M.L. 2014 Project Abstract
For the Period Ending June 30, 2018

PROJECT TITLE: Brown Marmorated Stink Bug Monitoring and Biocontrol Evaluation
PROJECT MANAGER: Robert Koch
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 04f-1 and M.L. 2017, Chp. 96, Sec. 2, Subd. 18

APPROPRIATION AMOUNT: $167,000
AMOUNT SPENT: $166,228
AMOUNT REMAINING: $772

Overall Project Outcome and Results

The brown marmorated stink bug (BMSB), a pest of numerous crops and nuisance household invader, continues to spread and increase in abundance in Minnesota. Biological control offered by tiny parasitic wasps that attack BMSB eggs is a promising tactic for sustainable management of this pest. This project aimed to evaluate and identify appropriate biological control agents for use against BMSB in Minnesota before the pest reaches damaging levels. This work was performed as a successful collaboration with USDA. In particular, we used laboratory methods for quantifying the temperatures at which insects freeze and die to examine the ability of the candidate biological control agents to survive cold winter conditions. Methods were developed to measure the response to cold temperature of several populations of two species of parasitic wasps of BMSB. Among these, the samurai wasp is the primary species of interest for biological control releases and is already known to occur in parts of the USA. Results indicated that the samurai wasp likely to survive cold winter conditions of much of Minnesota and is better able to survive exposure to cold than BMSB. Furthermore, results of a broader modeling effort showed a south to north gradient of climatic suitability for the samurai wasp in Minnesota ranging from high to marginal suitability. Therefore, based on comparison of specific cold hardiness parameters and more complex modeling, the samurai wasp is likely to survive if introduced to Minnesota, and could provide for more sustainable management of BMSB than the current use of insecticides. In addition, this project prepared the State for implementation of biological control against BMSB by identifying and optimizing cold storage methods and conditions for mass production of the samurai wasp for biological control releases against BMSB.

Project Results Use and Dissemination

Results of this project were disseminated to scientific and grower audiences. For scientific audiences, results on the ability of biological control agents for BMSB were published in scientific article (Nystrom Santacruz et al. 2017, https://doi.org/10.1016/j.biocontrol.2017.01.004) and publication of additional results related to cold storage and mass production of the samurai wasp will be published soon. In addition, research was disseminated through multiple oral presentations at conferences of the Entomological Society of America and seminars at the U of MN. To reach audience of growers and the general public, results of this research have been included in several extension presentations to growers and crop consultants. Furthermore, the research stimulated attention and interviews by local media

(http://www.startribune.com/local/262464751.html?page=1&c=y and http://kstp.com/article/stories/S3469363.shtml?cat=26). While working on this project, it was observed that there was a general lack of accessible information on the biology and management of BMSB and other stink bugs for crop and land managers. Therefore, we wrote and published an extension-friendly article on these topics in the Journal of IPM (Koch et al. 2017, https://doi.org/10.1093/jipm/pmx004), which also received considerable media attention.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan – Final Report

Date of Report: August 15, 2018

Final Report

Date of Work Plan Approval: June 4, 2014

Project Completion Date: June 30, 2018

PROJECT TITLE: Brown Marmorated Stink Bug Monitoring and Biocontrol Evaluation

Project Manager: Robert Koch

Organization: Univeristy of Minnesota

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Location: St. Paul, Ramsey County, Minnesota

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$167,000**
	Amount Spent:	**$166,238**
	Balance:	**$772**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 04f-1
M.L. 2017, Chp. 96, Sec. 2, Subd. 18

Appropriation Language:
$99,000 the second year is from the trust fund to the commissioner of agriculture and $167,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to monitor for brown marmorated stink bugs to identify problem areas, target biocontrol efforts, and evaluate the suitability of candidate biological control agents for use in Minnesota. This appropriation is available until June 30, 2018, 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, 2018: (3) Laws 2014, chapter 226, section 2, subdivision 4, paragraph (f), Brown Marmorated Stink Bug Monitoring and Bio-Control Evaluation.

I. PROJECT TITLE: Brown Marmorated Stink Bug Monitoring and Biocontrol Evaluation

II. PROJECT STATEMENT:

Our project seeks to install a framework for monitoring brown marmorated stink bug (BMSB) (*Halyomorpha halys*) within Minnesota to identify developing problem areas and target implementation of biological control. We also propose to evaluate the suitability of biological control agents identified by USDA for use in Minnesota.

BMSB in Minnesota

BMSB was first discovered in Minnesota in 2010 (St. Paul) and is now in Ramsey, Washington, Anoka, Winona, Hennepin, Chisago, Carver, Dakota and St. Louis counties. BMSB is a generalist plant pest attacking 300+ species of plants in natural, agricultural and horticultural settings, with potential to feed on many native plant species in Minnesota. Due to unpleasant odor, large size and sheer numbers, BMSB is a nuisance home invader worse than Asian lady beetles or box elder bugs in the eastern U.S. As BMSB populations build in Minnesota, indirect impacts to environment and natural resources are likely to occur through increased pesticide use in homes, yards, agricultural fields and orchards to control this pest. For example, when Midwestern soybean was invaded by the soybean aphid (*Aphis glycines*), insecticide use increased 130-fold in that crop. In addition to soybean, BMSB attacks many other field, fruit and vegetable crops. Insecticide use in orchards in the Mid-Atlantic region has already increased fourfold due to BMSB. Direct impacts of BMSB to environment and natural resources are likely due to its broad host range including native plant species and potential for rapid population growth. A Federal risk assessment determined: "Heavy feeding pressure by BMSB could also damage or reduce native plant species and impact biodiversity throughout the United States." The same Federal document also states: "…it is reasonable to expect that BMSB could displace and directly compete with native stink bugs…"

Monitoring for BMSB – MDA Component

Based on experience with BMSB in other areas of the country, we expect BMSB to first become a household nuisance and then become a significant plant pest. We expect these adverse impacts to occur 5-10 years from initial discovery. It has been 3 years since discovery in Minnesota; therefore, it is imperative that a proactive response be implemented now. Monitoring for BMSB at the landscape level should help to predict where problem areas are developing; alerting stakeholders within the area and allowing targeted efforts at biological control to protect natural and agricultural resources. Research on a trap and lure for BMSB is reaching the point where this approach is feasible.

Biological control – UMN Component

Management of this pest in eastern states has relied primarily on insecticide use. Biological control has proven to be an environmentally sound and economical alternative in some systems. It is necessary to evaluate and identify appropriate biological control agents for use against BMSB in Minnesota before populations reach damaging levels. Federal researchers are evaluating 35 populations of 4 species of parasitic wasps (*Trissolcus* spp.) known to attack BMSB eggs in South Korea, Japan and China. This work is aimed to determine the potential efficacy and safety of these species as biological control agents for BMSB. The ability of the candidate biological control agents to survive winter conditions in northern states, such as Minnesota, remains undetermined and will be necessary for advancement to implementation. This project will examine the ability of the most promising candidate biological control agents to survive winter and use modeling techniques to determine the suitability of Minnesota for establishment.

III. PROJECT STATUS UPDATES:

Amendment Request (10/22/2014)

To accommodate funding a graduate student on this project, my request is for your approval of a rebudget to transfer $8,174/year (x3 yrs) of the technician/grad student costs to a new budget line called "student assistance."

Amendment Approved: October 24, 2014

Project Status as of November 15, 2014:

The primary focus of these efforts is currently on *T. japonicus*, because this species appears to be potentially more effecting for BMSB suppression than other species under evaluation. Protocols were created and tested for project partners from USDA-ARS to send BMSB eggs that have been parasitized by the parasitic wasp *T. japonicus*, for maintaining *T. japonicus* in the MDA/MAES Containment Facility, and for acquiring measures of

cold hardiness (i.e., supercooling point and lower lethal temperature). Methods were developed on a population of *T. japonicus* originating from Beijing, China. Preliminarily, the mean unacclimated supercooling point of *T. japonicus* was -21.8°C, which is below the mean Minnesota winter temperature range of -14.4 to -18.9 °C. These methods are currently being used to evaluate the cold hardiness of four geographically-distinct populations of *T. japonicus* (Beijing, China; Nanjing, China; Puncheon, South Korea; and Tsukuba, Japan.). Data from this experiment continue to be summarized and analyzed for comparison of cold hardiness among the populations. The next step in the research plan is to focus on acclimating the insects to winter conditions through modification of light and temperature regimes to get more biologically relevant measures of insect cold hardiness.

Project Status as of May 15, 2015:

During this reporting period, project collaborators from USDA-ARS reported on the first detections of *T. japonicus* in the wild in North America near Beltsville, Maryland (http://www.stopbmsb.org/stink-bug-bulletin/asian-wasp-enemy-of-stink-bugs-found-in-the-united-states/). This detection of *T. japonicus* in North America increases the urgency of the project reported on in this report, as the ability of this species to survive winter temperatures remains unknown. Project-specific efforts during this reporting period continued to focus on evaluation of cold tolerance of unacclimated *T. japonicus* from four populations of from Asia (Beijing, China; Nanjing, China; Puncheon, South Korea; and Tsukuba, Japan). Super cooling point and lethal temperature were used as indices as cold tolerance. Methods developed for these measurements have proven effective. Efforts were also expanded to include two populations of an additional Asian parasitoid, *T. caltratus*. Future efforts will attempt to incorporate acclimated individuals of *T. japonicus*.

Project Status as of November 15, 2015:

During this reporting period, additional detections were made of *T. japonicus* in the wild. Of particular interest is the fact that it was also found on the West Coast (http://entomologytoday.org/2015/10/23/natural-born-stink-bug-killer-found-in-washington-state/). Project-specific efforts during this period included continued analysis of the previously collected cold hardiness data (i.e., super cooling point and lethal temperature) continued for the multiple populations of *T. japonicus* and *T. cultratus*. In addition, methods were developed for testing if environmental conditions (i.e., temperature and photoperiod) known to induce winter acclimation in BMSB have any effect on the cold hardiness of *T. japonicus*.

Project Status as of May 15, 2016:

During this reporting period, we performed an experiment to determine if the environmental conditions known to induce winter acclimation (i.e., increase cold tolerance) in BMSB will affect the cold tolerance of *T. japonicus*. Results of the experiment indicate that conditions known to induce winter acclimation in BMSB have no effect on the cold tolerance of *T. japonicus*. Therefore, the results reported on for previous reporting periods likely provide valid estimates of the cold tolerance of this insect. However, to more thoroughly assess the potential for cold acclimation in *T. japonicus*, we are planning an experiment to explore the effects of different combinations of temperature and day length on *T. japonicus* cold hardiness.

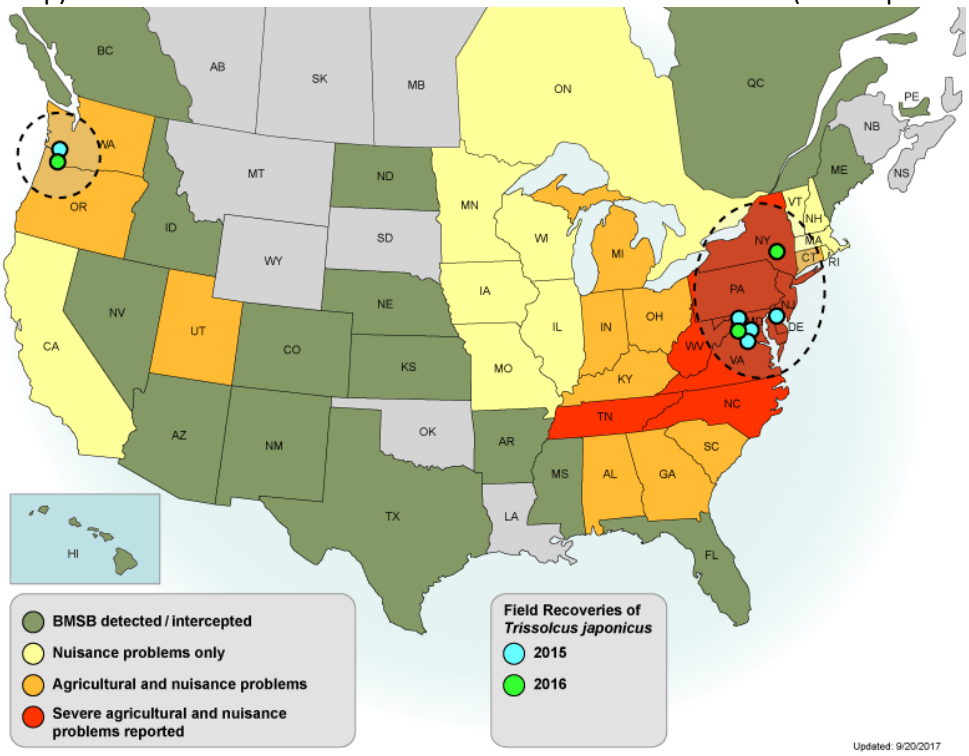
Project Status as of November 15, 2016:

During this period we compiled data and wrote a manuscript summarizes the results reviewed in the previous reports. The manuscript was submitted for publication to the journal, Biological Control. New research has begun to examine the effects of the interaction of time with cold temperature on survival of *T. japonicus*. Much of this reporting period has been focused on methods development for these new experiments, which will begin soon.

Amendment Request (12/01/2016)
A 12-month no-cost extension is being requested for the U of MN component of this project. It is projected that we will have about $30,000 remaining at the initially agreed upon end date for this project. This is due to salary savings that were experienced when staff was being billed only part-time during phases when equipment were not functioning or were unavailable, or certain methods were being developed. The equipment issues mentioned above have delayed progress on examination of the effect of time on the insect's survival at low temperatures. This extension will ensure this objective and the modeling objective are met or exceeded.
Ammendment Approved: 5/30/2017

Project Status as of July 15, 2017:
During this period, the scientific paper summarizing results from the first sets of experiments was published in the scientific journal, Biological Control. A set of follow-up experiments were initiated to examine survival of *T. japonicus* at cold, non-freezing temperatures, which simulate conditions that would be encountered if a "cold spell" were to occur during the growing season. These experiments also allow us to assess the potential for long-term cold storage of this organism, so that large numbers could be accumulated for release in biological control programs. Results of this experiment should be ready for presentation in the next report.

Project Status as of December 15, 2017:
The importance and relevance of this research continues to increase as more detections of *T. japonicus* (samurai wasp) have been made in the wild in the eastern and western U.S. (see map below from www.stopBMSB.org).



A second year of small-scale survey efforts for *T. japonicus* in forests and soybean fields in Minnesota failed to document this parasitic wasp in state. Much of this reporting period has been focused on conducting experiments. The first follow-up experiment designed to examine survival of *T. japonicus* at cold, non-freezing temperatures was completed. A second follow-up experiment to further examine this response was initiated.

Amendment Request (12/15/2017)
To accommodate the greater than expected need space to rear *T. japonicus*, BMSB (i.e., the food for T. japonicus) and plants (i.e., the food for BMSB) we have incurred more than the budgeted amount in the "other" category of the budget for this project. Therefore, my request is for your approval of a rebudget request to

transfer $5,751 of the personnel costs to the "other" category to cover these project specific insect- and plant-rearing costs.
Amendment Approved: [02/15/2018]

Overall Project Outcomes and Results:
The brown marmorated stink bug (BMSB), a pest of numerous crops and nuisance household invader, continues to spread and increase in abundance in Minnesota. Biological control offered by tiny parasitic wasps that attack BMSB eggs is a promising tactic for sustainable management of this pest. This project aimed to evaluate and identify appropriate biological control agents for use against BMSB in Minnesota before the pest reaches damaging levels. This work was performed as a successful collaboration with USDA. In particular, we used laboratory methods for quantifying the temperatures at which insects freeze and die to examine the ability of the candidate biological control agents to survive cold winter conditions. Methods were developed to measure the response to cold temperature of several populations of two species of parasitic wasps of BMSB. Among these, the samurai wasp is the primary species of interest for biological control releases and is already known to occur in parts of the USA. Results indicated that the samurai wasp likely to survive cold winter conditions of much of Minnesota and is better able to survive exposure to cold than BMSB. Furthermore, results of a broader modeling effort showed a south to north gradient of climatic suitability for the samurai wasp in Minnesota ranging from high to marginal suitability. Therefore, based on comparison of specific cold hardiness parameters and more complex modeling, the samurai wasp is likely to survive if introduced to Minnesota, and could provide for more sustainable management of BMSB than the current use of insecticides. In addition, this project prepared the State for implementation of biological control against BMSB by identifying and optimizing cold storage methods and conditions for mass production of the samurai wasp for biological control releases against BMSB.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Monitoring for BMSB
Description:
This activity will be carried out by MDA. See MDA work plan for project description and budget.

Summary Budget Information for Activity 1:

ENRTF Budget: $0
Amount Spent: $ 0
Balance: $

Activity Completion Date:

Outcome	Completion Date	Budget
1. *Volunteer BMSB monitoring network*	May each year	$0
2. *Collect data at regular intervals and publish in online map*	Sept. each year	$0

Activity Status as of November 15, 2014:

Activity Status as of May 15, 2015:

Activity Status as of November 15, 2015:

Activity Status as of May 15, 2016:

Activity Status as of November 15, 2016:

Final Report Summary:

ACTIVITY 2: Studies on overwintering potential of BMSB control agents in Minnesota

Description:

Biological control is a tactic under exploration for suppressing BMSB populations to reduce their direct and indirect impacts on the environment, economy and people of Minnesota. Several species of tiny parasitic wasps have been identified attacking BMSB in Asia and are under evaluation in USDA laboratories. These stingless wasps lay their eggs in the eggs of BMSB. The larvae of the stingless wasps then feed on and kill the BMSB eggs. The most effective of these stingless wasps in Asia is *Trissolcus japonicus*, which causes up to 70% parasitism in China. However, we are unsure how well any of these species of stingless wasp will survive the cold winter temperatures in Minnesota, and therefore are unsure about their potential impact on BMSB in Minnesota. Minnesota experiences considerably colder winter temperatures than other areas where BMSB is currently established, and where biological control agents may be released. We need to determine proactively which of candidate biological control agents will have a high chance of survival here, so that we can then focus our efforts for developmental of a biological control program against BMSB.

To assess how well these insects will survive Minnesota winters, we will use a series of laboratory studies to determine at what temperature the insects freeze (i.e., supercooling point), at what temperature they die (i.e., lower-lethal temperature) and how soon they die (i.e., lower lethal time). These biological parameters will then be used to predict the ability of these insects to survive MN winters. This work is critical now, because the process of identifying, testing and getting approval to use new biological control agents can take several years.

Summary Budget Information for Activity 2, UMN:

ENRTF Budget: $167,000
Amount Spent: $166,228
Balance: $772

Activity Completion Date: 06/30/2018

Outcome	Completion Date	Budget
1. *Measurement of the response to cold temperatures by candidate biological control agents of BMSB*	12/31/2017	$143,398
2. *Model for impact of winter temperatures on survival of candidate biological control agents of BMSB*	06/30/2018	$23,602

Activity Status as of November 15, 2014:

This status report covers the project period from July 1 to November 1, 2014. A graduate student was hired to assist with research on the cold hardiness of BMSB biocontrol agents. The primary focus of these efforts is currently on *T. japonicus*, because this species appears to be potentially more effecting for BMSB suppression than other species under evaluation. USDA-ARS is actively researching the efficacy (parasitism rates) and environmental safety of this species and others. Protocols were created and tested for project partners from USDA-ARS to send BMSB eggs that have been parasitized by the parasitic wasp *T. japonicus*, for maintaining *T. japonicus* in the MDA/MAES Containment Facility, and for acquiring measures of cold hardiness (i.e., supercooling point and lower lethal temperature). Shipment of parasitized BMSB eggs from USDA-ARS to the U of MN is permitted under permit attained from the USDA-APHIS. In the containment facility, the parasitized BMSB eggs are maintained in a growth chamber until emergence of adult *T. japonicus*. Soon after emergence, the *T. japonicus* are used for cold hardiness measures.

Methods were developed on a population of *T. japonicus* originating from Beijing, China. Supercooling point, the temperature at which the organism freezes, was measured using surface contact thermometry, in which the insect is placed in close contact with a thermocouple, the thermocouple with insect is then placed in a foam cube and then the foam cube is placed in a -80°C freezer. At this temperature, the foam cube provides a cooling rate of about 1°C per minute, which is the standard in scientific literature. Insect body temperatures are then recorded during cooling and the supercooling point is visualized as the lowest temperature attained prior to the release of latent heat, a physical process occurring when liquid turns to solid. Lower lethal temperature, the low

temperature at which a short duration of exposure results in death of the organism, was recorded with a similar experimental set up as described for the supercooling points. However, for this study, the insects are removed from the freezer upon reaching different low temperatures and are then assessed for survival. Preliminarily, the mean unacclimated supercooling point of *T. japonicus* was -21.8°C, which is below the mean Minnesota winter temperature range of -14.4 to -18.9 °C.

These methods, which have proven successful, are currently being used to evaluate the cold hardiness of four geographically-distinct populations of *T. japonicus* (Beijing, China; Nanjing, China; Puncheon, South Korea; and Tsukuba, Japan.). Data from this experiment continue to be summarized and analyzed for comparison of cold hardiness among the populations. The next step in the research plan is to focus on acclimating the insects to winter conditions through modification of light and temperature regimes to get more biologically relevant measures of insect cold hardiness.

Activity Status as of May 15, 2015:

This status report covers the project period from November 2, 2014 to May 15, 2015. Research from the USDA-ARS continues to indicate that *T. japonicus* holds the greatest potential for success at suppressing BMSB populations. The methods and protocols described above continue to prove effective for this research. Efforts to assess the cold tolerance of Asian parasitoids continued to focus on *T. japonicus* from four distinct populations (Beijing, China; Nanjing, China; Puncheon, South Korea; and Tsukuba, Japan), but expanded to include an additional parasitoid, *T. caltratus* (from two Japanese populations). For unacclimated *T. japonicus*, the mean supercooling points (i.e., temperature at which bodies freeze) ranged from -20° to -21° C and did not differ significantly among the populations. These results are similar to our preliminary results reported for the mean supercooling point of the Beijing population *T. japonicus*. Preliminary comparisons of lethal temperatures (i.e., temperature at which individuals die) to supercooling points show that unacclimated *T. japonicus* adults die before freezing, which is indicative of "chill intolerance." For *T. cultratus*, the mean supercooling points ranged from -18.2° and -18.8° C and did not vary significantly between the two populations. The supercooling points of *T. cultratus* were, however, higher than those of *T. japonicus*. The research team is meeting with statistical consultants at the U of MN to identify the most appropriate analyses for the supercooling and lethal temperature data. Results of these analyses will be reported on in a later progress report. Furthermore, the research team is with working closely with colleagues evaluating the cold tolerance of BMSB to try to identify potential light and temperature regimes (environmental conditions) for use in future experiments to try to acclimate *T. japonicus* to simulated winter conditions.

Figure 1: Experimental apparatus for evaluation of parasitoids of BMSB. Insects are placed in close proximity to thermocouples inside of small plastic tubes. The tubes are then specially designed foam cubes, which are placed in a -80°C freezer to cool insects at about 1°C per minute.

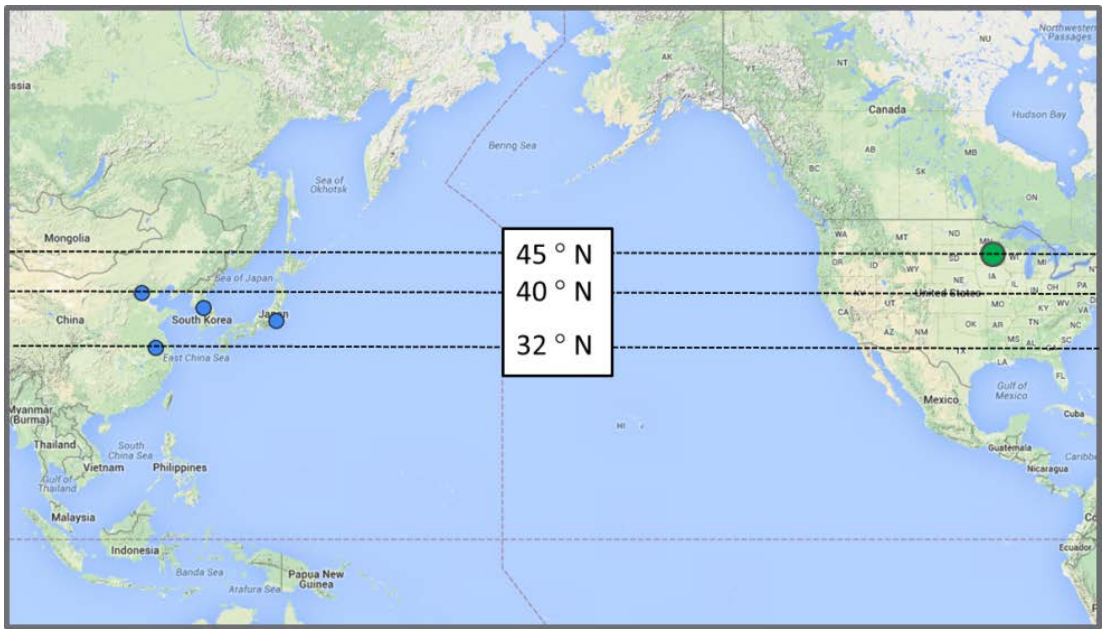


Figure 2: Map showing geographic origins of Asian populations of *T. japonicus* being examined for cold tolerance. Note: all these populations are from more southerly locations than Minnesota.

Figure 3: Adult *T. japonicus* parasitizing BMSB eggs in laboratory.

Activity Status as of November 15, 2015:

This status report covers the project period from May 16, 2015 to November 15, 2015. Analysis of data on the comparison of cold tolerance of *T. japonicus* among four distinct populations (Beijing, China; Nanjing, China; Puncheon, South Korea; and Tsukuba, Japan) and two populations of *T. cultratus* (two Japanese locations) is nearly complete. Across the populations of *T. japonicus*, the temperature required to kill 50% of the populations

ranged from -17.36 to -20.03°C and the temperatures at which 50% of the populations froze ranged from -21.42 to -22.04°C (Figure 4). Statistical comparisons of lethal temperatures (i.e., temperature at which individuals die) to supercooling points show that unacclimated *T. japonicus* adults die before freezing, which is indicative of "chill intolerance" (Figure 4). Across the populations of *T. cultratus*, the temperature required to kill 50% of the populations ranged from -19.86 to 19.88°C (Figure 5). These results will soon be written up for publication in a peer-reviewed scientific journal. However, the results mentioned above are from individuals that not acclimated to winter conditions, therefore resulting in a conservative estimate of the cold tolerance and potential geographic distribution of these potential biological control agents. An additional set of experiments are beginning to determine if the environmental conditions known to induce winter acclimation in BMSB will affect the cold tolerance of *T. japonicus*.

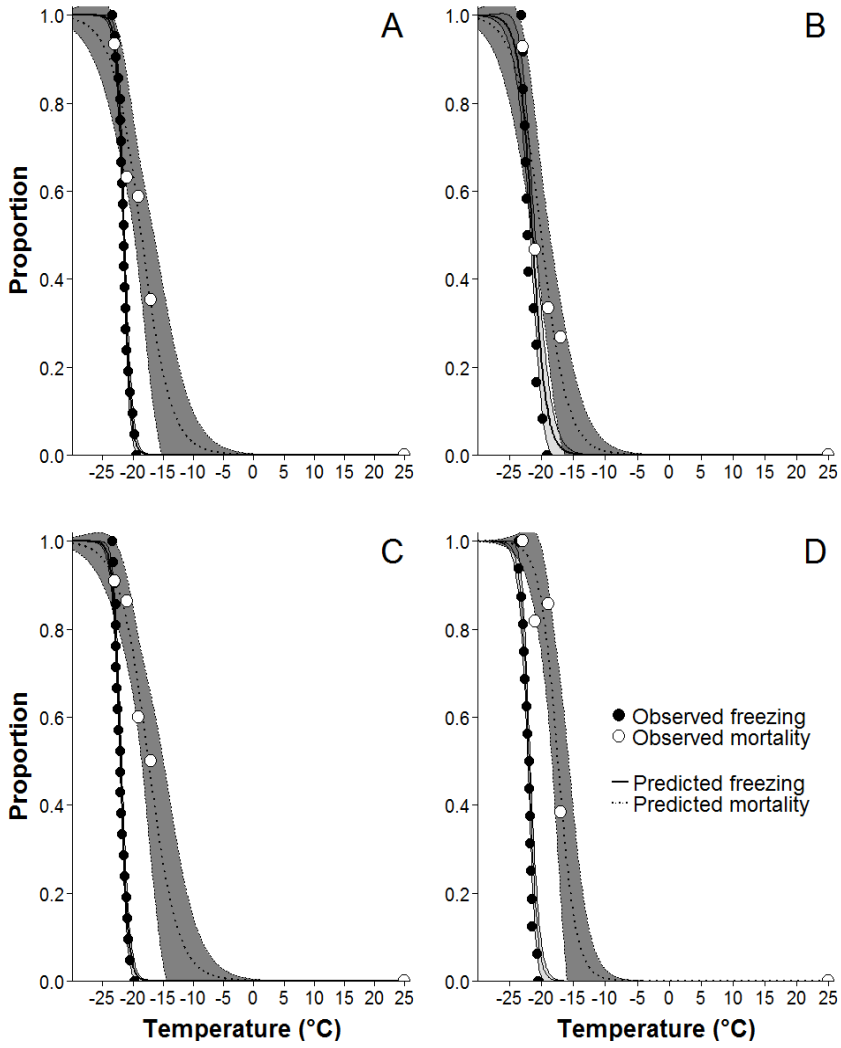


Figure 4: Observed (symbols), and predicted (lines ± 95% confidence bands) mortality and cumulative freezing in *Trissolcus japonicus* populations: (A) Beijing, China; (B) Nanjing, China; (C) Pucheon South Korea; (D) Tsukuba, Japan.

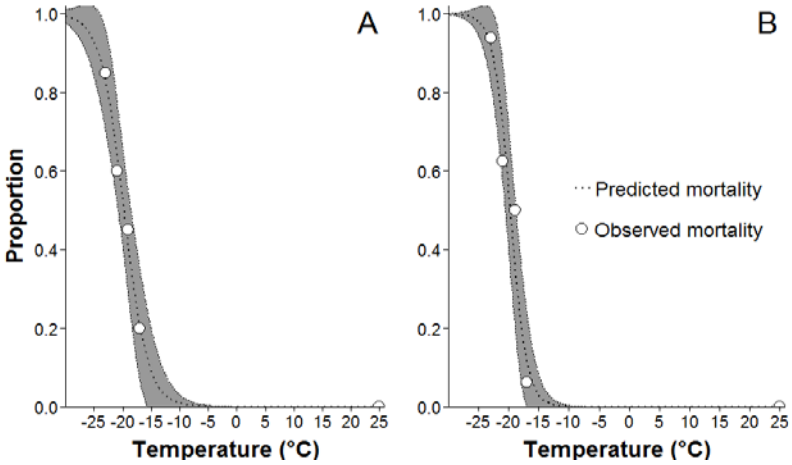


Figure 5: Observed (symbols), and predicted (lines ± 95% confidence bands) mortality in *Trissolcus cultratus* populations: (A) Japan1, (B) Japan2.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan – Final Report

Activity Status as of May 15, 2016:

This status report covers the project period from November 15, 2015 to May 15, 2016. An experiment was performed to determine if the environmental conditions known to induce winter acclimation (i.e., increase cold tolerance) in BMSB will affect the cold tolerance of *T. japonicus*. To start, the effect of temperature on mortality of a group of wasps from the Beijing population of *T. japonicus*, that we are calling the "parental" generation, was measured as described above (Figure 6A). Then, the progeny (F1 generation) from parental generation was split into two groups, which were randomly assigned to one of two different sets of rearing conditions (control conditions: 23±1.5 °C and 16:8 L:D; or cold conditions 18±1.5 °C and 12:12 L:D) from egg to adult life stages. It has recently been confirmed at the University of Minnesota that these cold conditions induce winter acclimation in BMSB (Cira et al., unpublished). The wasps were then reared under these conditions for two additional generations (generations F2 and F3) with the effect of temperature on mortality measured for each generation (Figure 6A, 6B and 6D). Statistical analyses showed no differences in cold tolerance among generations or between the rearing conditions. These results indicate that conditions known to induce winter acclimation in BMSB have no effect on the cold tolerance of *T. japonicus*. Therefore, the results reported on above for previous reporting periods likely provide valid estimates of the cold tolerance of this insect. However, to more thoroughly assess the potential for cold acclimation in *T. japonicus*, we are planning an experiment to explore the effects of different combinations of temperature and day length on *T. japonicus* cold hardiness.

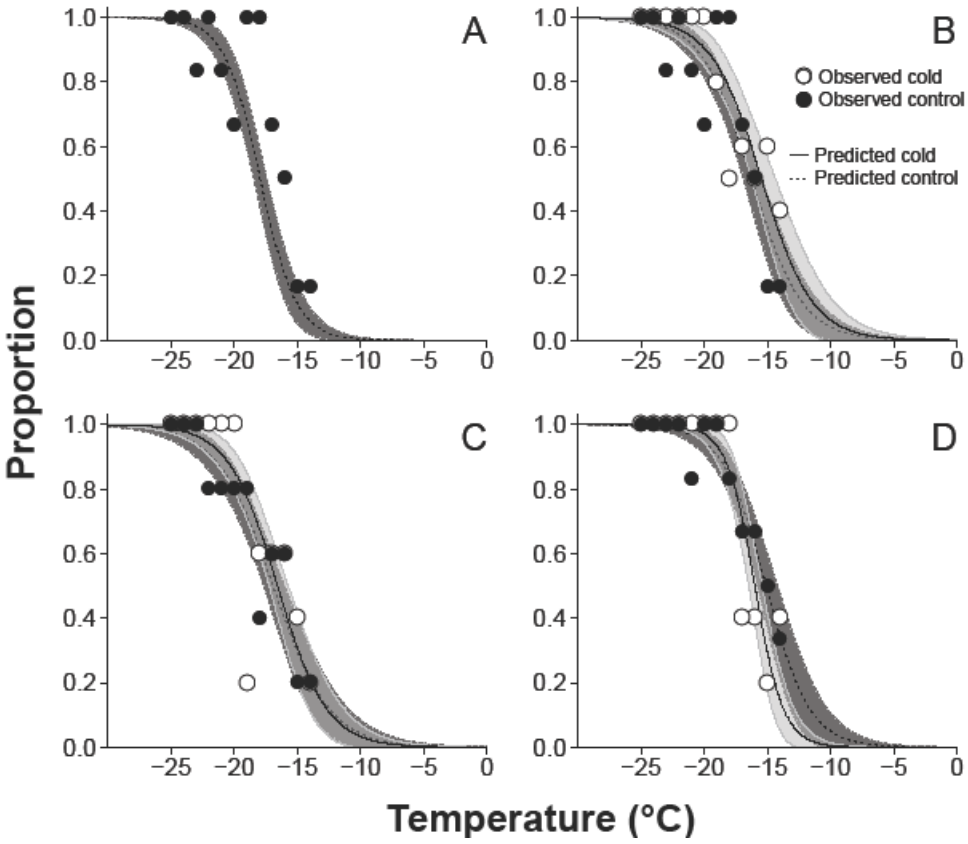


Figure 6. Observed and predicted mortality (± SE band) for *Trissolcus japonicus* reared on control (i.e., standard rearing) conditions and cold (i.e., lower temperature and shorter day length) conditions. Panels of this figure represent the different generations reared under these conditions (A: Parental; B: F1; C: F2; D: F3).

Activity Status as of November 15, 2016:

This status report covers the project period from May 15, 2016 to November 15, 2016. Results of the project described in previous reports were compiled and written as a manuscript that was submitted for publication in the journal Biological Control on September 16. The manuscript is currently under review by the journal. New research has begun to examine the effects of the interaction of time with cold temperature on survival of *T. japonicus*. Much of this reporting period has been focused on methods development for these new experiments. We were discouraged to learn that the equipment available in the MAES/MDA Containment Facility can only attain low temperatures of about 5°C for extended periods of time. Initially, we thought the equipment could attain temperatures as low as -5 to -8°C, which would be more relevant for assessment of winter survival. Despite this set back, we are continuing with plans to perform experiments at 5 to 8°C to simulate conditions that would be encountered if a "cold spell" were to occur during the growing season. These methods will also allow us to assess the potential for long-term cold storage of this organism, so that large numbers could be accumulated for release in biological control programs. Briefly, we have designed and tested experimental chambers that can be used for such experiments (Figure 7). These chambers provide adequate moisture and airflow to keep the insects alive and minimize growth of mold on the interior of the chambers. Actual experiments using this methodology will begin soon.

Figure 7: Chambers created to assess effect of interaction of time and cold on *T. japonicus* survival. Left: individual chambers created from plastic vials. Right: an array of chambers being evaluated in an environmental growth chamber.

Status as of July 15, 2017:

During the reporting period, experiments were initiated to examine lethal time in terms of survival of *T. japonicus* at cold, non-freezing temperatures, which simulate conditions that would be encountered if a "cold spell" were to occur during the growing season. These experiments also allow us to assess the potential for long-term cold storage of this organism, so that large numbers could be accumulated for release in biological control programs. For the first experiment, adult males and females of *T. japonicus* were set up to be reared in growth chambers under the following conditions: 23°C without food, 8°C without food, 23°C with food and 8°C with food. Survival of the wasp will be checked periodically over a period of 100+ days. Surviving females from each observation period will be transferred to standard conditions, mated and exposed to BMSB eggs to assess sublethal effects of the rearing conditions on their ability to parasitize the host.

Status as of December 15, 2017:

The first follow-up experiment to examine lethal time in terms of survival and sublethal effects after various durations of exposure to cold, non-freezing temperatures was completed. Adult males and females of *T.*

japonicus reared at 23°C without food (i.e., honey), 8°C without food, 23°C with food, and 8°C with food. Survival of the wasps was checked over a period of 170 days and females were mated and exposed to BMSB eggs for parasitism. The following graphs summarize results of this experiment. Results show that adults survived longer when reared at the lower temperature and when they had access to food. However, even though they survived better at the lower temperatures, female wasps maintained higher reproductive (parasitism) rates when reared at the warmer temperature and when they had access to food.

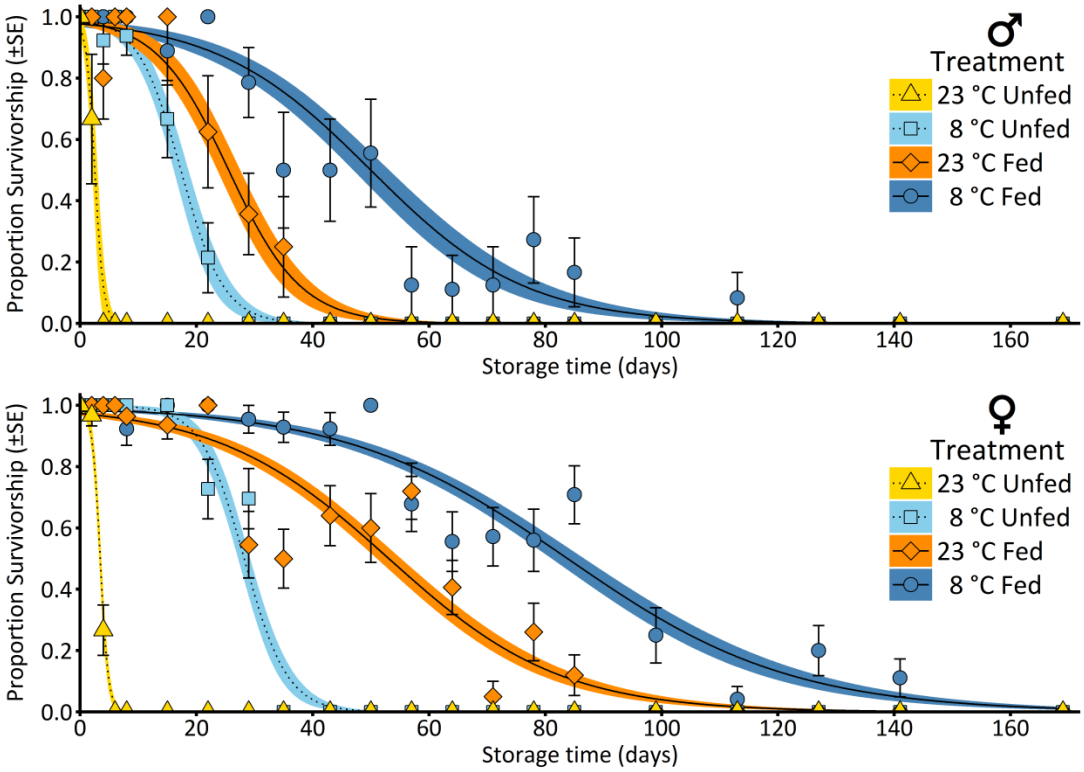


Figure 8: Survival of *T. japonicus* adults reared under different temperatures conditions and with or without food (i.e., honey).

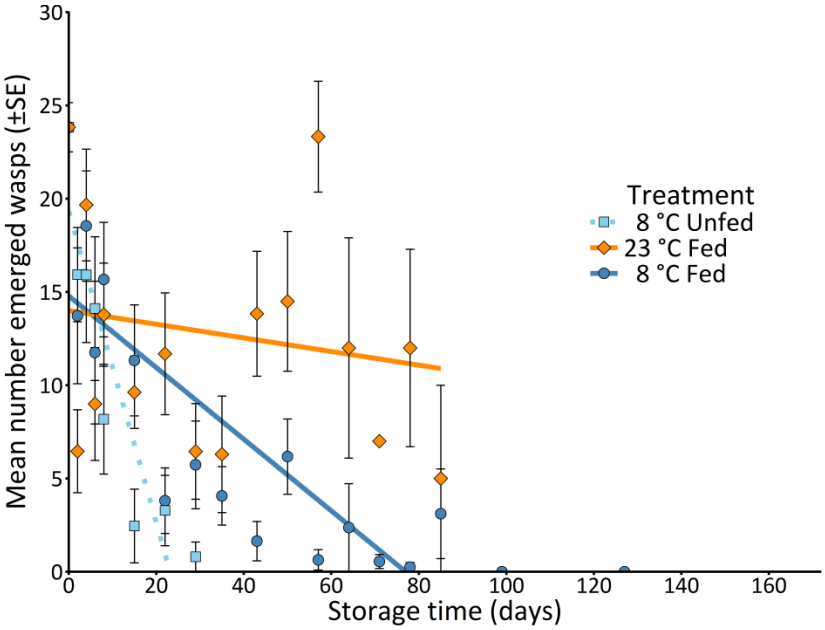


Figure 9: Reproduction (parasitism rates) of female *T. japonicus* adults reared under different temperatures conditions and with or without food (i.e., honey).

A second follow-up experiment to further examine effects of cold, non-freezing temperatures. In this experiment, adult *T. japonicus* males and females are being reared at four different temperatures (23°C, 18°C, 13°C, and 8°C) with access to food (i.e., honey). Similar to the previous experiment, we will assess survival and reproduction of these wasps overtime. Results of this experiment will be presented in the final report.

Final Report Summary:

This project was a successful collaboration between the University of Minnesota (U of MN) and the USDA to evaluate the cold hardiness of biological control agents of the brown marmorated stink bug (BMSB), as part of a larger effort to develop a biological control program for this pest. While this project was underway, an important biological control agent was documented for the first time in several eastern and western locations in the U.S., but not in Minnesota. At the U of MN, methods were developed to measure the response to cold temperature of parasitic wasps of BMSB. In particular, these methods measured the temperature at which wasps freeze (supercooling point), the temperature at which they die after short-term exposure to cold (lethal temperature), and the effects of long-term exposure to cold (lethal time). These methods were used to assess four geographically-distinct populations of the samurai wasp, *Trissolcus japonicus* from China, Korea and Japan, and two distinct Japanese populations of *T. cultratus*. *Trissolcus japonicus*, the primary species of interest for biological control releases and the species already known to occur in the USA, appears better able to survive exposure to cold than the targeted pest, BMSB. These results were published in the well-respected journal called Biological control (Nystrum Santacruz et al. 2017, https://doi.org/10.1016/j.biocontrol.2017.01.004). Furthermore, results of this cold hardiness research informed a larger modeling effort to determine the likelihood of *T. japonicus* surviving in different locations (Avila and Charles 2018, https://doi.org/10.1007/s10526-018-9866-8). The climatic suitability of Minnesota shows a south to north gradient ranging from high to marginal suitability. Therefore, based on comparison of specific cold hardiness parameters and more complex modeling, *T. japonicus* is likely to survive if introduced to Minnesota, and could provide for more sustainable management of this invasive BMSB.

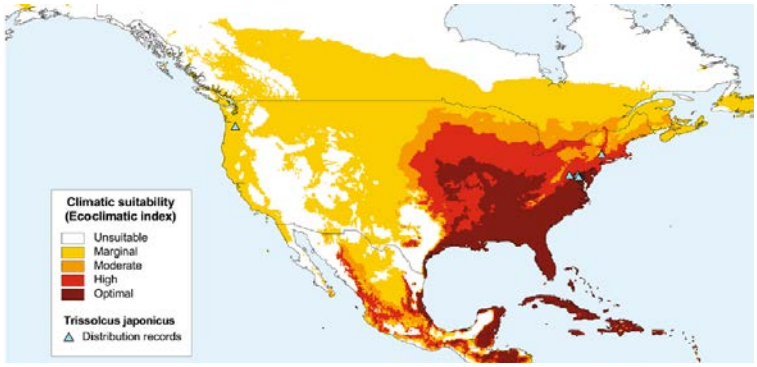


Figure 10: Excerpt from Avila and Charles (2018) showing climatic suitability of North America for survival of T. japonicus. Modeling results are partially based on cold hardiness parameters obtained for the present project.

Additional effort under this project focused on lethal time (or cold storage) have provided the U of MN with methods and knowledge for mass production and storage of T. japonicus when approval is granted for release of this insect as a biological control agent or when it is detected occurring in the wild in Minnesota. In particular, this research developed specific rearing containers and methods for storing this insect, and optimized storage conditions related to: effects of food availability and cold storage duration on adult *T. japonicus*; effect of cold storage temperature and duration on adult *T. japonicus*; effect of cold storage duration of BMSB egg masses on parasitism by adult *T. japonicus*; effects of cold storage temperature and duration on parasitized BMSB eggs; and effect of duration between parasitism and cold storage of BMSB eggs. Results of these experiments continue to be summarized and analyzed for publication. Objectives of this project were addressed and no unresolved problems were encountered.

V. DISSEMINATION:

Description:
The primary audience for this work will be producers and hobby growers of fruits and vegetables. Monitoring information for BMSB will help to anticipate problem areas before they develop. In addition to commercial and hobby growers, others will also benefit from this information due to the nuisance behavior of this insect to invade structures in the fall. Identifying areas where this may become problematic and providing that information in advance of the problem may help to avoid negative reactions among residents of these areas. Information will be disseminated to these audiences through direct email communication, web site updates, social media and news releases. The updates on the monitoring network and biological control status will be reported at relevant meetings and conferences throughout the year. We anticipate that this work will also result in an article in a scientific journal as well as presentations at national scientific meetings. However, ENRTF funds will not be used for travel to national meetings.

Status as of November 15, 2014:
This project has already media attention, with interviews provided for the Star Tribune on 6/9/2014 (http://www.startribune.com/local/262464751.html?page=1&c=y), KSTP on 6/10/2014 (http://kstp.com/article/stories/S3469363.shtml?cat=26) and WCCO-AM on 6/10/2014. An article was recently posted on the U of MN's Minnesota Crop News blog to provide an update on the status of BMSB in Minnesota and indicate that a proactive response is underway (http://blog.lib.umn.edu/efans/cropnews/2014/10/status-of-the-brown-marmorated.html). The primary audience for this blog is agricultural professionals. Initial results of this project will be presented at the annual meeting of the Entomological Society of America in Portland, Oregon on November 17, 2014. The title of the presentation will be: Cold tolerance of *Trissolcus japonicus* (Hymenoptera: Platygastridae), an egg parasitoid of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). This conference will be well-attended by entomologists from around the world.

Status as of May 15, 2015:
Initial results of this project were presented at the annual meeting of the Entomological Society of America in Portland, Oregon on November 17, 2014. The title of the presentation was be: "Cold tolerance of *Trissolcus japonicus* (Hymenoptera: Platygastridae), an egg parasitoid of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae)." This presentation was well-attended by entomologists.

Status as of November 15, 2015:

During this project period, further dissemination of results did not occur. However, during the next period, we will be able to report on further dissemination.

Status as of May 15, 2016:

Results of this project were presented by graduate student Erica Nystrom in a presentation entitled "Cold tolerance of *Trissolcus japonicus*, a potential biological control agent of *Halyomorpha halys*, the brown marmorated stink bug" at the annual meeting of the Entomological Society of America in Minneapolis, Minnesota from November 15-18, 2015. In addition, we will be presenting on results of this project to stakeholders at the Upper Midwest Invasive Species Conference. La Crosse, Wisconsin in October 2016.

Status as of November 15, 2016:

Results of this project were presented by graduate student Erica Nystrom in a public seminar entitled "Cold tolerance of *Trissolcus japonicus* and *T. cultratus*, two potential classical biological control agents of the brown marmorated stink bug" at the University of Minnesota on August 25, 2016. In addition, she presented results of this project in a presentation entitled "Cold tolerance of two potential biological control agents of the brown marmorated stink bug" to stakeholders at the Upper Midwest Invasive Species Conference. La Crosse, Wisconsin

in October 2016. Results of the project described in previous reports were compiled and written as a manuscript that was submitted for publication in the journal, Biological Control.

Status as of July 15, 2017:

During this period, we published an "extension friendly" review article on stink bug identification, biology and management in Midwestern U.S.: Koch, R.L., D.T. Pezzini, A.P. Michel and T.E. Hunt. 2017. Identification, biology, impacts and management of stink bugs (Hemiptera: Heteroptera: Pentatomidae) of soybean and corn in the midwestern United States. Journal of Integrated Pest Management 8(1): 1-14 (https://academic.oup.com/jipm/article-lookup/doi/10.1093/jipm/pmx004). The targeted audience of this publication is consultants, agency and extension staff, farmers, and other land managers. In addition, the scientific paper summarizing results from the first sets of experiments was published: Nystrom Santacruz, E., R.C. Venette, C. Dieckhoff, K. Hoelmer and R.L. Koch. 2017. Cold tolerance of *Trissolcus japonicus* and *T. cultratus*, potential biological control agents of Halyomorpha halys, the brown marmorated stink bug. Biological Control 107(1): 11-20. (http://dx.doi.org/10.1016/j.biocontrol.2017.01.004) In the short time since publication, this paper has already been cited at least four times in papers by other researchers around the world working with this insect. In addition, preliminary results of the follow-up research were presented at the 2017 meeting of the North Central Branch of the Entomological Society of America in a talk entitled, "*Trissolcus japonicus* survival and reproduction after varying exposure to constant low temperature: Implications for mass rearing of this potential biological control."

Status as of December 15, 2017:

Dr. Koch was invited to present on stink bugs at the Wisconsin Agri-Business Classic (January 2018 in Madison, WI) in a talk entitled, "Stink bugs as an emerging threat to crop production: Overview of their biology, impacts and management." Dr. Koch also presented on stink bugs and biological control with *T. japonicus* at Extension meetings during this reporting period.

Final Report Summary:

Results of this project were disseminated to scientific and grower audiences. For scientific audiences, results on the ability of biological control agents for BMSB were published in scientific article (Nystrom Santacruz et al. 2017, https://doi.org/10.1016/j.biocontrol.2017.01.004) and publication of additional results related to cold storage and mass production of biological control agents will be published soon. In addition, research was disseminated through multiple oral presentations at conferences of the Entomological Society of America and seminars at the U of MN. To reach audience of growers and the general public, results of this research have been included in several extension presentations to growers and crop consultants. Furthermore, the research stimulated attention and interviews by local media (http://www.startribune.com/local/262464751.html?page=1&c=y and http://kstp.com/article/stories/S3469363.shtml?cat=26). While working on this project, it was observed that there was a general lack of accessible information on the biology and management of BMSB and other stink bugs for crop and land managers. Therefore, we wrote and published an extension-friendly article on these topics in the Journal of IPM (Koch et al. 2017, https://doi.org/10.1093/jipm/pmx004), which also received considerable media attention.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

University of Minnesota

Budget Category	$ Amount	Explanation
Personnel:	$135,864	1 Grad Student (1.0 FTE):

	$6,282	Salary ($20,177/yr) + Fringe@16.8% & Tuition ($18,854/yr) + Student assistance ($8,174/yr) * 3 yrs 1 Undergraduate Student ($12.08/hr x 260 hrs) x 2 yrs
Equipment/Tools/Supplies:	$4,503	Cages & supplies for maintaining insect populations in lab: $1,501/yr * 3 yrs
Printing:	$1,000	Publication fees: 2 scientific articles * $500 / article
Other:	$19,351	Fee for use of space in quarantine facility, growth chambers & greenhouse.
TOTAL ENRTF BUDGET:	**$167,000**	

Explanation of Use of Classified Staff: N.A.

Explanation of Capital Expenditures Greater Than $5,000: N.A.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 3.2
UMN Grad Student: 1.0 FTE for 3 years
UMN Undergraduate Student Worker: 225 hrs/yr for 2 yrs

Number of Full-time Equivalents (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			
	$	$	
State			
	$	$	
TOTAL OTHER FUNDS:	**$**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

Receiving funds: Mark Abrahamson with MDA will lead the monitoring work (**receiving $99,000**). Dr. Robert Koch with U of MN will lead the work to evaluate potential biological control agents for suitability in Minnesota (**receiving $167,000**). Both organizations will provide in-kind equipment, facilities, and GIS/technical support.

Not receiving funds: For monitoring, we will draw volunteers from the various groups such as Master Gardeners, First Detectors and Producers. For evaluation of the biological control agents, Dr. Robert Venette with the USDA Forest Service will provide technical guidance on overwintering biology and cold hardiness. Dr. Kim Hoelmer of the USDA ARS will provide biological control agents for this work.

B. Project Impact and Long-term Strategy:

This project will put in place a monitoring network for BMSB that will likely prove useful for years to come and will be a first step towards implementation of biological control for BMSB, which is a critical need for proactively dealing with this pest in an economically and environmentally sustainable manner. This work will aid in selection of biological control agents for use in Minnesota. If one or more biological control agents show a high

likelihood for survival in Minnesota, the next step will be work on implementation of a control program after approval for release has been granted.

C. Spending History:

Funding Source	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13	M.L. 2013 or FY14

VIII. ACQUISITION/RESTORATION LIST: N.A.

IX. VISUAL ELEMENT or MAP(S):
See attached visual graphic.

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET:
N.A.

XI. RESEARCH ADDENDUM:
N.A.

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than 11/15/2014, 5/15/2015, 11/15/2015, 5/15/2016, 11/15/2016, 7/15/2017 and 12/15/2017. A final report and associated products will be submitted between June 30 and August 15, 2018.

Brown Marmorated Stink Bug Biocontrol Evaluation

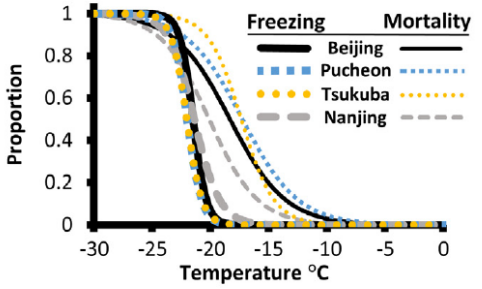
R. Koch (University of Minnesota), R. Venette (US Forest Service) & K. Hoelmer (USDA ARS)

Brown marmorated stink bug (BMSB) is an invasive crop pest and household invader.

Biological control with parasitic wasps attacking BMSB eggs was evaluated.
The samurai wasp is the most promising potential biological control agent for BMSB.

Samurai wasp is likely to survive Minnesota's cold winters and is better able to survive cold than BMSB

• Source locations of *Trissolcus* spp.



Freezing and mortality temperatures of *T. japonicus*

Methods were developed for cold storage and mass production of samurai wasp for biological control.

Environment and Natural Resources Trust Fund

M.L. 2014 Project Budget

Project Title: *Brown Marmorated Stink Bug Monitoring and Biocontrol Evaluation*

Legal Citation: *M.L. 2014, Chp. 226, Sec. 2, Subd. 04f-1*

Project Manager: *Robert Koch*

Organization: *University of Minnesota*

M.L. 2014 ENRTF Appropriation: $*167,000*

Project Length and Completion Date: *4 Years, June 30, 2018*

Date of Report: June 30, 2018

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***See MDA budget***			***Studies on overwintering potential of BMSB control agents in Minnesota***				
Personnel (Wages and Benefits)				$142,146	$142,146	$0	$142,146	$0
*1 Student (1.0 FTE): Salary ($20,177/yr) + Fringe@16.8%&Tuition ($18,854/yr) + Student assistance ($8,174/yr) * 3 yrs*								
1 Undergraduate Student ($12.08/hr x 260 hrs) x 2 yrs - $6,282 total								
Professional/Technical/Service Contracts								
Equipment/Tools/Supplies								
Cages & supplies for maintaining insect populations in lab: $1,501/yr * 3 yrs				$4,503	$4,503	$0	$4,503	$0
Capital Expenditures Over $5,000								
Printing								
Publication fees: 2 scientific articles * $500 / article				$1,000	$228	$772	$1,000	$772
Other								
Fee for use of space in quarantine facility & growth chambers: $400/mo * 34 months				$19,351	$19,351	$0	$19,351	$0
COLUMN TOTAL				**$167,000**	**$154,190**	**$12,810**	**$167,000**	**$772**