M.L. 2013, Chp. 52, Sec. 2, Subd. 06cB & M.L. 2016, Chapter 186, Section 2, Subdivision 18

Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE:	Improving Emerald Ash Borer Detection Efficacy for Control – Part B
PROJECT MANAGER:	Brian Aukema
AFFILIATION:	University of Minnesota
MAILING ADDRESS:	1980 Folwell Avenue
CITY/STATE/ZIP:	St Paul, MN 55108
PHONE:	(612) 625-5299
E-MAIL:	BrianAukema@umn.edu
WEBSITE:	http://www.forest-insects.umn.edu
FUNDING SOURCE:	Environment and Natural Resources Trust Fund
LEGAL CITATION:	M.L. 2013, Chp. 52, Sec. 2, Subd. 06cB & M.L. 2016, Chp 186, Sec. 2, Subd. 18

APPROPRIATION AMOUNT:	$ 360,000
AMOUNT SPENT:	$ 358,125
AMOUNT REMAINING:	$ 1,875

Overall Project Outcomes and Results

The emerald ash borer is an extremely challenging insect to manage because (1) there is a long lag phase between initial infestation and tree decline/mortality and (2) the insect is difficult to monitor and detect. There are several detection tools available, such as laboriously peeling the bark from branches harvested from trees, visual inspection of trees for evidence of woodpecker feeding, and attraction to purple prism traps hung in ash trees during periods of adult flight. We calibrated these detection tools to provide an estimate of the efficacy of these tools across different population densities of emerald ash borer. We found that visual evaluations to monitor trees for woodpecker damage are an effective method for identifying EAB at low densities prior to wide-spread tree decline. We found that 50 to 78% of trees at an infestation site will show signs of woodpecker damage before larval densities are high enough to cause irreparable damage to the tree. Visual inspections during leaf-off conditions are more inexpensive than other methods, and can be used by local communities to detect and respond to populations early.

We were able to use these project funds to leverage a federal grant to investigate the impact of strategic and targeted tree removals if emerald ash borer is detected early in a community. We published a scientific paper (Fahrner, Abrahamson, Venette, and Aukema 2017 "Strategic removal of host trees in isolated, satellite infestations of emerald ash borer can reduce population growth" Urban Forestry & Urban Greening 24:184-194) that found that removal of two thirds of the trees in the Twin Cities area where EAB was first detected in 2009 reduced populations by just over one half over the course of five years. These strategic removals slowed population growth considerably, and set populations back by at least one year. The most significant impact was achieved by targeting trees with evidence of woodpecker feeding.

Finally, studying potential tradeoffs between Minnesota's colder climate (than other places in emerald ash borer's range) and dispersal capacity, we found that overwintering location affects survival rates, but not energy reserves or flight capacity. In other words, Minnesota might be cold, but surviving insects do not appear to be less capable of dispersing in the spring.

Project Results Use and Dissemination

This was a joint partnership with the Minnesota Department of Agriculture. The primary audience for this work was disseminated to municipalities and other entities responsible for managing EAB at the local level.

Information was conveyed through meetings held throughout the year, both at MDA through the EAB Forum (bimonthly meeting) and also through conferences, meetings and workshops held around the state and also at professional and technical conferences.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2013 Work Plan Final Report

Date of Final Report: Oct 25, 2017

Final Report

Date of Work Plan Approval: June 11, 2013

Project Completion Date: June 30, 2017 **Is this an amendment request?** Yes

PROJECT TITLE: Improving Emerald Ash Borer Detection Efficacy for Control – Part B

Project Manager: Brian Aukema

Affiliation: University of Minnesota

Mailing Address: 1980 Folwell Avenue

City/State/Zip Code: St Paul, MN 55108

Telephone Number: (612) 625-5299

Email Address: BrianAukema@umn.edu

Web Address:

Location: Region: Statewide, Metro, Southeast

Counties: Statewide, Hennepin, Houston, Ramsey, Winona

Total ENRTF Project Budget:

ENRTF Appropriation:	**$360,000**
Amount Spent:	**$358,116**
Balance:	**$1,884**

Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06cB
M.L. 2016, Chapter 186, Section 2, Subdivision 18

Appropriation Language:
$600,000 the first year is from the trust fund to evaluate and implement options for effective detection of the presence of emerald ash borer. Of this appropriation, $240,000 is to the commissioner of agriculture and $360,000 is to the Board of Regents of the University of Minnesota. This appropriation is available until June 30, 2016, by which time the project must be completed and final products delivered.

Carryforward: (a) The availability of the appropriations for the following projects are extended to June 30, 2017: (6) Laws 2013, chapter 52, section 2, subdivision 6, paragraph (c), Improving Emerald Ash Borer Detection Efficacy for Control.

I. PROJECT TITLE: Improving Emerald Ash Borer Detection Efficacy for Control – Part B, U of M

II. PROJECT STATEMENT:

Emerald ash borer (EAB) was first discovered in Minnesota in 2009 in St Paul. It is now known to occur in four Minnesota Counties (Ramsey, Hennepin, Houston and Winona) as of September, 2012. Minnesota has more ash than any other area of the U.S. and ash is an important component of our rural and urban forests. Much work has been done to stem the spread of EAB throughout Minnesota including education, quarantine, detection surveys and biological control efforts. The likely consequence of taking no action against EAB is its rapid spread through most of the state and the resulting death of > 99% of the ash trees in those areas.

Detection is a key obstacle to controlling EAB. Minnesota has worked with the United State Department of Agriculture (USDA) to conduct detection surveys for EAB since 2003 using a variety of techniques – most recently large, purple traps. However, EAB detection tools have not been calibrated to provide an estimate of what population density of EAB they are able to detect. This is a critical information gap as EAB population density is a critical parameter in determining how and where to implement control measures.

This project will evaluate a range of detection tools and measure their ability to detect EAB at different population densities. We will also evaluate aspects of EAB biology that are critical in estimating dispersal and consequently, spread. We will use different detection techniques in and around EAB-infested areas in order to compare their ability to detect EAB. We will work with local governments to implement this work.

Through this project we will gain a better understanding as to the feasibility of using EAB detection surveys to inform EAB management for local governments or others.

III. PROJECT STATUS UPDATES:

Project Status as of November 15, 2013**:**
This project is off to a good start and on track with work goals and planned spending. Work has been initiated for both activities and is on schedule with targeted outcomes. No problems have been encountered to date that will delay or change the planned schedule of work. Specific details on work accomplished to date are provided under each of the activity sections below.

Project Status as of May 15, 2014**:**
No changes to report. Project is on track. See specific activities.

Project Status as of November 15, 2014**:** This project continues to go well. Winter mortality to emerald ash borer was surprisingly high such that low density populations – the most critical knowledge gap in sampling – have not grown as fast as initially expected. The MDA continues to collect data that will be analyzed as populations grow. One key question is the effect that early management intervention may have once low density populations are detected. We were able to successfully leverage this LCCMR on monitoring of EAB to a federal agency (USDA APHIS) for a $35,000 grant to study the efficacy of sanitation on the population growth of EAB once low density, satellite infestations of EAB are detected. For the current project, no problems have been encountered that will delay or change the planned schedule or scope of work. Specific details on work accomplished to date are provided under each of the activity sections below.

Project Status as of May 15, 2015**:** Project is proceeding as planned. Last year at this time we were most concerned about the lack of high density populations of EAB due to winter mortality, as it is important to develop these monitoring methods across a range of densities. However our fears were (unfortunately) alleviated with the expected rise in populations in four sites over the past year. The MDA report details our site replacement plans. A graduate student was recruited to work on Activity 2

Project Status as of November 15, 2015**:** Project is making very good progress. As noted in the MDA report, we are seeing increasing populations of EAB across the state, with several new finds. This project led to the first confirmed find in Duluth in a low-density infestation using branch sampling. Activity 2 (tradeoff between lipid content and dispersal) continues in full gear, although the delay in finding a suitable student until Jan 2015 necessitates a request for a no-cost extension to finish that activity (see below). Initial experiments were completed during the winter of 2014-2015. Preliminary results were analyzed, summarized, and presented at a regional professional conference.

Amendment Request (Nov 24, 2015): We are requesting a one year extension to this project to June 30, 2017 with no changes to budget or scope of work. Master's student Mr. Dylan Tussey was recruited to the project in January 2015 (18 months after project initiation). This extension will allow him to complete his thesis requirements. **[Ammendment Approved 12-08-15].**

Project Status as of May 15, 2016**:** Project continues to make good progress. (As, well, there have been several new finds of EAB in the past six months, as the insect continues to spread). The MDA completed plot sampling in the fall with a data exchange to the UMN team this spring. Activity 1 displays the start of spatial analysis of one of the sampling designs of one of the plots. In Activity 2, insects have been reared from logs that have overwintered in two climatically-distinct locations. These insects are now being analyzed on the flight mill as well as being subjected to physiological assays measuring lipid content. Results from the last progress report were presented at a national meeting in November.

Project Status as of Nov 15, 2016**:** All field data have been collected and passed from the MDA to the U. The next six months will look a lot like the past six months: data analysis. We reported on some spatial analysis in Activity 1 in the last progress report; we are finding, however, that there are few spatial relationships at the plot level scales that were collected. This is not an unexpected result; it simply reflects the biology of the insect. If a tree starts showing symptoms of emerald ash borer infestation, it is likely that all the trees in the 100m vicinity will show symptoms. In many ways, this simplifies data analysis in the next six months as we move toward finding the best sampling method at a given emerald ash borer density. In Activity 2, additional insects have been subjected to physiological assays measuring lipid content. Results were presented by the graduate student at two conferences.

Retroactive Amendment Request 10/25/17

In Activity 1, we ended with $1684 cost savings accumulated in *travel* (able to use lab vehicle for periods instead of exclusive UMN Fleet rental) and *other/publications* (published scientific article in journal without page charges). We request to redistribute this $1684 amount to *personnel*. We engaged our senior lab technician Aubree Kees at times to help the graduate student, as she had experience scouting emerald ash borer prior to coming to the University of Minnesota. That portion of graduate student time was moved to a federal project leveraged with LCCMR funds; the small overage in salary is due to differences in benefits rates.
In Activity 2, we ended with $1904 savings in *supplie*s (did not need to replace many temperature sensors), $3,389 in travel (combined travel with Activity 1 whenever possible and EAB populations remained close to metro area through life of project), and $1,000 in publications (Ent Soc of America unexpectedly dropped page charge fees from their journals). These amounts total $6,293. We request to redistribute $4,409 of these savings to personnel; a postdoctoral scholar with international expertise in dispersal of invasive species assisted with project completion as the graduate student completed his degree. These changes leave a balance of $1,884 unspent that we return to the ENRTF with project completed.

Overall Project Outcomes and Results

The emerald ash borer is an extremely challenging insect to manage because (1) there is a long lag phase between initial infestation and tree decline/mortality and (2) the insect is difficult to monitor and detect. There are several detection tools available, such as laboriously peeling the bark from branches harvested from trees, visual inspection of trees for evidence of woodpecker feeding, and attraction to purple prism traps hung in ash trees during periods of adult flight. We calibrated these detection tools to provide an estimate of the efficacy of these tools across different population densities of emerald ash borer. We found that visual evaluations to monitor trees for woodpecking damage are an effective method for identifying EAB at low densities prior to wide-spread tree decline. We found that 50 to 78% of trees at an infestation site will show signs of woodpecking damage before larval densities are high enough to cause irreparable damage to the tree. Visual inspections during leaf-off conditions are more inexpensive than other methods, and can be used by local communities to detect and respond to populations early.

We were able to use these project funds to leverage a federal grant to investigate the impact of strategic and targeted tree removals *if emerald ash borer is detected early in a community*. We published a scientific paper (Fahrner, Abrahamson, Venette, and Aukema 2017 "Strategic removal of host trees in isolated, satellite infestations of emerald ash borer can reduce population growth" Urban Forestry & Urban Greening 24:184-194) that found that removal of two thirds of the trees in the Twin Cities area where EAB was first detected in 2009 reduced populations by just over one half over the course of five years. These strategic removals slowed population growth considerably, and set populations back by at least one year. The most significant impact was achieved by targeting trees with evidence of woodpecker feeding.

Final, studying potential tradeoffs between Minnesota's colder climate (than other places in emerald ash borer's range) and dispersal capacity, we found that overwintering location affects survival rates, but not energy reserves or flight capacity. In other words, Minnesota might be cold, but surviving insects do not appear to be less capable of dispersing in the spring.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Implement detection surveys for EAB to evaluate efficacy of different detection techniques under different abundances of EAB

Description:
We will conduct detection surveys for EAB in and around infested areas. The purpose of working in these areas will be to measure the efficacy of different detection techniques. The techniques will include visual evaluation (low labor input), purple traps and / or EAB cadaver traps (moderate labor input) and removal and sampling tree branches (high labor input). We will also visually evaluate tree canopy and stem condition in these areas so as to relate the results of the detection work to tree health. We will gather data from trees felled by cooperators for EAB sanitation when possible to estimate EAB population density in these areas. This is a labor intensive task, but important to understanding the efficacy of the detection techniques (i.e., at what population density are they detecting EAB?).

MDA - Part A
This work will be coordinated by MDA who will hire one temporary employee for this task. The employee is anticipated to spend 80% of their time on this project. In addition, MDA staff funded by other EAB projects will collect information that will contribute to this project as well.

UMN - Part B
Sampling design and analysis will be coordinated by Drs. Aukema and Venette. One graduate student and one undergraduate student advised by Dr. Aukema will also work on sampling design and analysis as well as data collection. All sampling work will be coordinated by MDA with local government cooperators who will also assist by felling branches for sampling.

Summary Budget Information for Activity 1, UMN - Part B: **ENRTF Budget: $ 210,750**
Amount Spent: $ 210,750
Balance: $ 0

Activity Completion Date:

Outcome	**Completion Date**	**Budget**
1. Year 1 - Visual assessment of canopy condition in detection areas, associated data management and analysis	September 2013	$ 28,725 (UMN)
2. Year 1 **-** Branch and tree sampling in detection areas, visual assessment of stem condition, associated data management and analysis	April 2014	$ 30,908 (UMN)
3. Year 2 - Trap survey for EAB in detection areas, visual assessment of canopy condition, associated data management and analysis	September 2014	$ 39,008 (UMN)
4. Year 2 - Branch and tree sampling in detection areas, visual assessment of stem condition, associated data management and analysis	April 2015	$ 31,408 (UMN)
5. Year 3 - Trap survey for EAB in detection areas, visual assessment of canopy condition, associated data management and analysis	September 2015	$ 39,008 (UMN)
6. Year 3 - Branch and tree sampling in detection areas, visual assessment of stem condition, associated data management and analysis	April 2015	$ 31,408 (UMN)
7. Develop, print and distribute informational materials related to project	June 2016	$ 10,283 (UMN)

Activity Status as of November 15, 2013**:**

UMN

Data analysis has not yet commenced as the project has just begun. Activities have been restricted to advertising and hiring a technician to work on this part of the project. As part of onboarding process, the technician underwent training of crown ratings of infested ash in the Minneapolis/Seward neighborhoods. Training has gone well, as the candidate had prior experience with tree inventory work in preparation of emerald ash borer in another state prior to relocation to the University of Minnesota to join this project.

See MDA Project Report for description of progress for MDA work.

Activity Status as of May 15, 2014**:**

We designed a sampling plan with our MDA collaborators (see MDA report). The MDA has been taking a lead role in field data collections and detection surveys. Other than some data curation, the technician was temporarily assigned to another project as the bulk of this work will take place in the latter half of the project as we analyze sampling methods and detection efficacy as the infestation progresses.

See MDA Project Report for description of progress for MDA work.

Activity Status as of November 15, 2014**:** The majority of analysis will occur in the latter parts of the project once we have gathered as much data as possible. We were able to leverage this project for a federal grant to study the effects of early and aggressive management of EAB in satellite infestations in locations isolated from major influxes of surrounding beetles. This work integrates well with this project's focus on determining the best sampling methods for detecting low density populations of emerald ash borer. Two presentations were given that highlighted this work (see dissemination below), including one at the Upper Midwest Invasive Species Conference in Duluth in October. Another has been invited at the national Entomological Society of America (meeting next week) as LCCMR's investment in monitoring, detection, and development and implementation of biological control for emerald ash borer continues to garner national attention in the forest health community. (Project funds are not being used to travel out-of-state).

See MDA Project Report for description of progress for MDA work.

Activity Status as of May 15, 2015: The MDA report summarizes results of visual and branch sampling methods to date. We are now beginning to see the range of densities needed for good statistical "calibration" of these sampling techniques, with between 0-75 larvae per square meter. We have met to determine replacement strategies for the increasing numbers of trees being removed due to mortality. On the modeling side we have begun implementing some of these early techniques (e.g., wood-pecking) with the federal grant leveraged above to determine *which* trees provide the best population-wide management if removed when appropriately detected. We are trying to determine if the effort to remove woodpecked trees, for example, is worth the investment in finding them, and if so, how much time might it buy?

See MDA Project Report for description of progress for MDA work.

Activity Status as of November 15, 2015: We continue work on modeling removal of early-detected trees in concert with the federal grant that was leveraged to quantify the delay in population expansion from removing infested trees. A focus has been to estimate the number of emerald ash borer larvae on the landscape. We have been focusing on the region of the core infested area of the Twin Cities from 2009-present to estimate a scenario *without* any management intervention (Fig. 1). This is useful to determine in future steps how many ash borers may have been/will be removed once trees were/are a) detected and b) removed at different time steps in the inevitable population increase.

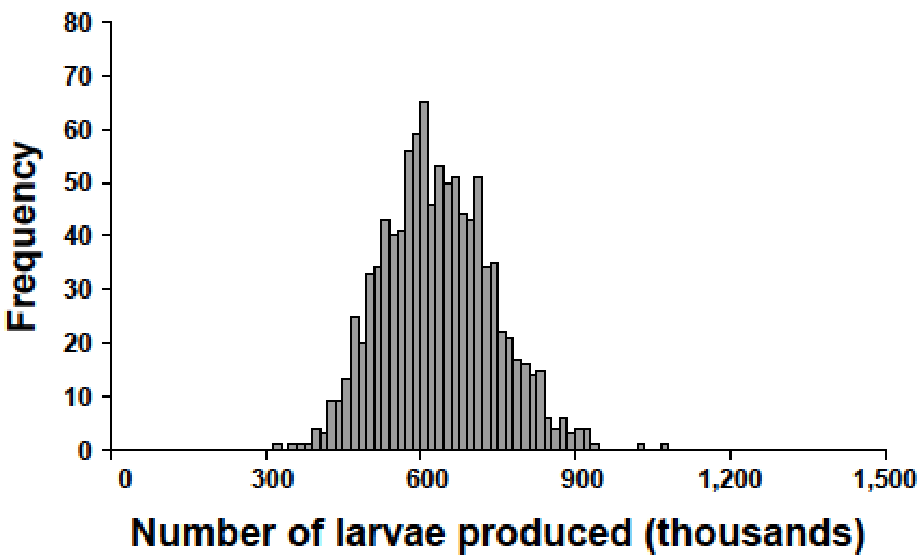


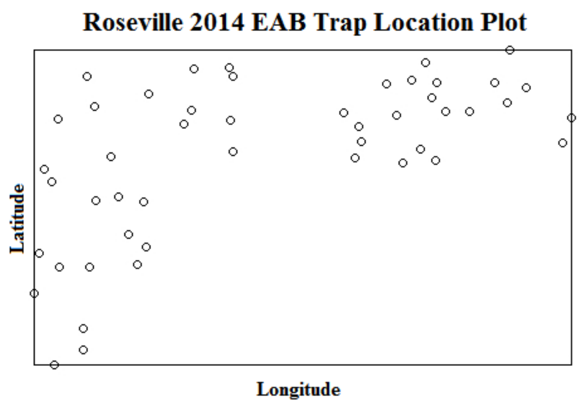
Fig. 1: Estimate of number of emerald ash borers that would have been produced 2009-2014 without any management activities, Twin Cities, MN

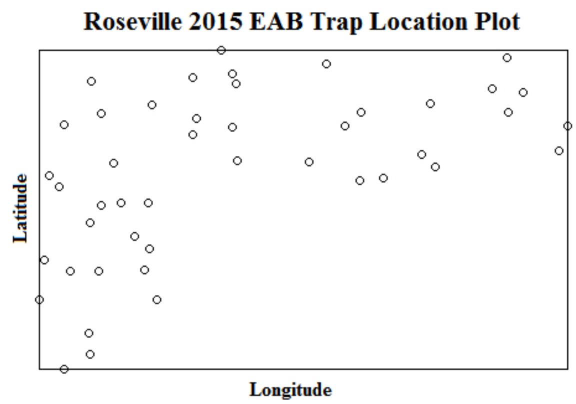
Initial estimates (above) indicate that just in the core area of the metro, there would have been upwards of 600,000 insects by now if no management activities had taken place. Our initial estimates of numbers of insects removed by management (once detected) indicate that sanitation may be effective if performed in isolation from other infested area, but the delay in population growth may not last more than 4 years. Nevertheless, this may buy cities or municipalities valuable time to formulate other long term control strategies. We have drafted a manuscript that will be submitted to a peer-reviewed journal during the next period. Work will also continue on analysis of best detection methods at different EAB densities from the field data once the winter data is collected from our MDA partners.

Activity Status as of May 15, 2016:

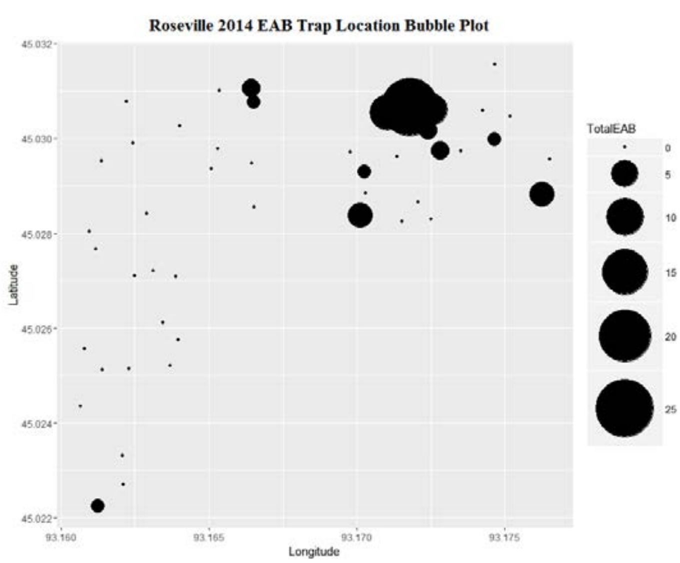
The emerald ash borer (EAB) detection data package from 2013-2016 gathered by our MDA friends was received this spring. Data is currently being process and analyzed. These data include branch sampling, trap catches, visual assessment of EAB damage, gallery sampling and whole tree sampling from 2013-2016 from multiple sites in Minnesota, ranging from Duluth to Great River Bluffs State Park, and several sites in the metro area including Fort Snelling, Roseville and others.

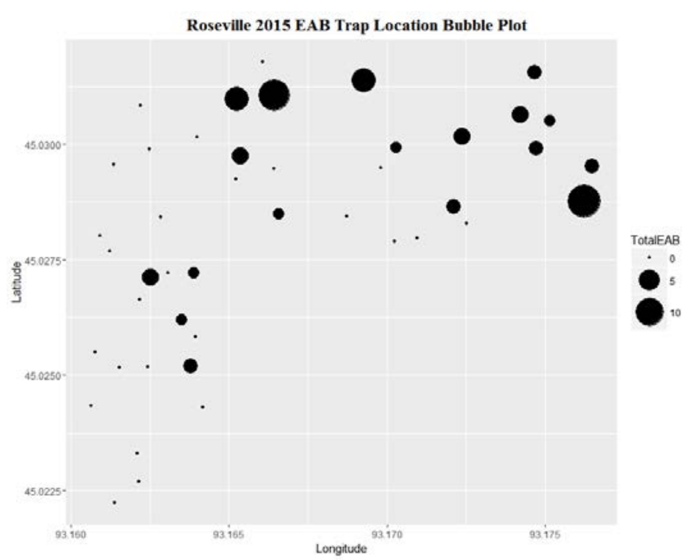
Spatial analysis is being used to compare the efficacy of sampling techniques at each site, over multiple years. For example, the graphs below show the spatial distribution of the EAB traps used in 2014 and 2015 at the Roseville study site.



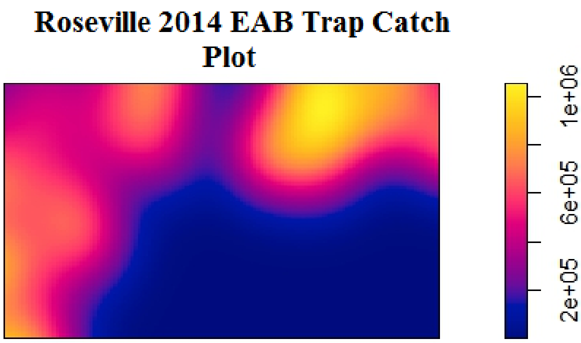


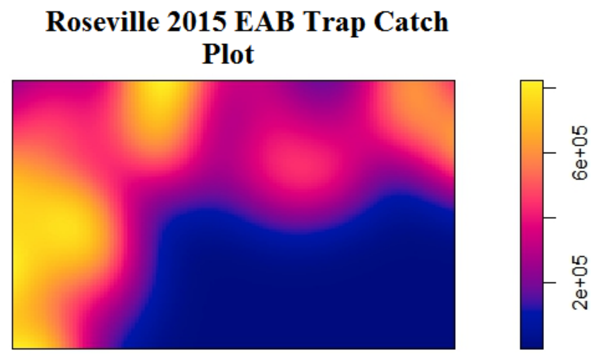
We use bubble plots, below, to view the spatial point pattern of EAB trap captures for each trap in the study area. The circle radii represent the total number of EAB captured in each trap throughout the given year. Here we can see differences in the quantity of total EAB caught and the location of trap catches from one year to another.

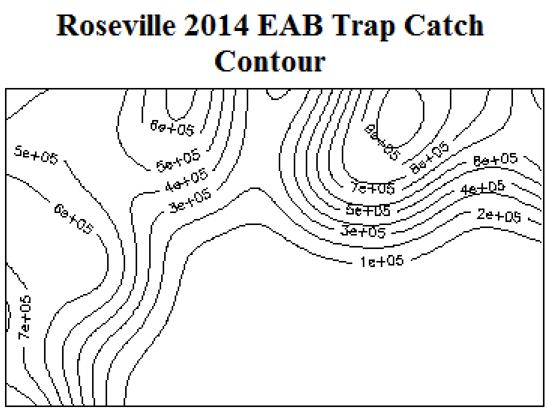


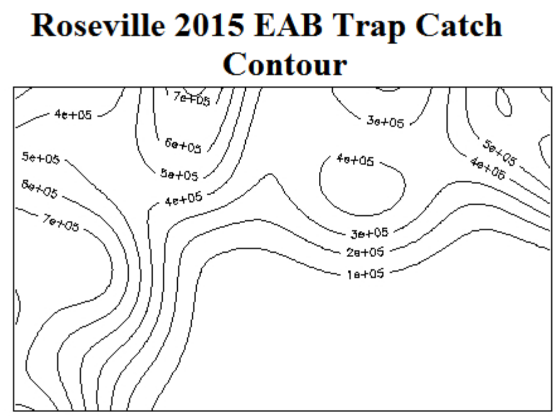


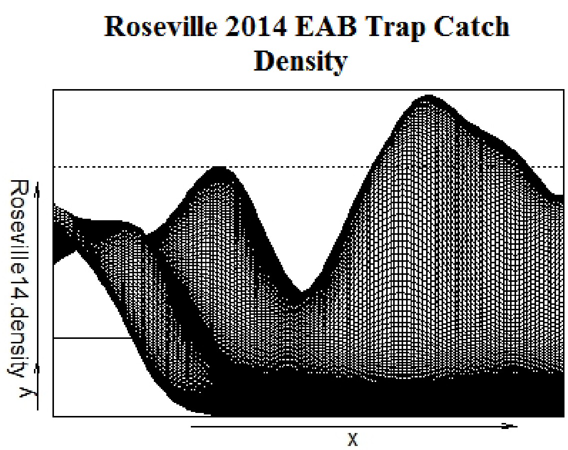
Spatial point process analysis is being used to depict the spatial density of the total number of EAB caught in traps throughout the year in 2014 and 2015 at the Roseville study site. The response variable has been rescaled for analytical reasons, but this provides the reader an illustration of some of the techniques being used (next page).

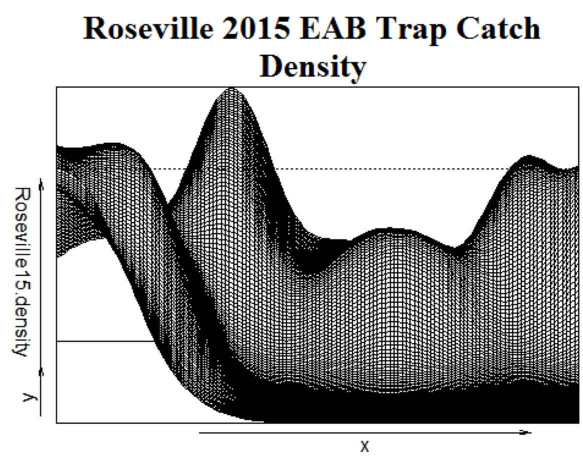










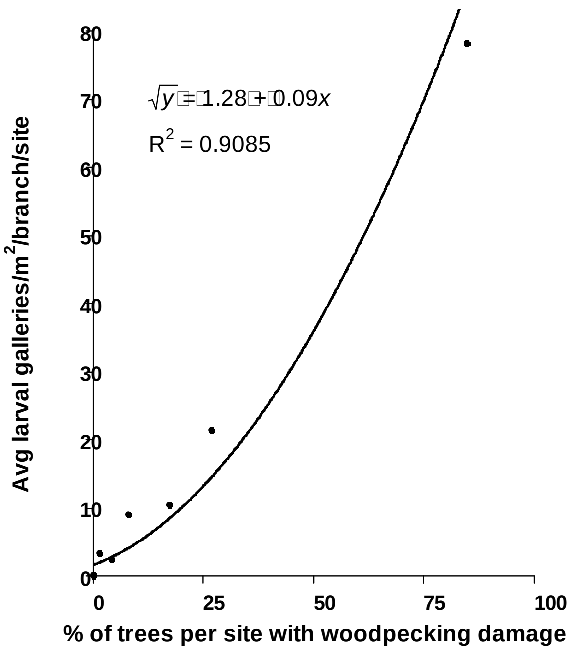


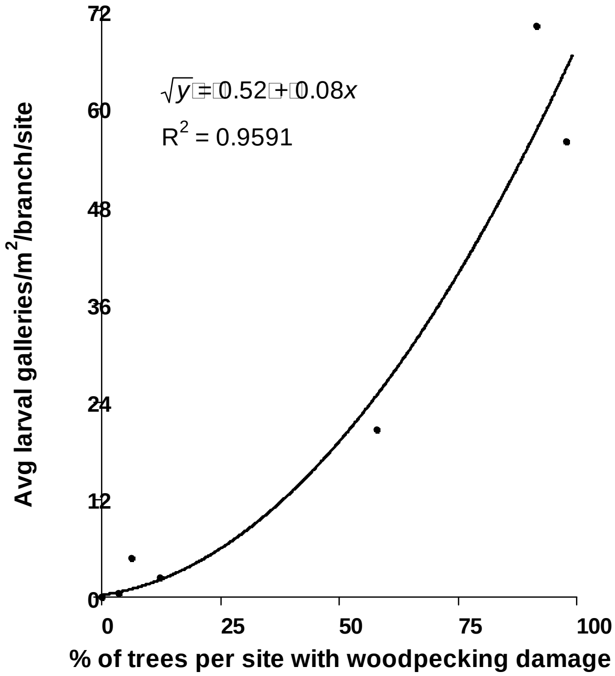
From these steps of interpolating surfaces we can move into further quantitative analyses.

Activity Status as of November 15, 2016:

We continue to analyze data on the relationships between branch sampling, trap catches, visual assessment of EAB damage, gallery sampling and whole tree sampling from 2013-2016 from multiple sites in Minnesota, ranging from Duluth to Great River Bluffs State Park, and several sites in the metro area including Fort Snelling, Roseville and others. The data from 2013-2014 is behaving better than the 2015 data.
We include two graphs below to show the relationship between woodpecking damage and the average number of galleries of EAB larvae in a tree. Canopy dieback typically becomes apparent once densities reach approximately 25 insects per square yard of phloem.

2013 2014

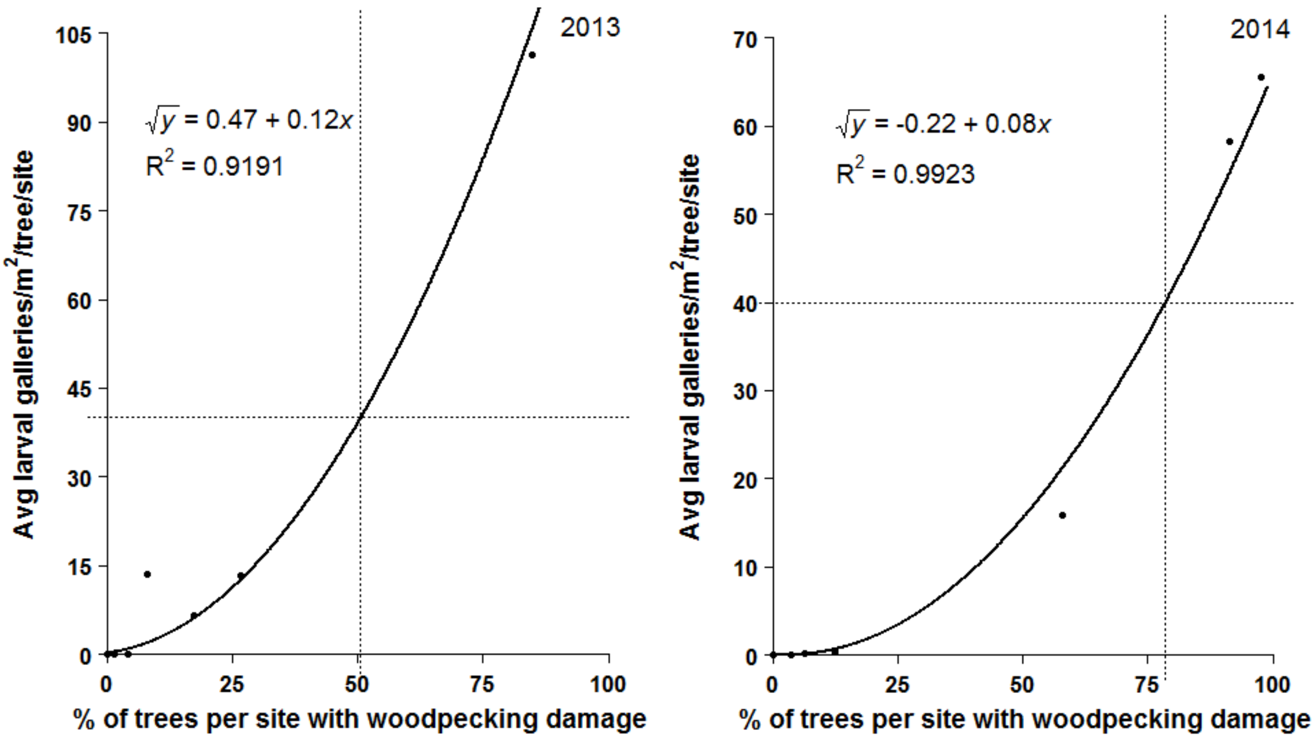




We are extending these site-level regression analyses to other measures of EAB population density such as EAB trap catches.

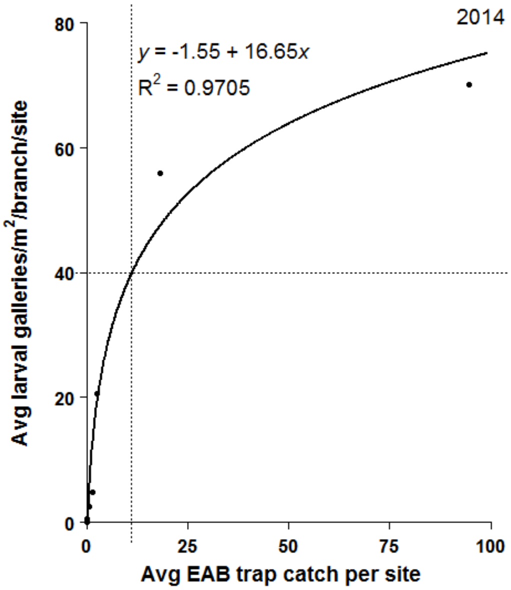
Final Report Summary:

We found that visual inspection of woodpecking damage was the best method of detecting low density infestations of EAB. In the final phase of the project, we correlated woodpecking damage with the average number of larval galleries in the whole tree (an extension of branch sampling, see above). We were pleased that the relationships remained quite strong (see next page).



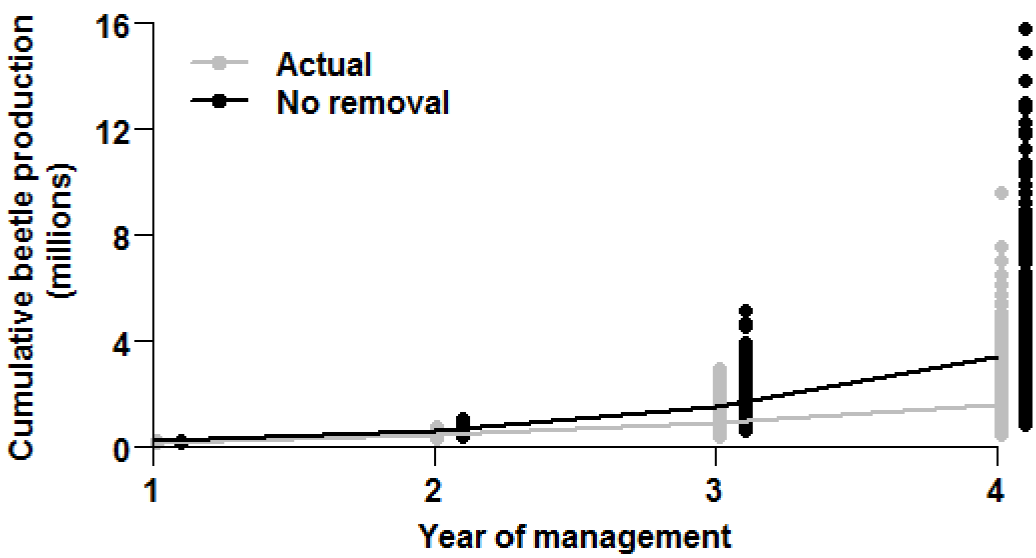
The cross hatching on the figure shows where 50% percent (2013) and 76% (2014) of trees surveyed with evidence of woodpecker feeding intersect with an average density of 40 galleries of emerald ash borer larvae per square meter of bark surface. Forty larvae per square meter is recognized as the point above where a tree may no longer be treatable, and death becomes inevitable.

For comparison, we include a graph of the 2014 data of purple prism catches on the next page. The graph shows that once as few as 10 adult EAB are captured in the traps, the surrounding trees are already likely to be well-infested and untreatable. Based on the eight sites, this sampling technique is precise (i.e., the line fits very well) but primarily indicates that if traps are catching EAB in high numbers, most of the surrounding trees are already heavily infested.



We found that these relationships broke down in 2015 as populations in some of the high density sites became too high.

Using federal funds, we were able to estimate the impact of tree removal on growth rates of the insect in the Twin Cities metro area. We were excited to prove that removal of two thirds of the trees in the Twin Cities area where EAB was first detected in 2009 reduced populations by just over one half over the course of five years. These strategic removals slowed population growth considerably, and set populations back by at least one year. The most significant impact was achieved by targeting trees with evidence of woodpecker feeding. Below we reproduce one graph from the paper.



Caption: Cumulative beetle production under *actual* versus *no removal* scenarios across four years (2010-2013) of management in Minneapolis-Saint Paul, MN, USA

This work was published in Fahrner, Abrahamson, Venette, and Aukema 2017 “Strategic removal of host trees in isolated, satellite infestations of emerald ash borer can reduce population growth” Urban Forestry & Urban Greening 24:184-194.

ACTIVITY 2: Implement field and laboratory experiments to examine factors affecting dispersal distances and winter survival of EAB.

Description:
We will measure the effect of winter cold on dispersal by measuring the fat content of beetles held under different temperature regimes. It is possible that beetles held at lower temperatures will have lower lipid reserves and therefore shorter dispersal ability. This is an important consideration when predicting spread rates of EAB in different areas of the state. We will also model the relationship between air temperature and the temperature within trees where EAB overwinter. This is a critical gap in our understanding of the impact of winter on EAB. This work will be conducted by Dr. Venette, one graduate student and one undergraduate assistant. Initial work on the overwintering biology of EAB is being completed by Dr. Venette as a result of the ENRTF project "Ecological and Hydrological Impacts of Emerald Ash Borer" which was initiated in July 2010. That work investigated the effect of host (green ash vs black ash) on the supercooling point and lower lethal temperature of EAB. The proposed project would take the next step to investigate the impact of non-lethal cold temperatures on the ability of EAB to disperse. This is an important component in understanding how Minnesota winters will affect the rate of spread and ultimately the impact of EAB.

Summary Budget Information for Activity 2, UMN – Part B: **ENRTF Budget: $ 149,250**
Amount Spent: $ 147,366
Balance: $ 1,884

Activity Completion Date:

Outcome	**Completion Date**	**Budget**
1. Measure effect of cold on EAB lipid content and create model.	June, 2016	$ 73,375
2. Measure relationship between air and within-tree temperatures and create model.	June, 2016	$ 75,875

Activity Status as of November 15, 2013**:**
We have secured logs infested with emerald ash borer larvae to study, but have not yet conducted any cold temperature testing. Testing will begin this winter.

Activity Status as of May 15, 2014**:**
Surveys during the North Dakota EAB field visit hosted by MDA (see Dissemination events in MDA report) indicated that cold temperatures killed between 60-70% of overwintering larvae under field conditions this winter. As we continue to gain an understanding of mortality-temperature relationships, we will be able to begin lipid content analyses.

Activity Status as of November 15, 2014**:**
No winter cold temperature testing was completed during this past summer. Graduate student Lindsey Christiansen, trained during the first phase of the LCCMR EAB biocontrol grant (2011-2014), is finishing her work on mortality-temperature relationships of EAB in different hosts.

Activity Status as of May 15, 2015:
Dylan Tussey was admitted as a Master's student to the Entomology graduate program at the University of Minnesota. Dylan will be investigating the sublethal effects of cold on emerald ash borer. He began in January 2015.

Lipid extraction methods. Preliminary research has focused on the refinement of methods to extract lipids from different developmental stages of emerald ash borer. This insect develops from an egg through four instars. The final (fourth) instar undergoes distinct morphological changes without molting. These additional "stages" are commonly known as “J-stage” and “fat-head” larvae. The fat-head stage immediately precedes pupation and subsequent adult emergence. Emerald ash borer typically overwinter as J-stage (most common) or as first or second (early) instars or third or fourth (late) instars.

We rely on a petroleum ether method of extraction that has been used to extract lipids from bark beetles (McKee and Aukema 2015[1]). However, later instar larvae and adults are considerably larger than bark beetles, so the objective was to determine the length of time necessary to extract all lipids.

Three insect sources were used for these studies. First, lipids were extracted from air-dried, preserved adult specimens of adult emerald ash borer. These individuals had been collected in 2013. Second, lipids were extracted from adult beetles that had been collected in June 2014 from infested trees harvested from Great River Bluffs State Park. These insects were collected within 72 hours of emergence, weighed, and immediately frozen at -80°C. Finally, larvae were collected in March 2015 from ash logs that originated at two sites in St. Paul, MN. Larvae were categorized as early instar (1st & 2nd), late instar, J-stage, or fathead and immediately weighed.

All insects were oven-dried at approximately 60°C for 24 hours. Dry weights were obtained by weighing insects within one hour of removal from the oven to minimize rehydration from ambient humidity.

Lipid extractions for adults and larvae were performed using 300mL of petroleum ether circulating through an extraction column and condenser with a round-bottomed flask heated to ~45° C. Individuals were placed in modified 0.5 mL microcentrifuge tubes with labels. When necessary, larger larvae were bisected to fit in the microcentrifuge tubes. Bisected larvae were recorded and compared to whole larvae for differences. Extractions ran with two flushes of the extractor column per hour. In general, total extraction time was determined by extracting lipids for a predetermined period, drying the insects for 24 hours at 60° C, and weighing the insects. This general process was repeated multiple times with the same individuals. The dry weights of individuals before and after each extraction were compared, and extractions continued until no significant weight difference ($P<0.05$; paired t-test) was observed.

After the final lipid extraction, lipid weight was determined by subtracting the final post-lipid-extraction, dry weight from the initial dry weight. Percent lipid content for each EAB was calculated by dividing the mass of lipid by the initial dry weight.

Total extraction time was determined to be 16 hours for adults and early-instars with no significant difference in lipid weights between 16 h and 20 h (P-value: 0.358). J-stages, fat-heads, and late instars require longer extraction times. Figure 1 shows the mean± SE of percent lipid of dry mass of adults and larvae.

Figure 1. Lipid content of emerald ash borer life stages.

[1] McKee, F. and B.H. Aukema. 2015. Influence of temperature on the reproductive success, brood development and brood fitness of the eastern larch beetle *Dendoctonus simplex* LeConte. Agricultural and Forest Entomology 17: 102-112.

Sublethal effects of cold exposure. Infested ash trees were harvested from Great River Bluffs State Park (Winona County) and two locations in St. Paul during January and February 2015. Adult beetles are currently being collected from these logs for tests to be conducted this spring and summer.

Activity Status as of November 15, 2015: A total of 152 adult emerald ash borer adults were used in this part of the study. We began by examining the effect of feeding on lipid content and the effect of lipid content on flight capacity. Green ash trees that were naturally infested with emerald ash borer larvae were cut from two sites in St. Paul and one in Great River Bluffs State Park. Cut logs were held in cardboard rearing tubes to allow adult beetles to emerge naturally. Beetles were collected daily, and the sex of each individual was immediately determined. Beetles were placed in plastic rearing containers that had been fitted with a floral pick. The pick kept a terminal leaflet of *Fraxinus uhdei* fresh for adult consumption. *Fraxinus uhdei* is a, tropical evergreen ash that grows well in the greenhouse, and thus, has become a standard host for rearing emerald ash borer in the laboratory. After feeding, the leaves are scanned to determine how much area is missing (i.e., consumed by the insects) .Leaves were replaced as necessary, Beetles were assigned randomly to different feeding treatments. The treatments were a predetermined period of feeding. All emerald ash borers are then weighed when they finish eating to obtain fresh weights. Approximately one-half of the total number of adults are frozen to measure lipid content. The other half are put on the laboratory flight mill for 24 hours. At the end of 24 hours, they are weighed again and frozen to determine lipid content. From flight mill recordings, we can determine the number of bouts of flight, flight velocity, and flight distance, among other flight characteristics. Lipid extraction takes place as above.

We are finding that three patterns are emerging:

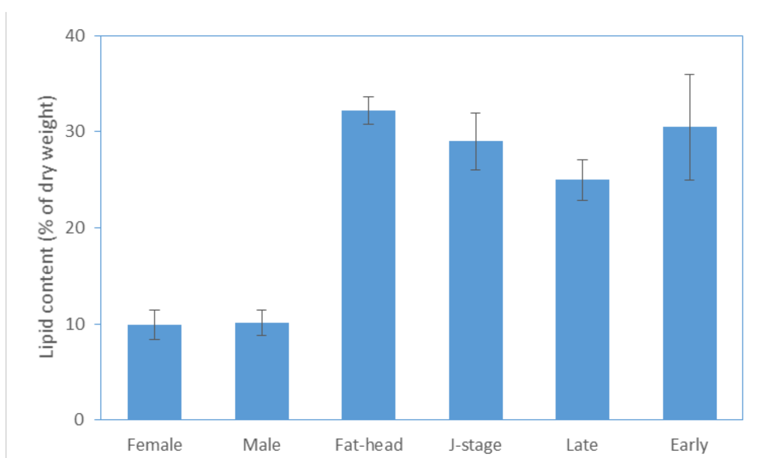
- Male and female beetles have similar lipid contents
- Adult feeding does increase dry weight, but not necessarily lipid content
- Emerald ash borers that consume more food tend to fly shorter distances

Additional details appear in the attached poster.

These results are being presented at the Entomological Society of America meeting next week.

More infested green ash was collected from St. Paul, MN to repeat this study during the winter of 2015-2016.

Activity Status as of May 15, 2016:



We are currently in the process of repeating and expanding the first year of the study by taking three complementary approaches. First, in a repeat of the study that was conducted in the spring of 2015, we are evaluating the relationship between energy reserves, adult feeding, and flight capacity. We initially focused exclusively on lipids because the literature suggests this energy source fuels flight in many insects. However, glycogen may also be an important fuel source, so we will expand our biochemical analyses to measure quantities of this fuel in adult insects. These studies are essential to establish relationships between fuel levels and flight capacity. For this study, infested trees were harvested in Minneapolis and St. Paul in late fall of 2015 and held outdoors on the St. Paul campus. Early this spring, the logs were placed in rearing tubes to allow us to collect emerging EAB adults. Emergence began in late April.

In the second study, we are evaluating the impact of different winter temperature regimes on lipid content of EAB larvae, and possibly glycogen if our methods will allow. Infested ash trees were harvested in Minnesota and Ohio. Under permits from USDA APHIS, in early January, half of the logs from Minnesota were taken to Ohio, and half of the logs from Ohio were brought to Minnesota. Logs were held in unheated restricted spaces in both locations. In March, logs were peeled to collect EAB larvae. Supercooling point, a measure of the temperature at which an insect begins to freeze, was measured for each larva. Larvae were preserved for later lipid analysis. (The costs of harvesting these trees, transporting the trees, and collecting larvae were paid by the USDA Forest Service – Northern Research Station; the biochemical analysis is an add-on to this larger experiment.)

The third study is a hybrid between the previous two. The study is designed to test the effects of winter severity and foliage feeding on energy levels (lipids and glycogen) and flight capacity of EAB adults. Infested logs were harvested in Minneapolis and St. Paul. Approximately 30 logs were held outdoors in St. Paul and another 30 were held outdoors in Grand Rapids, MN. All logs were returned to St. Paul. Average temperatures in Grand Rapids were approximately 3°C colder than in St. Paul, while the average daily low was 9°C colder. Logs have been placed in emergence tubes to collect adult EAB. We will measure lipid an glycogen levels on adults from both locations after allowing them to feed on ash leaves for 0 – 14 days and before or after flight.

Activity Status as of November 15, 2016:

We continued leaf-feeding assays and presented our results at two conferences (see dissemination). Adult feeding did not increase lipid content, a common measure of energy reserves. We did find, however, that there was a slight increase in sugar and glycogen content of the insects when they fed on more ash leaves. The insects feeding behavior was very similar independent of which site the insects had overwintered. Regardless of whether the insects overwintered in logs in St. Paul or the colder location of Grand Rapids, the insects fed at the same rate when they emerged from the logs. Insects are typically apt to fly for several days post-emergence, but insects that have flown have lower glycogen contents. We are now working to elucidate how colder temperatures may or may not limit spread though different mechanisms of reduced movement vs. strict mortality of individual insects.

Final Report Summary:

We have found that cold temperatures do not limit spread by virtue of reduced movement or dispersal capacity in the spring, contrary to our original hypothesis. Instead, it appears that cold temperatures simply affect ultimate survival, but those that do survive the winter show no ill effects that would be manifested in reduced flight. Adult glycogen content declined with flight and increased only slightly with feeding. Overwintering location affected survival rates, but not energy reserves or flight capacity. These results suggest that flight capacity of *A. planipennis* is largely determined at emergence.

We are submitting this work to a peer-reviewed journal and will forward the eventual publication to LCCMR for inclusion in project files.

V. DISSEMINATION:

Description:

The primary audience for this work will be municipalities and other entities responsible for managing EAB at the local level. There are many opportunities to address this audience through meetings held throughout the year, both at MDA through the EAB Forum (bimonthly meeting) and also through conferences and meetings held

around the state throughout the year. MDA is often invited to provide information about EAB at these meetings and conferences which is likely to continue in the future.
We anticipate that this work will result in the development of guidelines or documents meant to convey the findings of this work and what it means for local level management of EAB. In addition, we expect that this work will result in articles in scientific journals as well as presentations at national scientific meetings. However, ENRTF funds will not be used for travel to national meetings. Significant findings through this work may be communicated through the news media as well as social media.

Status as of November 15, 2013**:**

See MDA Project Report for description.

Status as of May 15, 2014**:**

See MDA Project Report for description.

Status as of November 15, 2014**:**
In addition to the presentations listed in the MDA report, this work was highlighted by the project lead at the

- Southern Forest Insect Work Conference, July 22-25, Charleston, SC
- Upper Midwest Invasive Species Conference, October 22-24, Duluth, MN

Status as of May 15, 2015**:**
In addition to the presentations listed in the MDA report, this work was highlighted by the project lead at the
Entomological Society of America Annual Meeting, Nov 16-19, Portland, OR

Status as of November 15, 2015**:**
In addition to the presentations listed in the MDA report, this work was highlighted at:

Tussey, D. A., B.H. Aukema, and R.C. Venette. 2015. Effects of feeding on lipid content and dispersal capability of adult *Agrilus planipennis*. North Central Forest Pest Workshop, Mosinee Indian Reservation, Keshena, WI, Sept 24-27, 2015 (Poster; see attached).

Tussey, D.A., B.H. Aukema, and R.C. Venette. 2015. Effects of age and feeding on lipid content and flight capacity of *Agrilus planipennis*. Department of Entomology, University of Minnesota, St. Paul, MN, November 10, 2015. (1st prize for poster presentation).

This research will be presented at the upcoming national meeting of the Entomological Society of America.

Activity Status as of May 15, 2016:
Tussey, D.A., B.H. Aukema, and R.C. Venette. 2015. Effects of age and feeding on lipid content and flight capacity of *Agrilus planipennis*. National meeting of the Entomological Society of America, Minneapolis, MN, November 16, 2016 (Poster)

Tussey, D.A. 2016. Effects of winter severity on emerald ash borer nutrition levels and flight performance. Department of Entomology seminar series, University of Minnesota, St. Paul, MN. May 10, 2016

Activity Status as of Nov 15, 2016:

- Upper Midwest Invasive Species Conference, October 18 – Joint presentation with MDA collaborators; Mark Abrahamson provided a 20 minute presentation on the project and summarized the results demonstrating that all sampling methods were useful for detecting EAB before significant tree damage at study sites.

- Graduate student Dylan Tussey presented work from Activity 3 (cold tolerance) at the International Congress of Entomology in Orlando, Florida September 24-27, 2016. No project funds were used for this travel.

Final Report Summary:

We worked closely with the MDA to disseminate this work; see the sibling MDA project summary for details. The primary audience for this work was disseminated to municipalities and other entities responsible for managing EAB at the local level. Information was conveyed through meetings held throughout the year, both at MDA through the EAB Forum (bimonthly meeting) and also through conferences and meetings held around the state throughout the year and also at professional and technical conferences.

In addition to regional spring EAB meetings (Blaine, Rochester, Duluth), summaries of these results were recently provided in presentations to 170 attendees at the Workshop on the Future of Ash Forests, July 25-27, 2017 in Duluth.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget:

University of Minnesota

Budget Category	$ Amount	Explanation
Personnel:	~~$ 348,000~~ $ 354,093	One person (Dr. Aukema) for 3 years of faculty summer salary = $64,000 • 1.6 month/year + benefits One ~~Two~~ 3 year FTE graduate students = $240,000 • mean salary of $21,300 + fringe + tuition @ $13,300 = $40,000/year/student Two undergraduate students = $44,000 • $12/hour for 14 weeks at 40 hours/week + 8% benefits for 2 students for 3 years We were able to engage additional personnel on the project (lab technician, postdoc) due to reduced graduate student time (shifting to leveraged and related federal project).
Equipment/Tools/Supplies:	~~$ 2,500~~ $ 596	Temperature sensors for recording within tree winter temperatures ~25 @ $100 each
Travel Expenses in MN:	~~$ 7,500~~ $ 4,991	Vehicle rental and fuel = $1,500 • Mileage for vehicle rental and fuel at $500 /year for 3 years – as described above in the MDA budget, the most cost efficient means of travel will be utilized Meals and Lodging = $6,000 • Approximately 15 days of travel/year for 3 years for 4 employees - 2 undergrad students, 2 grad students, and approximately 5 days of travel/year for 3 years for 2 of the co-principal investigators
Other:	~~$ 2,000~~	Publications including approximately 2 journal articles

	$ 320	($500-$1,000 each), scientific meeting posters (2 @ $200 each) Cost savings were realized by new poster printer in-house after start of project and reduced publication fees.
TOTAL ENRTF BUDGET:	**$ 360, 000**	**Balance of $ 1,884 returned to ENRTF**

Explanation of Use of Classified Staff:
N/A

Explanation of Capital Expenditures Greater Than $3,500:
N/A

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:
MDA Coordinator: 3 years @ 32 hours / week = 4,992 total hours
UM Faculty Advisor: 3 years @ 1.6 months / year = 832 total hours
Graduate Students: 2 students for 3 years @ 2080 hours year = 12,480 total hours
Undergraduate Student Workers or Technicians: 2 students for 3 years @ 14 weeks per year = 3,360 total hours
Total Hours = 16,672
Total FTE's = 16,672 hours / 2080 hours per year = 8.02

Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation:
N/A

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state - federal APHIS grants 2015-2016	$0	$63,480	After the project started, we successfully leveraged project funds for these grants. We used these funds for an extensive computational analysis of the efficacy of removing trees when detected early.
State			
Field equipment, lab equipment and lab space, computing/software, GIS and data management ($40,000 for U of M), graduate student advising and research management ($100,000 at U of M)	$140,000	$	
TOTAL OTHER FUNDS:	**$140,000**	**$63,480**	

VII. PROJECT STRATEGY:

A. Project Partners:

Receiving funds: Improving EAB detection is a collabrotive effort between MDA (**receiving $240,000**) and University of Minnesota (**receiving $360,000**). MDA will oversee Part A of the project and coordinate detection work among project partners and cooperators. U of M will oversee Part B of the project and lead research efforts for both evaluating EAB detection efficacy and evaluating the impact of temperature on dispersal capability of EAB. Other EAB projects at MDA will be leveraged to support this work where common goals are found. Both MDA and U of M will supply in-kind support through facilities, IT support, equipment and intellectional input.

Cooperators on this project will include entities with EAB infestations on or adjacent to their jurisdiction such as the cities of St Paul, Minneapolis and Shoreview, Ramsey County, DNR and DOT. We will work with cooperators to implement detection activities within their jurisdictions – particularly in the removal of branches for EAB sampling. Some cooperators may be able to donate their time for this work in-kind, other cooperators will be reimbursed for their services using ENRTF funds (**$75,000** total among all cooperators for the entire project – these funds will be passed through from the amount designated for MDA).

Not receiving funds: US Forest Service will provide in-kind support through use of facilities, equipment and intellectual input. Some cooperators at the local level will provide in-kind support through the use of staff and equipment as described above. Like other EAB work within Minnesota, the progress of this project will be shared with a wide group of stakeholders including federal and state agencies, local governments and industry groups.

B. Project Impact and Long-term Strategy:

A more thorough understanding of the capabilities and limitations of detection techniques for EAB will provide a more solid basis for local governments and other entities in making management decisions related to EAB. For instance, current recommendations on when to begin chemical treatment for EAB indicate that trees within 10-15 miles of known EAB infestations are at significant risk of becoming infested and should be considered for treatment. However, our experience in Minnesota indicates that a much tighter buffer should be considered around infested trees which would potentially lead to fewer chemicals used but with greater impact due to concentrating efforts where they are truly needed.

Municipalities are at great risk from EAB due to the heavy reliance on ash in urban areas. Currently, there are no guidelines based on quantitative studies as to what the most efficacious technique for EAB detection is, and what the results from using a given technique mean. Consequently, municipalities are left without good information for detecting EAB and consequently without good information for making decisions related to EAB management.

The outcomes from this project should provide municipalities and other local land managers in Minnesota with the information they need to more confidently assess the presence/absence or distribution of EAB in their community and as a result to plan the most appropriate management actions.

C. Spending History:
N/A

VIII. ACQUISITION/RESTORATION LIST:
N/A

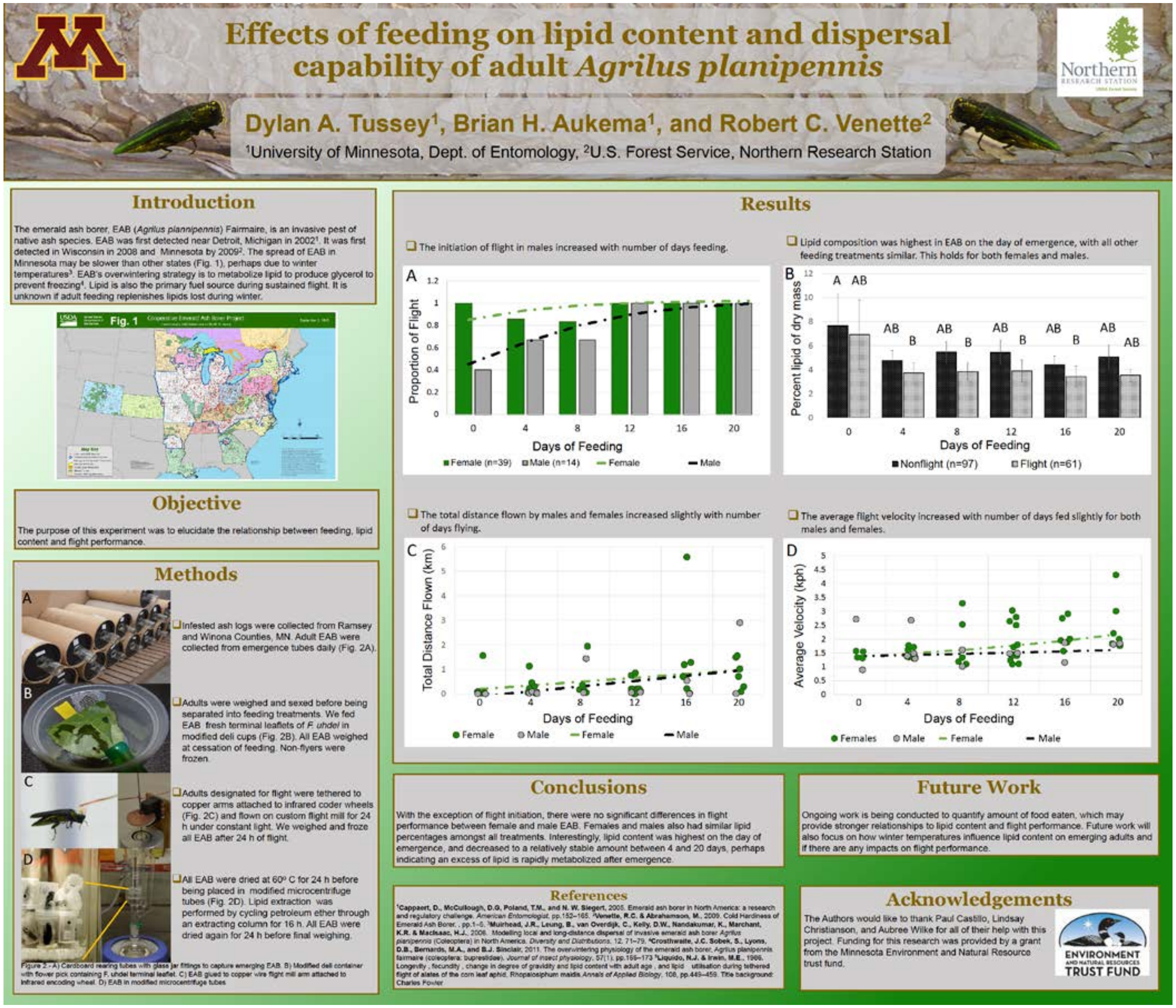
IX. MAP(S):
N/A

X. RESEARCH ADDENDUM:
N/A

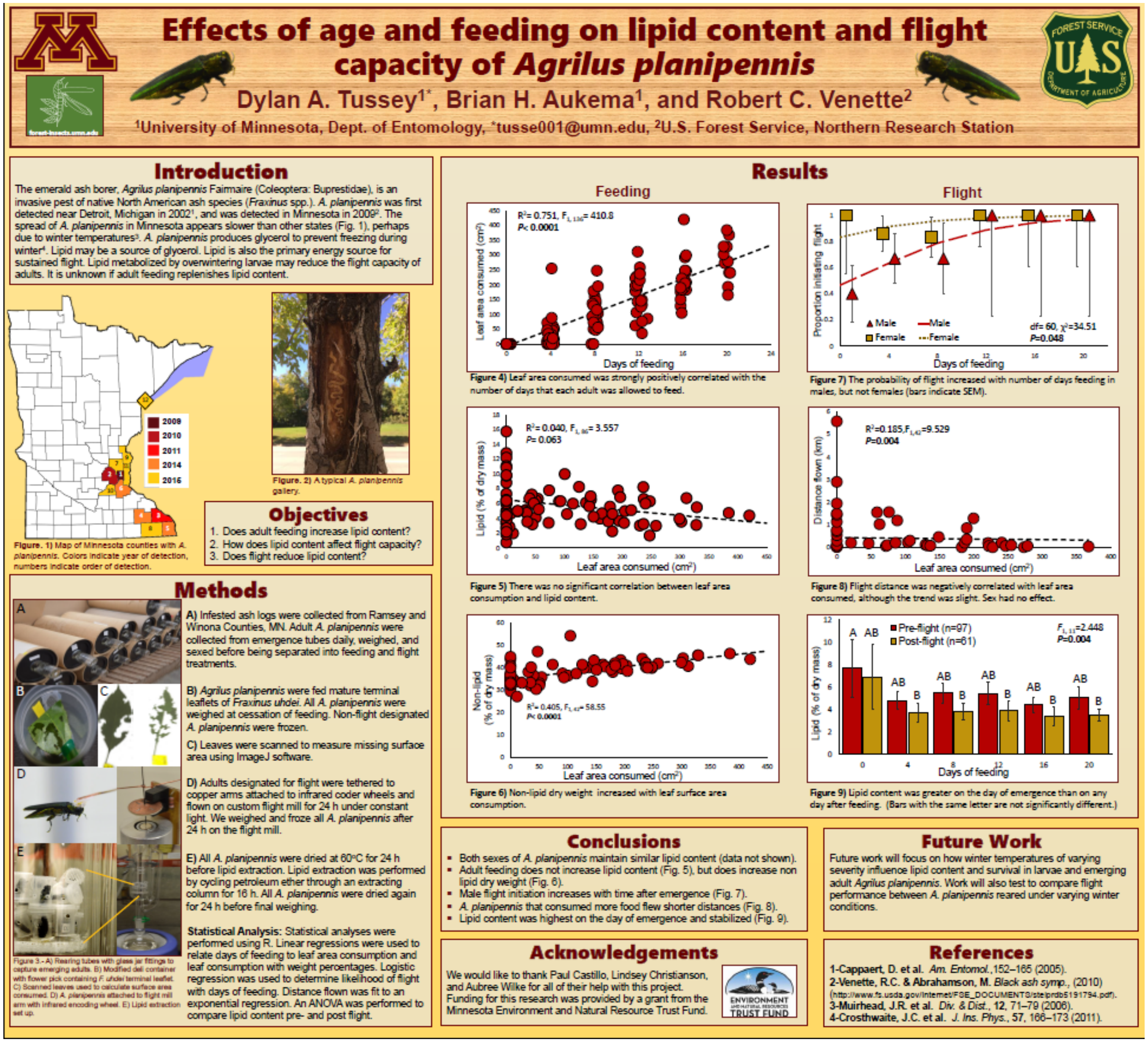
XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than November 15, 2013, May 15, 2014, November 15, 2014, May 15, 2015, and November 15, 2015. A final report and associated products will be submitted between June 30 and August 15, 2016 as requested by the LCCMR.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2013 Work Plan Final Report



Presented at the North Central Forest Pest Workshop to forest health professionals from the Upper Midwest and central Canada.



Presented at the national meeting of the Entomological Society of America to experts on emerald ash borer, insect physiology, and insect management.

Attachment A: Budget Detail for M.L. 2013 Environment and Natural Resources Trust Fund Projects

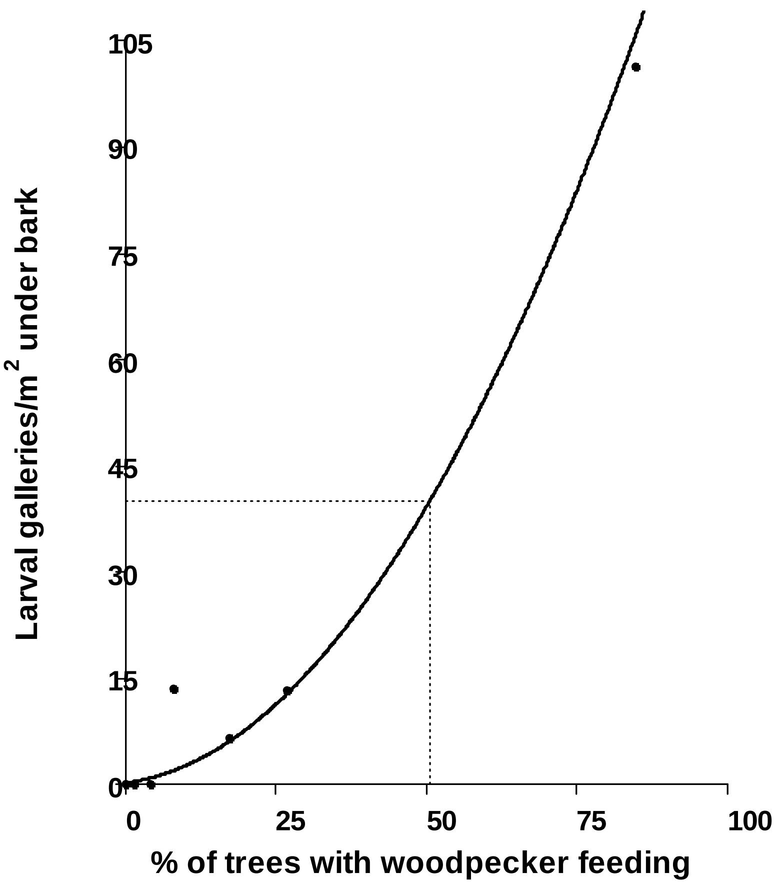
Project Title: Improving Emerald Ash Borer Detection Efficacy for Control
Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06cB
Project Managers: Brian Aukema @ U of M
M.L. 2013 ENRTF Appropriation: $ 600,000 between MDA ($240,000) and U of M ($360,000)
Project Length and Completion Date: 3 year project with one year approved extension to June 30, 2017

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Revised Activity Budget 08/31/17	Amount Spent	Balance	Activity 2 Budget	Revised Activity Budget 08/31/17	Amount Spent	Balance	TOTAL ORIGINAL BUDGET	TOTAL REVISED BUDGET	TOTAL SPENT	TOTAL BALANCE
BUDGET ITEM	**Implement detection surveys for EAB to evaluate efficacy of different detection techniques under different abundances of EAB.**				**Implement field and laboratory experiments to examine factors affecting dispersal distances and winter survival of EAB**							
Personnel (Wages and Benefits) - Direct appropriation to U of M												
- U of M Faculty Advisor: (79% salary, 21% fringe), 13% FTE (estimated $64,000) - U of M Graduate Students: Two students (47% salary, 53% fringe including tuition), 200% FTE (estimated $120,000) - U of M student workers: Two students/techs (92% salary, 8% fringe), 54% FTE (estimated $22,000)	~~$206,000~~	$207,684	$207,684	$0	~~$142,000~~	$146,409	$146,409	$0	$348,000	$354,093	$354,093	$0
Equipment/Tools/Supplies - Direct appropriation to U of M												
Temperature sensors for recording within tree winter temperatures - 25 @ ~100 each				$0	~~$2,500~~	$596	$596	$0	$2,500	$596	$596	$0
Travel expenses in Minnesota - Direct appropriation to U of M												
- Vehicle rental and fuel (estimated $750) - Meals and lodging for 2 graduate students and 2 undergraduate students (15 days of travel per year for 3 years) and approximately 5 days of travel per year for 3 years for 2 co-principal invesigators (estimated $3,000	~~$3,750~~	$2,746	$2,746	$0	~~$3,750~~	$2,245	$361	$1,884	$7,500	$4,991	$3,106	$1,885
Other - Direct appropriation to U of M												
Publications include approximately 2 journal articles ($500 - $1000 each), scientific meeting posters (2 @ $200 each)	~~$1,000~~	$320	$320	$0	~~$1,000~~	$0		$0	$2,000	$320	$320	$0
COLUMN TOTAL	**~~$210,750~~**	**$210,750**	**$210,750**	**$0**	**~~$149,250~~**	**$149,250**	**$147,366**	**$1,884**	**$360,000**	**$360,000**	**$358,116**	**$1,884**

Improving Emerald Ash Borer Detection Efficacy for Control

Detecting new infestations of emerald ash borer is hard. Really hard.

Woodpeckers feed on developing larvae, and can find them faster than people notice the infested trees. The best way to find new infestations of emerald ash borer is to measure what percentage of local trees have feeding. If it's less than 50%, we now know that there will likely be less than 40 larvae per square meter under the bark, and the tree may be saved.



MINNESOTA DEPARTMENT OF AGRICULTURE

FOREST SERVICE U.S. DEPARTMENT OF AGRICULTURE

UNIVERSITY OF MINNESOTA