Emerald Ash Borer Biocontrol at Great River Bluffs State Park, Winona County, MN



Biological control agent release totals by site and year between 07/01/11 and 06/30/14

Site	Latitude	Longitude	Year	<i>Spathius agrili</i>	<i>Tetrastichus planipennis</i>	<i>Oobius agrili</i>	Total
2nd Street NE	44.99477	-93.26373	2013	0	1,887	0	1,887
Como Park	44.97898	-93.14738	2013	0	1,286	191	1,477
			2014	0	1,334	150	1,484
E. River Pkwy 1	44.95877	-93.21427	2011	0	702	260	962
			2012	252	934	1,346	2,532
E. River Pkwy 2	44.95643	-93.20991	2011	745	1,587	571	2,903
			2012	2,118	3,006	1,664	6,788
E. River Pkwy 3	44.95190	-93.20251	2013	0	1,549	1,324	2,873
			2014	0	625	250	875
Fort Snelling Upper Post Area	44.88626	-93.19165	2012	915	1,642	0	2,557
			2013	0	0	480	480
Ft. Snelling Hwy 5 Hillside	44.88491	-93.18779	2013	0	2,867	807	3,674
			2014	0	835	0	835
Great River Bluffs State Park 1	43.92874	-91.38466	2011	521	1,103	0	1,624
			2012	840	166	340	1,346
			2013	0	1,342	1,692	3,034
Great River Bluffs State Park 2	43.93897	-91.40949	2011	1,023	2,663	0	3,686
			2012	2,465	586	338	3,389
			2013	0	1,205	766	1,971
Great River Bluffs State Park 3	43.93150	-91.38989	2012	697	1,228	690	2,615
			2013	0	23,008	200	23,208
Houston Release 1	43.52080	-91.23666	2012	1,212	1,114	622	2,948
Hwy 26	43.53996	-91.28052	2013	0	1,499	630	2,129
			2014	0	1,015	0	1,015
Lake Winona	44.03872	-91.65294	2014	0	1,015	0	1,015
Lamoille	43.99587	-91.46054	2011	234	282	0	516
			2012	171	270	0	441
			2013	0	3,583	548	4,131
Langford Park	44.97725	-93.19489	2011	570	271	394	1,235
			2012	1,489	2,556	971	5,016
Northwestern College	45.03636	-93.16732	2013	0	2,435	616	3,051
			2014	0	500	350	850
Shoreview 1	45.11235	-93.17918	2011	888	1,536	338	2,762
			2012	809	1,653	967	3,429
			2013	0	260	0	260
Summit & Dale	44.94212	-93.12364	2011	736	1,983	0	2,719
			2012	729	2,520	974	4,223
			2013	0	260	0	260
Tower Hill Park	44.96891	-93.21265	2011	926	1,900	694	3,520
			2012	1,437	2,075	664	4,176
W. River Pkwy 1	44.95657	-93.21661	2011	1,307	1,026	926	3,259
			2012	2,124	2,072	1,665	5,861
W. River Pkwy 2	44.95102	-93.20656	2013	0	1,398	1,343	2,741
			2014	0	1,210	50	1,260
Total				22,208	81,988	22,821	127,017

Ash health data were collected at all sites. Data fields included crown class, tree diameter, dominance in the canopy, number of epicormic shoots, EAB exit holes, bark splits and woodpecker feeding damage. These data collection will continue in Phase 2.

Crown class is an indicator ash health. After tree leaf out, tree canopies are visually rated on a scale of 1-5. A crown class of 1 is a healthy tree with a robust canopy. Ratings of 2, 3, and 4 indicate canopy decline with 4 showing the most decline. A dead tree is rated as a 5. The following visual rating images were excerpted from Appendix C of the 2013 Emerald Ash Borer Biological Control Release and Recovery Guidelines by USDA-APHIS-ARS-FS, Riverdale, Maryland.



The table below shows mean canopy class rating and standard error of the mean by site and year for biological control agent release trees for 2011 - 2013. The number of trees changes at some sites by year. The standard number of release trees per site is 12. Trees are removed at some Twin Cities sites either because they are infested or as part of a structured removal. Removal of infested trees is likely to influence the mean crown class rating. The number of release trees was increased at some sites to facilitate biological control agent dispersal within the sites. Data are not available for all sites for all years because some sites were not initiated until 2012 or 2013.

Site	Year	# Trees	Mean	Std Error
2nd Street NE	2013	12	1.3	0.18
Como Park	2013	10	1.4	0.17
E. River Pkwy 1	2011	12	1.6	0.26
	2012	10	1.4	0.31
	2013	9	2.1	0.35
E. River Pkwy 2	2011	12	1.7	0.22
	2012	10	1.3	0.21
	2013	8	1.9	0.13
E. River Pkwy 3	2013	9	1.6	0.24
Fort Snelling Upper Post Area	2012	12	1.0	0.00
	2013	9	1.2	0.15
Ft. Snelling Hwy 5 Hillside	2013	12	1.2	0.11
Great River Bluffs State Park 1	2011	12	1.6	0.29
	2012	12	1.0	0.00
	2013	12	1.7	0.19
Great River Bluffs State Park 2	2011	12	1.2	0.11
	2012	12	1.2	0.11
	2013	12	1.8	0.18
Great River Bluffs State Park 3	2012	20	1.7	0.02
	2013	20	2.2	0.15
Houston Release 1	2011	12	1.4	0.26

Site	Year	# Trees	Mean	Std Error
	2012	12	1.3	0.14
	2013	11	1.6	0.29
Hwy 26	2013	12	1.4	0.19
Lamoille	2011	4	1.0	0.00
	2012	12	1.0	0.00
	2013	16	1.0	0.00
Langford Park	2011	12	1.6	0.19
	2012	12	1.2	0.11
	2013	6	1.3	0.21
Northwestern College	2013	12	1.6	0.23
Shoreview 1	2011	12	1.3	0.13
	2012	11	1.0	0.00
	2013	12	1.0	0.00
Summit & Dale	2011	12	1.0	0.00
	2012	11	1.0	0.00
	2013	8	1.5	0.19
Tower Hill Park	2011	12	2.3	0.30
	2012	6	1.2	0.17
W. River Pkwy 1	2011	12	2.5	0.19
	2012	12	2.4	0.26
W. River Pkwy 2	2013	9	1.2	0.22

The work accomplished in Phase 1 setup a strong implementation program. We initiated parasitoid releases as soon as EAB was found in an area. This gave the parasitoids the best opportunity to have an impact on EAB populations. We will continue releases and documenting changes in ash health in Phase 2. We will focus more effort on recovering parasitoids in the field and determining whether parasitoids are establishing.

V. DISSEMINATION:

Description: We will communicate about EAB biological control research and implementation with the public, land managers, and researchers. The web will be used for communication with all www.mda.state.mn.us/en/plants/pestmanagement/eab/eabbiocontrol.aspx and will be updated annually. Communication with the public will be via news media (print, television, and radio) and social media such as Facebook and Twitter. We will communicate updates with land managers at the multi-agency EAB Forum (meets 4 times/year) and in trade publications such as “The Scoop” published by the Minnesota Nursery Landscape Association. Research findings will be presented at University of Minnesota seminars, the 2012 Minnesota-Wisconsin invasive species conference, and a national Entomological Society of America meeting (LCCMR funding will not be used for meetings). After project completion, research papers will be submitted for publication.

Status as of May 31, 2012:

We conducted extensive outreach and education through many channels.

- Scientific presentations (ENRTF dollars were not used for these meetings)
 - Hanson, A.A., Venette, R.C. 2011. Thermocouple design for emerald ash borer parasitoids. Poster submitted and presented (March 14, 2011) at the 2011 Entomological Society of America – North Central Branch Meeting General Session in Minneapolis, MN.
 - Hanson, A. A., Venette, R.C., Hutchison, W.D. 2011. Cold hardiness of emerald ash borer parasitoids *Spathius agrili* and *Tetrastichus planipennis*. Oral presentation

(November 14, 2011) at the 2011 Entomological Society of America National Meeting Student Competition in Reno, NV.

- Web
 - We updated the EAB biocontrol webpage (link above) and added a page on EAB biological control research at www.mda.state.mn.us/en/plants/pestmanagement/eab/eabbiocontrol/eabwaspresearch.aspx
 - In collaboration with MDA's general EAB program, an interactive map of confirmed EAB infestations and biological control release sites in Minnesota is now available at <http://gis.mda.state.mn.us/maps/eab.htm>
- Media coverage
 - There was print, television, radio, and social media coverage of biological control agent releases and our research projects. All major state media covered our stories and a few media from Wisconsin and North Dakota also covered EAB biological control stories.
 - Media events were held in Winona and at the Summit & Dale (St. Paul) infestations
 - Media ran stories on our ash health, EAB, and EAB bioagent monitoring study and the sticky traps using cadavers preliminary study
- Events
 - Open houses in the Twin Cities, Winona, and La Crescent
 - Social events are excellent opportunities to discuss EAB and EAB biocontrol with the public. We had informational tables with EAB activities and bioagent samples at:
 - Ice cream socials at Tower Hill Park and Falcon Heights
 - Cinco de Mayo in West St. Paul
 - Other events where EAB biocontrol was discussed include:
 - Shade Tree Short Course in New Brighton
 - Northern Green Expo in Minneapolis
 - Home and Landscape Expo in Minneapolis
 - Home and Garden Show in Minneapolis
 - DNR Forestry Expo in Duluth
 - Logger Training in Tower and Grand Rapids
- Training
 - Minnesota Forest Pest First Detectors receive training on EAB biocontrol. This group of skilled volunteers communicates about invasive species topics within their communities.
- Other
 - We hosted an interactive webinar to discuss plans for the upcoming field season with collaborators.
 - We provided regular updates to the EAB Forum

Status as of November 30, 2012:

Scientific presentations

- Hanson, Anthony A.; Venette, Robert C. 2012. Cold tolerance of introduced emerald ash borer parasitoids. In: 67th annual meeting of the North Central Branch of the Entomological Society of America; 2012 June 3-6; Lincoln, NE. Abstract. Available at <http://esa.confex.com/esa/2012ncb/webprogram/Paper63275.html> . (Accessed September 10, 2012).
- Fahrner, S.J. 23 October, 2013. Presentation of thesis proposal and preliminary research results, Department of Entomology, University of Minnesota.
- Hanson, Anthony A.; Venette, Robert C. 2012. Will introduced emerald ash borer parasitoids overwinter in the Upper Midwest? Upper Midwest Invasive Species Conference; 2012 October 31; LaCrosse WI. Abstract available at <http://www.umisc2012.org/uploads/1/0/7/5/10750703/abstracts2012final.pdf> (Accessed November 29, 2012).

- Hanson, Anthony A.; Venette, Robert C. 2012. Effects of cold tolerance on potential distributions of introduced emerald ash borer parasitoids. In: 60th annual meeting of the Entomological Society of America; 2012 November 12; Knoxville, TN. Abstract available at <http://esa.confex.com/esa/2012/webprogram/Paper67442.html>. (Accessed November 29, 2012) **Received President's Prize.**
- Entomological Society of America Nov 11-14, Knoxville, TN
Fahrner, S.J., Lelito, J.P., Aukema, J.E., and B.H. Aukema. Flight capacity of *Tetrastichus planipennisi*, an introduced parasitoid of emerald ash borer *Agrilus planipennis*. In: 60th annual meeting of the Entomological Society of America; 2012 November 12; Knoxville, TN.
- North Central Forest Pest Workshop Sept 24-27, Sault Ste. Marie, ON
Fahrner, S.J., Lelito, J.P., Aukema, J.E., and B.H. Aukema. Gaining inference on the dispersal capabilities of *Tetrastichus planipennisi*, a classical biological control agent of emerald ash borer

Media

- There was print, television, radio, and social media coverage of our research projects. The stories received approximately 900,000 views via news outlets.

Events

- EAB biocontrol presentation for the City of Shoreview open house regarding EAB management.
- EAB biocontrol presentation for the DNR's newly formed EAB response team in Winona County.
- EAB biocontrol presentation/implementation update at the Upper Midwest Invasive Species Conference.
- Staffed a table with information on EAB biocontrol at the Heartwood Heritage festival in the Hamline-Midway neighborhood of St. Paul.
- EAB biocontrol informative display at the Minnesota State Fair located in the DNR invasive species exhibit. Display included microscopes for a close up look at samples of EAB and its natural enemies to inform the public how tiny the parasitic wasps really are.

Training

- EAB detection training at Fort Snelling Golf Course for over 50 arborists from Iowa. Included examples of EAB bioagents and their role in management.
- EAB detection training at Fort Snelling Golf Course for Minneapolis Parks and Recreation forestry division. Included examples of EAB bioagents and their role in management.
- EAB detection training at Fort Snelling Golf Course for industry and government employees that deal with management of EAB on private or public property.
- EAB informative tour at Great River Bluffs State Park for the Upper Midwest Invasive Species Conference participants. Highlighted on the tour was MDA's EAB biocontrol implementation strategy within the park.

Other

- EAB Management meetings in Shoreview and St. Paul were held to bring together current affected communities and adjacent ones in the Twin Cities to discuss what management has taken place thus far and what is planned for the future. Updates were provided on the status of EAB biocontrol for the cities of Shoreview, Minneapolis and St. Paul.
- EAB biocontrol is featured during the month of March for MISAC's annual 2013 invasive species calendar.

Status as of May 31, 2013:

Scientific papers and presentations

- Hanson, Anthony A. 2013. Cold tolerance of Chinese emerald ash borer parasitoids and implications for biological control in the Upper Midwest. Seminar. Department of Entomology, University of Minnesota. 04 April 2013. St. Paul, MN.
- Fahrner, Samuel J. 2013. A general-to-specific introduction to forest entomology: flying parasitoids, starving budworms, and some dying trees. Department of Biology - University of Iowa, Iowa City. April 5, 2013. Invited speaker.
- Hanson, Anthony A. 2013. Cold tolerance of emerald ash borer parasitoids: *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), *Spathius agrili* Yang (Hymenoptera: Braconidae), and

Tetrastichus planipennisi Yang (Hymenoptera: Eulophidae). Master's Thesis. Department of Entomology, University of Minnesota.

- Hanson, Anthony A. and Venette, Robert C. 2013. Thermocouple design for measuring temperatures of small insects. CryoLetters (Submitted).
- Hanson, A.A., Venette, Robert C., and Lelito, Jonathan P. 2013. Cold tolerance of Chinese emerald ash borer parasitoids: *Spathius agrili* Yang (Hymenoptera: Braconidae), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae). Biological Control (Submitted).

Media

- Minnesota Bound television program recorded a story on EAB and included biological control. The story will be aired at a future date to be determined.
- Media covered the EAB tours listed below.

Events and Tours

- The Senate and House Committees involving agriculture toured MDA's lab on January 16th and 23rd 2013 respectively. EAB was highlighted and legislators learned about monitoring and managing EAB infestations in Minnesota.
- MDA had a booth at the Prairie Enthusiast Conference on March 16, 2013 at Mankato State University. The booth contained examples of EAB bioagents and information on their role in EAB management.
- Demonstration of flight mill to visiting scientist Dr. Jack Gray, University of Saskatchewan, April 16, 2013
- EAB detection field tours were held on multiple dates in April and May at Langton Lake Park in Roseville and Great River Bluffs State Park near Winona. Tours were very well attended and open to everyone at no cost. They included examples of EAB bioagents and their role in management.

Status as of November 29, 2013:

Scientific papers and presentations

- North Central Forest Pest Workshop, Sept 23-26, 2013, Frontenac, MN. Fahrner, S.J., Lelito, J.P., and B.H. Aukema. Dispersal capacity of *Tetrastichus planipennisi*, an introduced parasitoid of emerald ash borer *Agrilus planipennis*.
- International Union of Forestry Research Organizations Sections 7.03.05 & 7.03.07 on Population Dynamics of Bark and-Wood Boring Insects, Sep 15-19, 2013, Banff, Alberta, Canada (used travel scholarship money and matching funds; no LCCMR project funds), Fahrner, S.J., Lelito, J.P., and B.H. Aukema. Temperature-mediated dispersal of host and parasitoid: Improving release strategies for *T. planipennisi* in the biological control of emerald ash borer *Agrilus planipennis*.
- Ecological Society of America Aug 4-9, 2013, Minneapolis, MN; Fahrner, S.J., Lelito, J.P., Aukema, J.E., and B.H. Aukema. Flight capacity of *Tetrastichus planipennisi*, an introduced parasitoid of emerald ash borer *Agrilus planipennis*.

Media

- The Star Tribune published the story "In the Twin Cities, emerald ash borer faces war in the streets" on 07/03/13. Other media also ran the story.
- Jon Osthus was interviewed live by KUMD (103.3 FM Duluth) about EAB and biocontrol.
- The US Army Corps of Engineers interviewed Jon Osthus on 09/11/13 for a video about their ACOE's role with EAB biocontrol on Mississippi River lands that they manage on 09/11/13.
- Minnesota Public Radio aired an update on EAB biological control at Great River Bluffs State Park.

Events

- EAB biocontrol was exhibited at the Heartwood Festival (Hamline-Midway area of Twin Cities) on 06/01/13.
- National Ag in the Classroom discussed EAB and biocontrol on 06/26/13.
- EAB biocontrol was exhibited at the Slice of Shoreview event 07/26-28/2013.

- EAB biocontrol was exhibited at Farm Fest 08/06-08/13.
- EAB biocontrol was exhibited at the Minnetonka EAB Open House on 08/07/13.

Final Report Summary:

Scientific papers and presentations

- Venette, R.C., and M. Abrahamson. 2014. Cold weather impacts on emerald ash borer: state of the science. Minnesota Shade Tree Advisory Committee. Eden Prairie, MN. February 20, 2014.
- Venette, R.C., L.D.E. Christianson, and A.A. Hanson. 2014. Cold tolerance of emerald ash borer and its parasitoids: tales from the north. Invited Presentation: Forest Entomology Symposium. 69th Annual Meeting of the North Central Branch of the Entomological Society of America. Des Moines, IA. March 10, 2014.
- Osthus, J.M. and M.A. Chandler. 2014. Biological control of the emerald ash borer. Minnesota Shade Tree Short Course. Roseville, MN. March 18, 2014.
- Venette, R.C., and M. Abrahamson. 2014. Cold weather impacts on emerald ash borer: state of the science. Plenary Session. Minnesota Shade Tree Short Course. Roseville, MN. March 19, 2014.

Training

- Monika Chandler trained volunteers to identify and report EAB at the Master Naturalist monthly meeting Quarry Hill Nature Center in Rochester on 02/19/14.
- Jonathan Osthus and Monika Chandler presented information on EAB and Oriental Bittersweet to Xcel Energy vegetation management contractors that cover the Upper-Midwest at their annual training on 03/12/14.

Events (add dates) and Tours

- A booth on terrestrial invasive species at the Home and Garden Show at the Minneapolis Convention Center was staffed by MDA. EAB and EAB biocontrol was a main topic of interest for those that visited the booth (02/26/14-03/02/2014).
- A booth on EAB and EAB biocontrol at the EAB Symposium held by Rainbow Tree Care in Roseville was staffed by MDA (03/05/14-03/06/14).
- Jonathan Osthus helped staff the **PlayCleanGo** booth at the Midwest Mountaineering Outdoor Adventure Expo on 04/25/14. Attendees were very receptive to the messages on ways to prevent spreading terrestrial invasive species when recreating outdoors.
- The North Dakota Forest Service traveled to Minnesota for an EAB discussion and tour at Ft Snelling State Park on 04/8/2014.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget:

Budget Category	\$ Amount	Explanation
Personnel:	\$ 464,724	<p>U of M: One 2 year part-time faculty (1 mo/yr) mean salary \$8,200/mo plus fringe benefits @ 7% for examining parasitoid establishment and dispersal (Activity 2). The total is \$17,500 and is for Dr. Aukema's summer salary because he has a 9 month appointment that does not include the summer field season.</p> <p>U of M: Two 3 year full-time graduate students mean salary \$28,500/yr plus fringe benefits @ 25% for bioagent cold-hardiness (Activity 1) and examining parasitoid establishment and dispersal (Activity 2). The total is \$214,000.</p> <p>U of M: Two 3 year part-time school year and full-time summer field season undergraduate students mean wages \$15/hr plus fringe benefits @ 7.6%, 2 students for Activity 1 and 2 for Activity 2 (40 wks @ 20 hr/wk & 12 wks in summer @ 40hrs/wk). The total is \$41,200.</p> <p>MDA: One 2.7 year full-time Research Scientist 1 mean salary \$42,500/yr plus fringe benefits @ 49% for EAB biocontrol implementation (Activity 3). This is a new, unclassified position within the Plant Protection Division.</p>
Capital Equipment:	\$ 14,000	<p>U of M: 2 ultralow precision temperature freezers @ \$7,000 each for Activity 1. The precision freezers can be set to and hold constant lower temperatures than standard freezers are necessary to test the cold-hardiness of the biological control agents. Varying levels of time at specific temperatures will be tested. Running two freezers simultaneously is necessary to perform the tests within the project timeframe. After this project is completed, the freezers will continue to be used for invasive species related activities.</p>
Equipment/Tools/Supplies:	\$ 13,800	<p>Equipment for MDA: One rangefinder @ 300 for Activity 3.</p> <p>Tools and Supplies for U of M (12,000) and MDA (1,500): Activity 1 supplies include thermocouple wire (\$250/yr), thermocouple connectors (\$130/yr), PTFE tubing (\$140/yr), 8 channel data logger (\$330/yr), rearing containers (\$750/yr), petri dishes (\$150/yr). Activity 2 supplies include insect rearing tubes (\$500/yr), field supplies such as insect collection traps and containers (\$750/yr). All activity supplies include tools related to bark peeling such as draw knives and chisels (\$500/yr), and miscellaneous (\$1,000/yr) such as DBH tapes (for measuring tree size), spray paint, and tree tags.</p>
Travel Expenses in MN:	\$ 29,500	<p>Travel expenses for U of M for Activities 1 (\$3,000) and 2 (\$10,900) are \$13,900. Travel expenses for MDA are \$15,600.</p> <p>Vehicles: Vehicle rental for Activities 2 and 3 during the summer field season. (Activity 2: One 3 mo. vehicle rental (\$700/mo for 6 mo. for 2 yr - includes milage) and fuel (\$200/mo for 6 mo/yr for 2 yr) and Activity 3: One 3 mo. vehicle rental (\$700/mo for 3 mo. for 3 yr - includes milage)</p>

		and fuel (\$200/mo for 3 mo/yr for 3 yr)). MDA's vehicle pool will be used for travel vehicles during the non-field season. Meals and lodging for all 3 activities (Activity 1: Approx. 6 days of travel/yr each for 3 yr for 1 undergrad student, 1 grad student, and the PI; Activity 2: Approx. travel/yr for 3 yr for 1 undergrad student (3 days), 1 grad student (6 days), and the PI (3 days); Activity 3: Approx. travel/yr for 3 yr for 1 EAB biocontrol coordinator (10 days) and the PI (6 days))
TOTAL ENRTF BUDGET:	\$500,000	

Explanation of Use of Classified Staff: N/A

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:

One 2 year part-time faculty (1 mo/yr) = 346 hrs
 Two 3 year full-time graduate students = 2080*2*3 = 12,480 hrs
 Two 3 year part-time undergraduate students = 1280*2*3 = 7,680 hrs
 One 2.7 year full-time Research Scientist 1 = 2080*2.7 = 5,616
 Total hours: 26,122
 Total FTEs = 26,122/2080 = 12.56

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
	\$	\$	
State (in-kind)			
Field equipment, lab equipment and lab space, computing/software, GIS and data management (\$40,000 for U of M, \$15,000 for MDA), graduate student advising and research management (\$100,000 at U of M), project coordination and overseeing EAB biocontrol implementation (\$15,000 at MDA)	\$ 170,000	\$	
TOTAL OTHER FUNDS:	\$ 170,000	\$	

VII. PROJECT STRATEGY:

A. Project Partners: Receiving funds: EAB biological control research and implementation will be a joint U of M and MDA endeavor. U of M will lead research and receive funds for the research projects: Assessing bioagent cold-hardiness and method development for bioagent monitoring. MDA will lead implementation and receive funds for coordinating Minnesota's EAB biocontrol program. MDA will provide labor to support research and implementation. Both institutions will provide in-kind equipment, facilities, intellectual input, and GIS/technical support. **Not receiving funds:** We will collaborate with Dr. Luke Skinner (DNR), USDA EAB biocontrol researchers, other federal and state agencies, counties, municipalities, and private landowners. The US Forest Service will not receive funds but will provide facilities.

B. Project Impact and Long-term Strategy: All three biological control agent species were released and recovered in Michigan. We are confident that these species will establish in southern Minnesota which has a similar climate to the areas of biological control agent release and recovery. However, northern Minnesota is colder than Michigan so we are not sure that EAB and its biological control

agents will survive northern winters. Understanding their winter survival potential would inform biological control agent release decisions.

EAB biocontrol is still too new for conclusions regarding efficacy. Although EAB can spread and kill ash trees at high rates, the movement potential of parasitoids once released is less well known, especially in new environments like Minnesota. Understanding rates of establishment and spread will permit judicious use of biological control agents as new sites with EAB are detected.

Implementing EAB biological control is very time and labor intensive. Site selection, data collection, coordination with project partners, and outreach are involved. Biological control agents are in short supply due to the limitations of production and demand thereby increasing the need for strategic releases. Based upon the experience in Michigan and other states, we learned that EAB can spread and destroy ash trees very quickly. An efficient and forceful implementation strategy for Minnesota should be developed and enacted immediately. Management recommendations resulting from research should be incorporated into the strategy as they become available.

C. Spending History:

Funding Source	M.L. 2005 or FY 2006-07	M.L. 2007 or FY 2008	M.L. 2008 or FY 2009	M.L. 2009 or FY 2010	M.L. 2010 or FY 2011
Forest Service (supplies and salary for Activity 1)					8,000
University of Minnesota (salary to initiate Activity 2)					2,500
Minnesota Department of Agriculture (salary to initiate bioagent releases, Activity 3)					3,000

These funds were spent prior to LCCMR fund availability.

VIII. ACQUISITION/RESTORATION LIST: NA

IX. MAP(S): NA

X. RESEARCH ADDENDUM: (attached)

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than May 31, 2012, November 30, 2012, May 31, 2013 and November 29, 2013. A final report and associated products will be submitted between June 30 and August 15, 2014 as requested by the LCCMR.

Attachment A: Budget Detail for M.L. 2011 (FY 2012-13) Environment and Natural Resources Trust Fund Projects											
Project Title: Emerald Ash Borer Biocontrol Research and Implementation											
Legal Citation: M.L. 2011, 1st Special Session, Chapter 2, Article 3, Subd. 6b											
Project Manager: Monika Chandler, Minnesota Department of Agriculture, 651-201-6537, Monika.Chandler@state.mn.us											
M.L. 2011 (FY 2012-13) ENRTF Appropriation: \$ 500,000											
Project Length and Completion Date: 3 years, 06/30/2014											
Final Report: submtited August 15, 2014 with budget amendment request submitted September 17, 2014											
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 09/17/14	Amount Spent	Balance	Revised Activity 2 Budget 09/17/14	Amount Spent	Balance	Revised Activity 3 Budget 09/17/14	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Assess biological control agent winter survival potential</i>			<i>Examining parasitoid establishment and dispersal</i>			<i>Coordinate biological control implementation</i>				
Personnel (Wages and Benefits)											
Personnel at U of M for Activities 1 (\$127,600) and 2 (\$145,100) total is \$272,700. Personnel at MDA is \$170,000.	136,102	136,102	0	152,937	152,937	0	175,685	175,685	0	464,724	0
U of M: One 2 year part-time faculty (1 mo./yr) mean salary \$8,200/mo plus fringe benefits @ 7% for examining parasitoid establishment and dispersal (Activity 2). The total is \$17,500 and is for Dr. Aukema's summer salary because he has a 9 month appointment that does not include the summer field season.					23,514						
U of M: Two 3 year full-time graduate students mean salary \$28,500/yr plus fringe benefits @ 25% for bioagent cold-hardiness (Activity 1) and examining parasitoid establishment and dispersal (Activity 2). The total is \$214,000.		91,074			105,418						
U of M: One 1.5 year full-time technician mean wages \$15.30/hr plus fringe benefits @ 39.6% for Activities 1 and 2		35,244			15,483						
U of M: Two 3 year part-time school year and full-time summer field season undergraduate students mean wages \$15/hr plus fringe benefits @ 7.6%, 2 students for Activity 1 and 2 for Activity 2 (40 wks @ 20 hr/wk & 12 wks in summer @ 40hrs/wk). The total is \$41,200.		9,784			8,522						

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 09/17/14	Amount Spent	Balance	Revised Activity 2 Budget 09/17/14	Amount Spent	Balance	Revised Activity 3 Budget 09/17/14	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Assess biological control agent winter survival potential</i>			<i>Examining parasitoid establishment and dispersal</i>			<i>Coordinate biological control implementation</i>				
MDA: One 2.7 year full-time Research Scientist 1 mean salary \$42,500/yr plus fringe benefits @ 49% for EAB biocontrol implementation (Activity 3). This is a new, unclassified position within the Plant Protection Division.								135,000			
MDA: One part-time school year and full-time summer field season undergraduate students mean wages \$15/hr plus fringe benefits @ 7.6% for EAB biocontrol implementation (Activity 3). This is a new, unclassified position within the Plant Protection Division.								40,685			
Equipment/Tools/Supplies											
Equipment/Tools/Supplies at U of M for Activities 1 (\$20,000) and 2 (\$6,000) total is \$26,000. Equipment/Tools/Supplies at MDA is \$1,800.											
Capital equipment over \$3,500 Capital equipment: 2 ultralow precision temperature freezers @ \$7,000 each for Activity 1. The precision freezers can be set to and hold constant lower temperatures than standard freezers are necessary to test the cold-hardiness of the biological control agents. Varying levels of time at specific temperatures will be tested. Running two freezers simultaneously is necessary to perform the tests within the project timeframe. After this project is completed, the freezers will continue to be used for invasive species related activities.	12,131	12,131	0							12,131	0
Equipment: One rangefinder @ 300 for Activity 3							0	0	0	0	0
<u>Services (equipment repair)</u>				1,917	1,917	0				1,917	0

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 09/17/14	Amount Spent	Balance	Revised Activity 2 Budget 09/17/14	Amount Spent	Balance	Revised Activity 3 Budget 09/17/14	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Assess biological control agent winter survival potential</i>			<i>Examining parasitoid establishment and dispersal</i>			<i>Coordinate biological control implementation</i>				
Activity 1 supplies include thermocouple wire (\$250/yr), thermocouple connectors (\$130/yr), PTFE tubing (\$140/yr), 8 channel data logger (\$330/yr), rearing containers (\$750/yr), petri dishes (\$150/yr). Activity 2 supplies include insect rearing tubes (\$500/yr), field supplies such as insect collection traps and containers (\$750/yr). All activity supplies include tools related to bark peeling such as draw knives and chisels (\$500/yr), and miscellaneous (\$1,000/yr) such as DBH tapes (for measuring tree size), spray paint, and tree tags.	1,994	1,994	0	4,068	4,068	0	1,574	1,574	0	7,636	0

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget 09/17/14	Amount Spent	Balance	Revised Activity 2 Budget 09/17/14	Amount Spent	Balance	Revised Activity 3 Budget 09/17/14	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Assess biological control agent winter survival potential</i>			<i>Examining parasitoid establishment and dispersal</i>			<i>Coordinate biological control implementation</i>				
Travel expenses in Minnesota											
Travel expenses for U of M for Activities 1 (\$3,000) and 2 (\$10,900) are \$13,900. Travel expenses for MDA are \$15,600.											
Vehicles: Vehicle rental for Activities 2 and 3 during the summer field season. (Activity 2: One 3 mo. vehicle rental (\$700/mo for 6 mo. for 2 yr - includes milage) and fuel (\$200/mo for 6 mo/yr for 2 yr) and Activity 3: One 3 mo. vehicle rental (\$700/mo for 3 mo. for 3 yr - includes milage) and fuel (\$200/mo for 3 mo/yr for 3 yr)). MDA's vehicle pool will be used for travel vehicles during the non-field season.				650	650	0	7,858	7,858	0	8,508	0
Meals and lodging for all 3 activities: Activity 1: Approx. 6 days of travel/yr each for 3 yr for 1 undergrad student, 1 grad student, and the PI; Activity 2: Approx. travel/yr for 3 yr for 1 undergrad student (3 days), 1 grad student (6 days), and the PI (3 days); Activity 3: Approx. travel/yr for 3 yr for 1 EAB biocontrol coordinator (10 days) and the PI (6 days)	373	373	0	2,429	2,429	0	2,282	2,282	0	5,084	0
COLUMN TOTAL	\$150,600	\$150,600	\$0	\$162,000	\$162,000	\$0	\$187,400	\$187,400	\$0	\$500,000	\$0