

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

AFFILIATION: University of Minnesota

MAILING ADDRESS: 1980 Folwell Ave.

CITY/STATE/ZIP: St. Paul, MN 55108

PHONE: 612-624-6771

E-MAIL: koch0125@umn.edu

WEBSITE:

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
Mailing Address: 1980 Folwell Avenue
City/State/Zip Code: St. Paul
Telephone Number: (612) 624-6771
Email Address: koch0125@umn.edu
Web Address:

Location: St. Paul, Ramsey County, Minnesota

Total ENRTF Project Budget:	ENRTF Appropriation:	\$167,000
	Amount Spent:	<u>\$166,238</u>
	Balance:	<u>\$772</u>

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. caltratus*. 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)

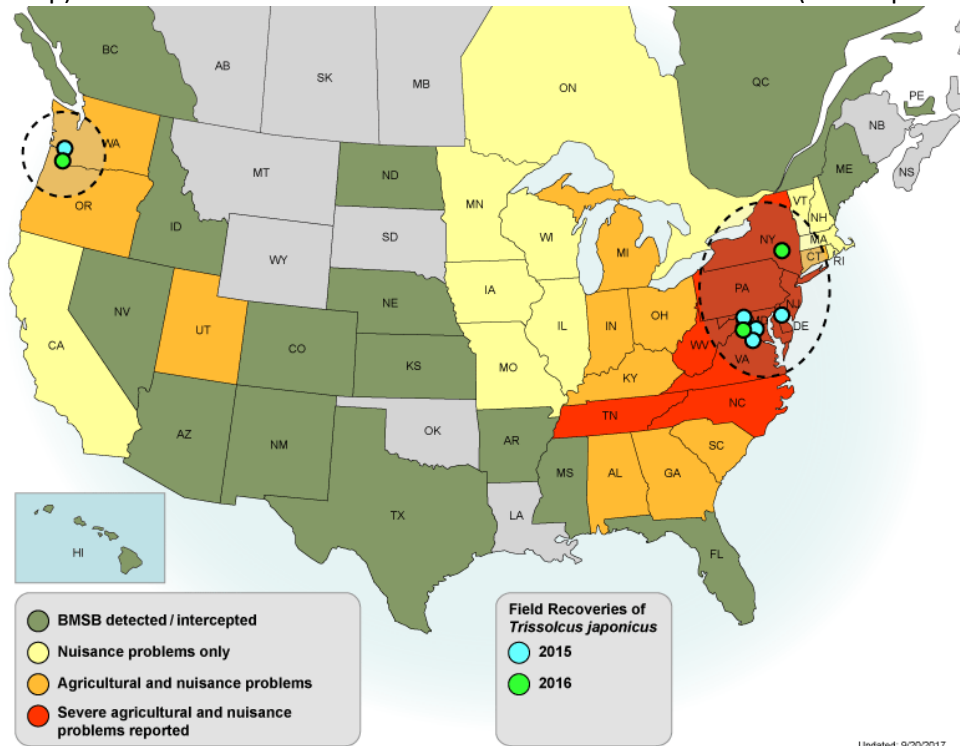
A 12-month no-cost extension is being requested for the U of MN component of this project. It is projected that we will have about \$30,000 remaining at the initially agreed upon end date for this project. This is due to salary savings that were experienced when staff was being billed only part-time 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. cultratus* (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 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.

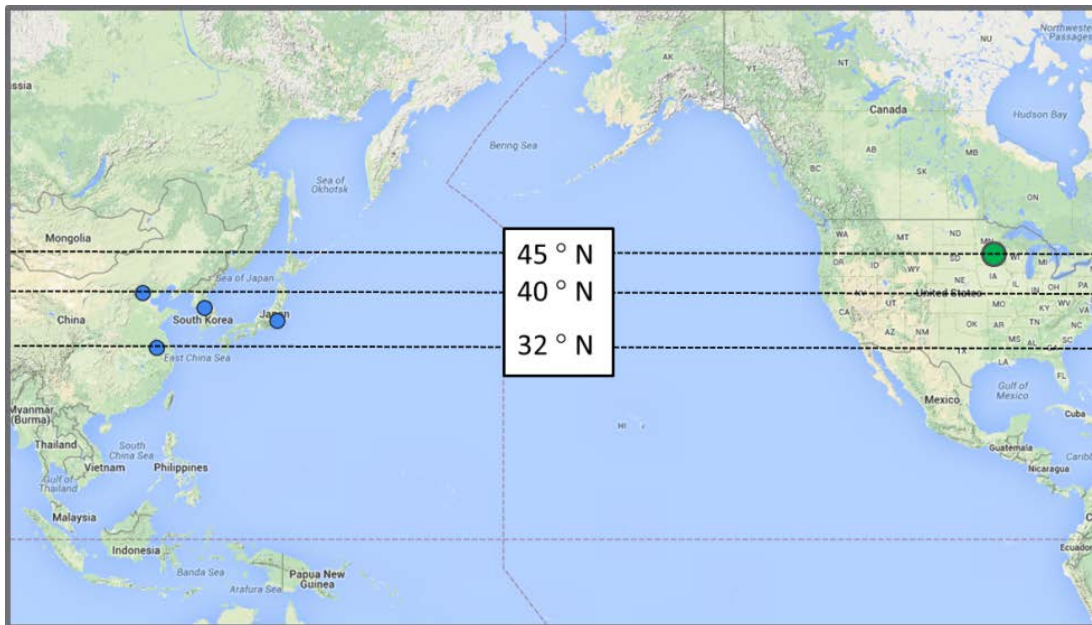


Figure 2: Map showing geographic origins of Asian populations of *T. japonicus* being examined for cold tolerance. Note: all these populations are from more southerly locations than Minnesota.



Photo credit: Christine Dieckhoff

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*.

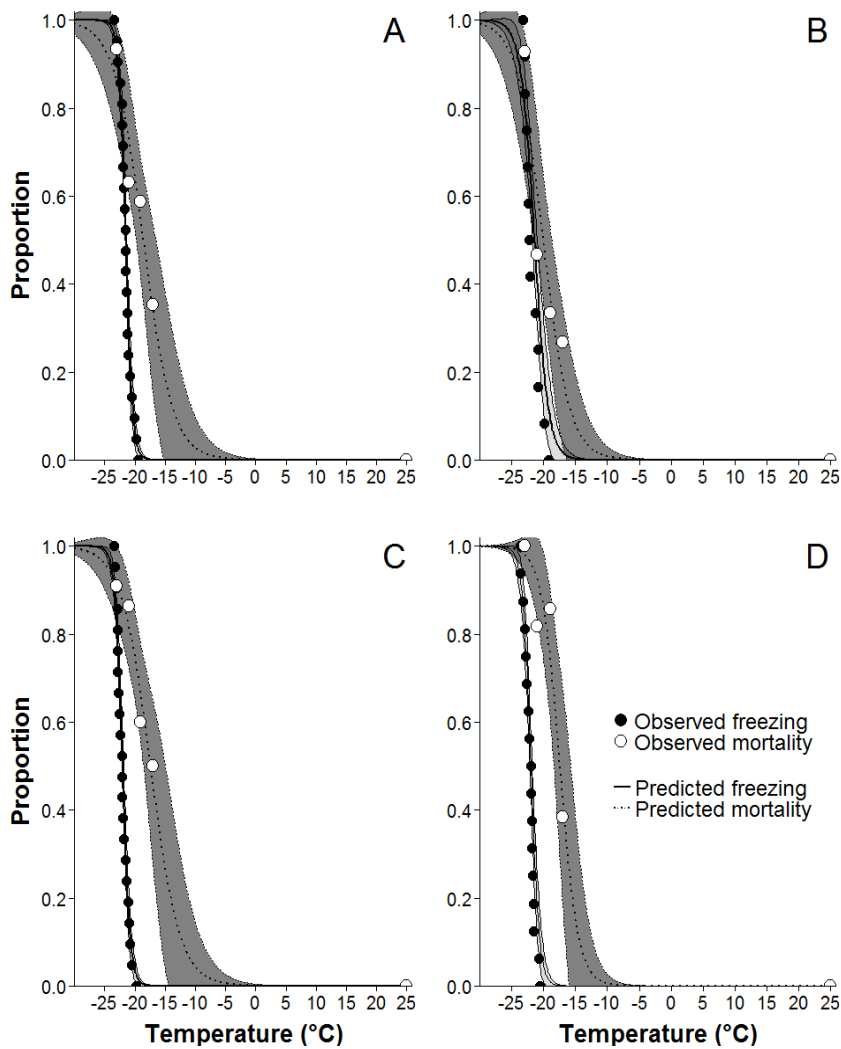


Figure 4: Observed (symbols), and predicted (lines ± 95% confidence bands) mortality and cumulative freezing in *Trissolcus japonicus* populations: (A) Beijing, China; (B) Nanjing, China; (C) Pucheon South Korea; (D) Tsukuba, Japan.

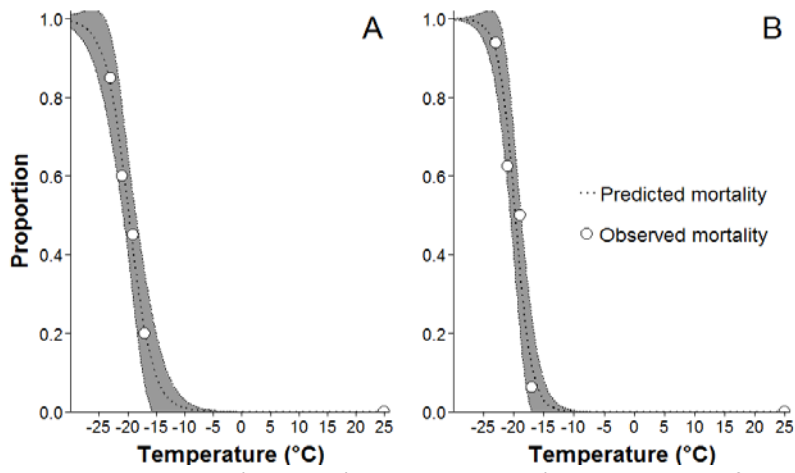


Figure 5: Observed (symbols), and predicted (lines \pm 95% confidence bands) mortality in *Trissolcus cultratus* populations: (A) Japan1, (B) Japan2.



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.

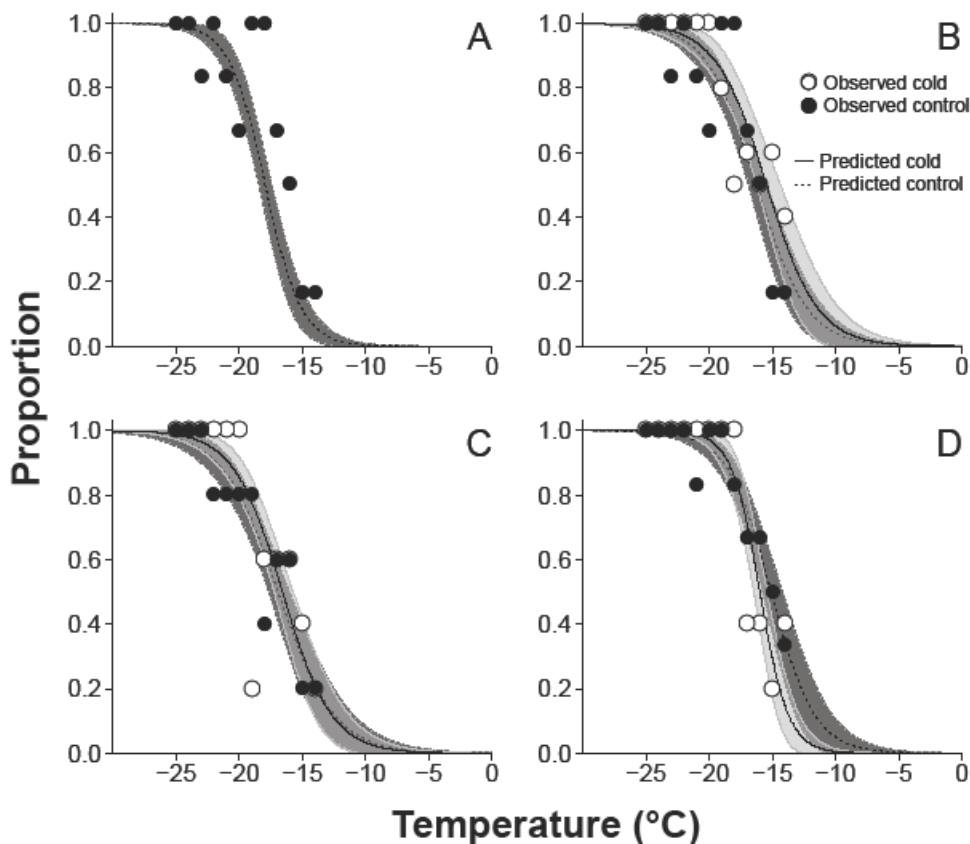


Figure 6. Observed and predicted mortality (\pm SE band) for *Trissolcus japonicus* reared on control (i.e., standard rearing) conditions and cold (i.e., lower temperature and shorter day length) conditions. Panels of this figure represent the different generations reared under these conditions (A: Parental;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

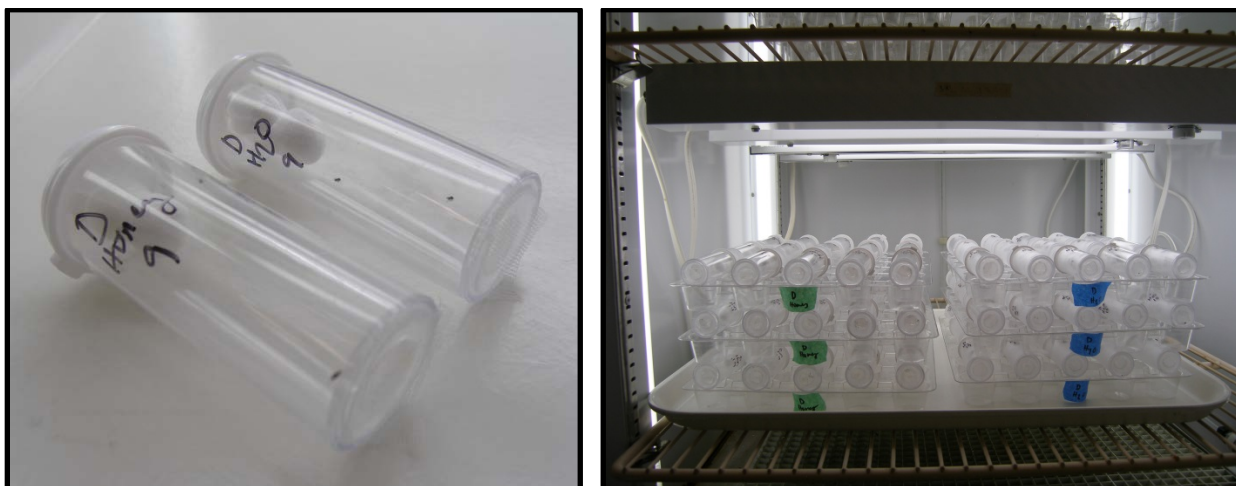


Figure 7: Chambers created to assess effect of interaction of time and cold on *T. japonicus* survival. Left: individual chambers created from plastic vials. Right: an array of chambers being evaluated in an environmental growth chamber.

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.

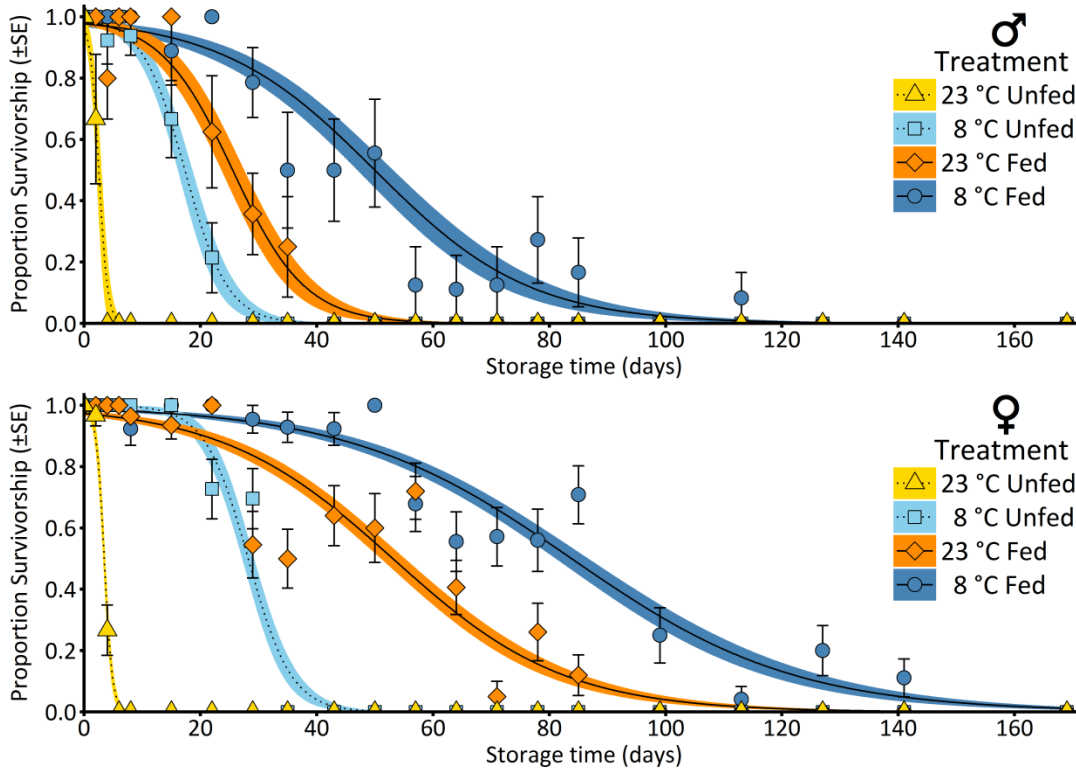


Figure 8: Survival of *T. japonicus* adults reared under different temperatures conditions and with or without food (i.e., honey).

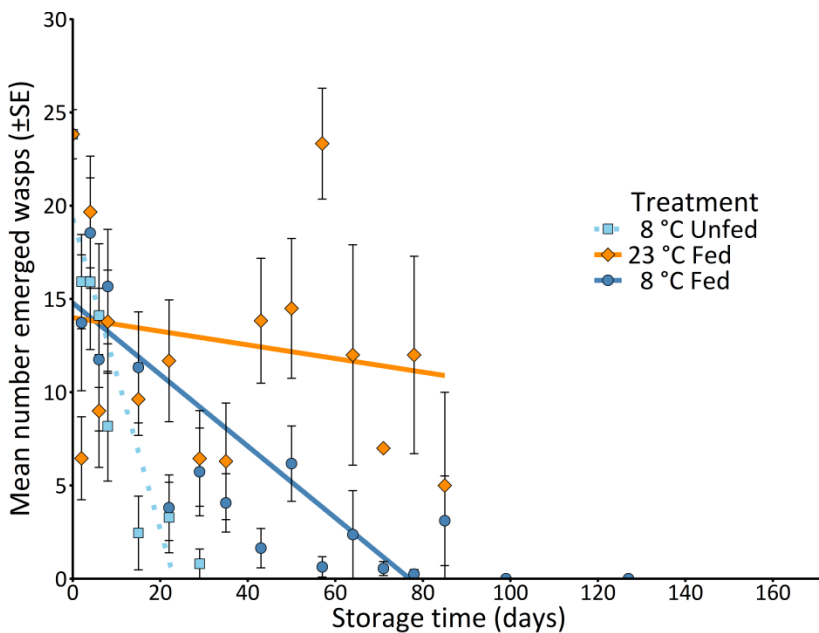


Figure 9: Reproduction (parasitism rates) of female *T. japonicus* adults reared under different temperatures conditions and with or without food (i.e., honey).

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.

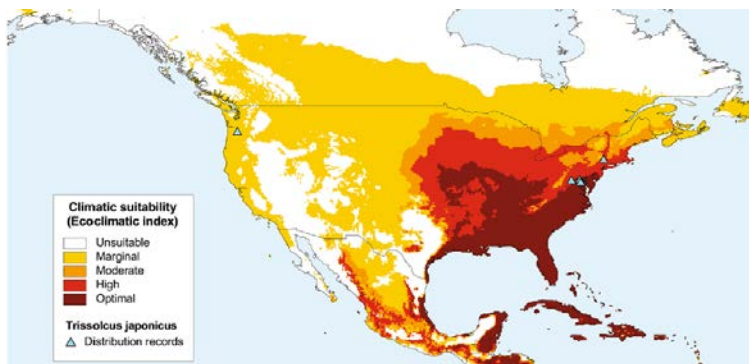


Figure 10: Excerpt from Avila and Charles (2018) showing climatic suitability of North America for survival of *T. japonicus*. Modeling results are partially based on cold hardiness parameters obtained for the present project.

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: Platygasteridae), 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: Platygasteridae), 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 & <u>greenhouse</u> .
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 Equivalent (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 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			
	\$	\$	
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.

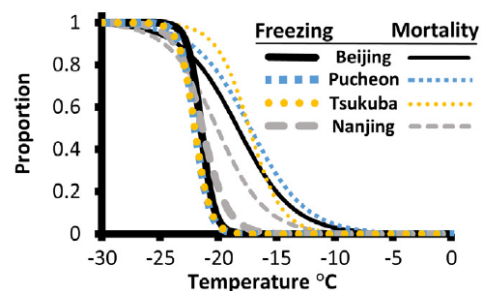


Biological control with parasitic wasps attacking BMSB eggs was evaluated. The samurai wasp is the most promising potential biological control agent for BMSB.

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: <i>Brown Marmorated Stink Bug Monitoring and Biocontrol Evaluation</i>								
Legal Citation: <i>M.L. 2014, Chp. 226, Sec. 2, Subd. 04f-1</i>								
Project Manager: <i>Robert Koch</i>								
Organization: <i>University of Minnesota</i>								
M.L. 2014 ENRTF Appropriation: \$167,000								
Project Length and Completion Date: <i>4 Years, June 30, 2018</i>								
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	<i>See MDA budget</i>			<i>Studies on overwintering potential of BMSB control agents in Minnesota</i>				
Personnel (Wages and Benefits)				\$142,146	\$142,146	\$0	\$142,146	\$0
<i>1 Student (1.0 FTE): Salary (\$20,177/yr) + Fringe @16.8%&Tuition (\$18,854/yr) + Student assistance (\$8,174/yr) * 3 yrs</i>								
<i>1 Undergraduate Student (\$12.08/hr x 260 hrs) x 2 yrs - \$6,282 total</i>								
Professional/Technical/Service Contracts								
Equipment/Tools/Supplies								
<i>Cages & supplies for maintaining insect populations in lab: \$1,501/yr * 3 yrs</i>				\$4,503	\$4,503	\$0	\$4,503	\$0
Capital Expenditures Over \$5,000								
Printing								
<i>Publication fees: 2 scientific articles * \$500 / article</i>				\$1,000	\$228	\$772	\$1,000	\$772
Other								
<i>Fee for use of space in quarantine facility & growth chambers: \$400/mo * 34 months</i>				\$19,351	\$19,351	\$0	\$19,351	\$0
COLUMN TOTAL				\$167,000	\$154,190	\$12,810	\$167,000	\$772

Identification, Biology, Impacts, and Management of Stink Bugs (Hemiptera: Heteroptera: Pentatomidae) of Soybean and Corn in the Midwestern United States

Robert L. Koch,^{1,2} Daniela T. Pezzini,¹ Andrew P. Michel,³ and Thomas E. Hunt⁴

¹Department of Entomology, University of Minnesota, 1980 Folwell Ave., Saint Paul, MN 55108 (koch0125@umn.edu; pezzi004@umn.edu), ²Corresponding author, e-mail: koch0125@umn.edu, ³Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University, 210 Thorne, 1680 Madison Ave. Wooster, OH 44691 (michel.70@osu.edu), and ⁴Department of Entomology, University of Nebraska, Haskell Agricultural Laboratory, 57905 866 Rd., Concord, NE 68728 (thunt2@unl.edu)

Subject Editor: Jeffrey Davis

Received 12 December 2016; Editorial decision 22 March 2017

Abstract

Stink bugs (Hemiptera: Heteroptera: Pentatomidae) are an emerging threat to soybean and corn production in the midwestern United States. An invasive species, the brown marmorated stink bug, *Halyomorpha halys* (Stål), is spreading through the region. However, little is known about the complex of stink bug species associated with corn and soybean in the midwestern United States. In this region, particularly in the more northern states, stink bugs have historically caused only infrequent impacts to these crops. To prepare growers and agricultural professionals to contend with this new threat, we provide a review of stink bugs associated with soybean and corn in the midwestern United States. Descriptions and images of common stink bug species are provided as a diagnostic aid. The biologies and impacts of stink bugs to crops are discussed, with particular attention to differences among species. Based primarily on information from southern states, scouting, thresholds, and insecticide-based management of these pests are discussed. It is hoped that this review will provide stakeholders sufficient information for management of these pests, until more region-specific research can be performed on stink bugs in soybean and corn in the midwestern United States.

Key words: *Chinavia hilaris*, *Euschistus servus*, *Euschistus variolarius*, *Halyomorpha halys*, *Podisus maculiventris*

The midwestern United States is the top *Glycine max* L. Merrill (soybean)- and *Zea mays* L. (corn)-producing region of the United States (National Agricultural Statistics Service [NASS] 2015). In this region, the stink bug (Hemiptera: Heteroptera: Pentatomidae) fauna is relatively diverse, comprising 45–57 species per state (McPherson 1982, Packauskas 2012, Rider 2012, Sites et al. 2012, Swanson 2012, Koch et al. 2014) and includes species known to be pests of corn and soybean in some regions (McPherson and McPherson 2000, Panizzi et al. 2000).

The significance of stink bugs in the midwestern United States is increasing. First, an invasive species of Asian origin, *Halyomorpha halys* (Stål) (brown marmorated stink bug), is invading the region (Tindall et al. 2012, Koch 2014). This species was first collected in North America in Pennsylvania in 1996 (Hoebeke and Carter 2003) and has since spread throughout much of the continental United States (Leskey et al. 2012a, Rice et al. 2014). *Halyomorpha halys* is a pest of many crops, including soybean and corn (Leskey et al. 2012a, Lee et al. 2013, Rice et al. 2014). Economically significant infestations of this pest in fruit, vegetable, and field crops had been limited primarily to the mid-Atlantic region of the United States

(Leskey et al. 2012a, Rice et al. 2014) but are expanding westward into the midwestern United States (<http://www.stopbmsb.org/where-is-bmsb/state-by-state/>). As populations of this species increase in the midwestern United States, increasing frequencies of economically significant infestations are likely. Since 2012, *H. halys* has been regularly found in Ohio soybean fields and sometimes at economic levels with other stink bug species (Michel et al. 2013). Another invasive species, *Piezodorus guildinii* (Westwood) (redbanded stink bug), is currently established in the southeastern United States and may be expanding its range northward (Tindall and Fothergill 2011). *Piezodorus guildinii* is a significant pest of soybean (McPherson and McPherson 2000, Panizzi et al. 2000). In addition to the spread of new invasive species, the abundance of native stink bug species appears to be increasing in the midwestern United States (Hunt et al. 2011, 2014; Michel et al. 2013). In particular, *Chinavia hilaris* (Say) (green stink bug), *Euschistus servus* (Say) (brown stink bug), *Euschistus variolarius* (Palisot de Beauvois) (onespotted stink bug), and *Thyanta custator accerra* McAtee (redshouldered stink bug) have been increasing in abundance and frequency for the past several years (Michel and Hunt, personal observations).

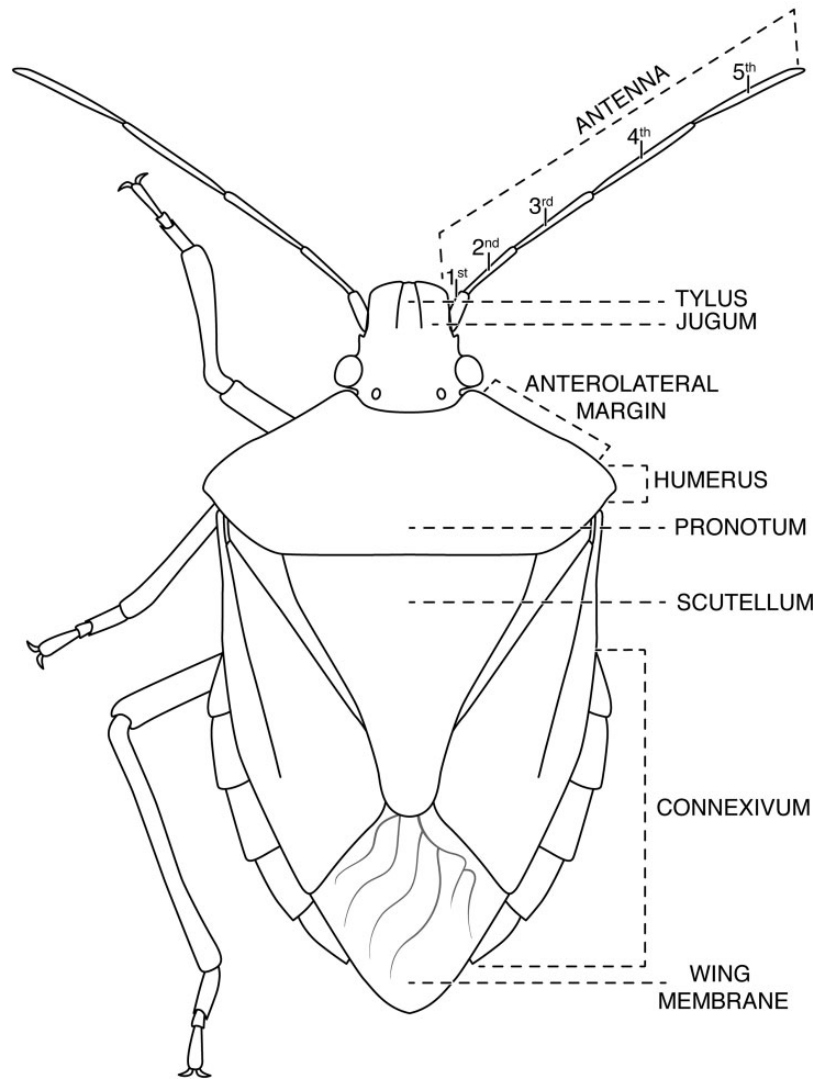


Fig. 1. Line drawing of stink bug adult showing body parts important for discrimination of species common in soybean and corn in the midwestern United States (image credit: A.K. Tran).

Owing to the growing attention stink bugs are receiving in the midwestern United States and the lack of a recent, comprehensive resource accessible to agricultural professionals, agency and Extension staff, and producers, we compiled this review of the identification, biology, impacts, and management of stink bugs in soybean and corn in the midwestern United States.

Identification

General

Stink bugs vary in shape and size, but are generally described as having round or oval (sometimes shield-shaped) bodies, a well-developed and often triangle-shaped scutellum, piercing-sucking mouthparts, and five-segmented antennae (Slater and Baranowski 1978, McPherson 1982, Panizzi et al. 2000; Figs. 1 and 2). At least 24 species or subspecies of stink bugs could potentially be encountered in soybean and corn in the midwestern United States (Table 1). This list was created by developing and cross-referencing lists of species of stink bugs known to occur in the midwestern United States [based on McPherson (1982), Packauskas (2012), Rider (2012),

Sites et al. (2012), Swanson (2012), and Koch et al. (2014)] and species of stink bugs reported from soybean and corn [based on McPherson (1982), Panizzi and Slansky (1985), McPherson and McPherson (2000), and several more recent crop-specific publications (see table footnotes)]. Some of these species have less extensive geographic ranges and do not occur in all parts of the midwestern United States (e.g., *P. guildinii* is more of a southern species). Depending on geography within the midwestern United States or species of interest, several diagnostic keys for stink bug identification are available, including McPherson (1982) for stink bugs and relatives of northeastern North America, McPherson and McPherson (2000) for stink bugs of economic importance, Rider (2012) for stink bugs and relatives of North Dakota, Paiero et al. (2013) for stink bugs and parent bugs of Ontario, Packauskas (2012) for stink bugs of Kansas, and Swanson (2012) for stink bugs and relatives of Michigan. The electronic key by Paiero et al. (2013) is particularly user friendly with many color photographs.

Among the species potentially encountered in soybean and corn in the midwestern United States (Table 1), several are encountered with frequency. Koch and Pahs (2014, 2015) and Koch and Rich (2015) recently performed surveys of the stink bugs associated with

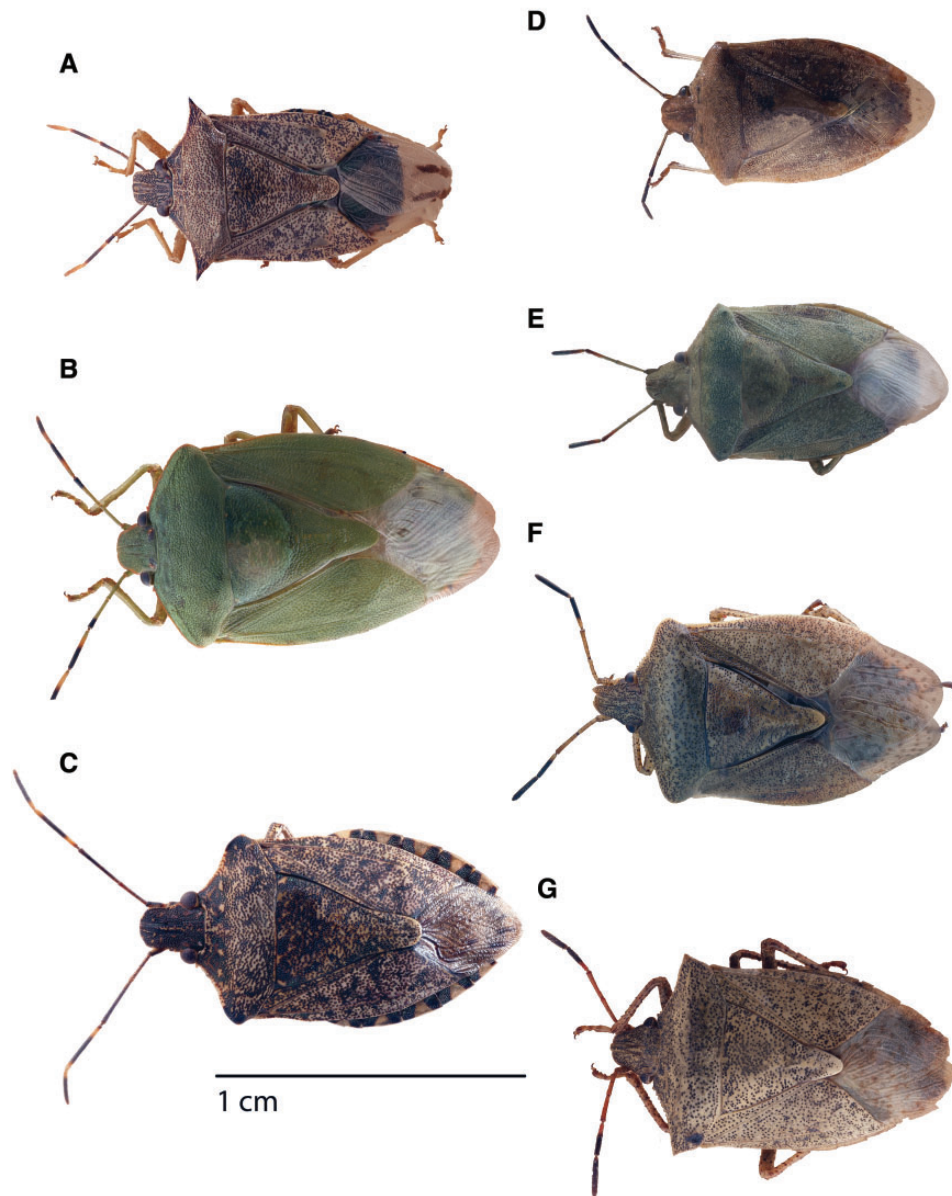


Fig. 2. Stink bug adults commonly encountered in soybean and corn of the midwestern United States: (A) *Podisus maculiventris*, (B) *Chinavia hilaris*, (C) *Halyomorpha halys*, (D) *Thyanta custator accerra* (green), (E) *Thyanta custator accerra* (brown), (F) *Euschistus servus euschistoides*, and (G) *Euschistus variolarius* (photo credits: C. Kurtz and C. Phillips, and modified by D. Pezzini).

soybean and corn in Minnesota. In Minnesota soybean, *E. variolarius*, *Euschistus servus euschistoides*, and *C. hilaris* comprise 68–90% of stink bug adults (Koch and Pahn 2014, Koch and Rich 2015). In Minnesota corn, *E. variolarius* and *E. servus euschistoides* comprised 95–100% of stink bug adults (Koch and Pahn 2015). A predatory species, *Podisus maculiventris* (spined soldier bug) is also observed in corn and soybean (Koch and Pahn 2014, 2015; Koch and Rich 2015). Further work is needed to characterize the stink bug community associated with these crops in other states in the region. Understanding of stink bug anatomy (i.e., body parts) is important for identification of these species (Fig. 1).

Euschistus servus (Brown Stink Bug)

Euschistus servus occurs throughout the midwestern United States and much of North America (McPherson 1982), and is the most

common species of *Euschistus* in the United States (Slater and Baranowski 1978). This species is composed of two subspecies (McPherson 1982). *Euschistus servus euschistoides* occurs in the northern United States (Fig. 2), and *E. servus servus* occurs in the southern United States. These two subspecies are known to interbreed and create a hybrid population, where their two populations meet in a swath extending from roughly Kansas to Maryland (McPherson 1982). These subspecies and the hybrid are similar in size (11.0–15.0 mm long; McPherson 1982) and color (brown or light brown). However, the subspecies can be distinguished from each other based on close examination of the head, antennae, and edge of the abdomen (i.e., connexivum). The tip of the head of *E. servus euschistoides* appears notched because the jugs are longer than the tylus, whereas the tip of the head of *E. servus servus* does not appear notched, because the jugs and tylus are equal or nearly equal in length (Paiero et al. 2013). In addition, the last two

Table 1. Plant-feeding stink bugs potentially encountered in soybean and corn of the midwestern United States (“X” indicates reported presence of a species in a particular crop)

Scientific name	Common name	Soybean	Corn
<i>Banasa dimidiata</i> (Say) ^{a,b,c,d,e}		X ^g	
<i>Banasa euchlora</i> Stål ^{a,b,c}		X ^g	
<i>Chinavia hilaris</i> (Say) ^{a,b,c,d,e}	Green stink bug	X ^{f,g,h,i,j,k,l}	X ^{f,h,i,j}
<i>Chinavia pennsylvanica</i> (Gmelin) ^{c,d,e}		X ^g	
<i>Chlorochroa persimilis</i> Horvath ^{a,b,c,d,e}		X ^l	X ^{f,h,i,j}
<i>Coenus delius</i> (Say) ^{a,b,c,d,e}		X ^{f,l}	
<i>Cosmopepla lintneriana</i> Kirkaldy ^{a,b,c,d,e}		X ^l	X ^j
<i>Euschistus ictericus</i> (L.) ^{b,c,d,e}		X ^{f,g,k,l}	X ^{i,o}
<i>Euschistus servus</i> (Say) ^{a,b,c,d,e}	Brown stink bug	X ^{f,g,h,i,j,k,l}	X ^{f,h,i,j,o}
<i>Euschistus tristigmus</i> (Say) ^{a,b,c,d,e}	Dusky stink bug	X ^{f,g,h,i,j,k,l}	X ^{i,o}
<i>Euschistus variolarius</i> (Palisot de Beauvois) ^{a,b,c,d,e}	Onespotting stink bug	X ^{f,g,h,i,j,l}	X ^{f,g,o}
<i>Halyomorpha halys</i> (Stål) ^{c,d,e}	Brown marmorated stink bug	X ^m	X ^m
<i>Holcostethus limbolarius</i> (Stål) ^{a,b,c,d,e}		X ^{f,g,l}	X ^f
<i>Hymenarcys nervosa</i> (Say) ^{a,c,d}		X ^{f,g,j}	X ⁿ
<i>Mecidea major</i> Sailer ^{a,c,e}		X ⁱ	
<i>Mormidea lugens</i> (F.) ^{a,b,c,d,e}		X ^{g,l}	
<i>Murgantia histrionica</i> (Hahn) ^{a,b,c,e}	Harlequin bug		X ^f
<i>Neottiglossa undata</i> (Say) ^{b,c,d,e}		X ^l	
<i>Oebalus pugnax</i> (F.) ^{a,c,d,e}	Rice stink bug	X ^{f,g,i,k}	X ^{f,i,j}
<i>Piezodorus guildinii</i> (Westwood) ^c	Redbanded stink bug	X ^{g,h,i,k}	
<i>Proxys punctulatus</i> (Palisot de Beauvois) ^c		X ^{f,g}	
<i>Thyanta calceata</i> (Say) ^{c,d}		X ^{f,g}	
<i>Thyanta custator accerra</i> McAtee ^{a,b,c,d,e}	Redshouldered stink bug	X ^{f,g,i,j,k,l}	X ^{f,j}
<i>Thyanta pallidovirens</i> (Stål) ^a		X ^g	

References for occurrence in midwestern United States:

^a Packauskas (2012),

^b Rider (2012),

^c Sites et al. (2012),

^d Swanson (2012),

^e Koch et al. (2014)

References for crop associations:

^f McPherson (1982),

^g Panizzi and Slansky (1985),

^h McPherson and McPherson (2000),

ⁱ Tillman (2010),

^j Suh et al. (2013),

^k Temple et al. (2013),

^l Koch and Pahs (2014),

^m Rice et al. (2014),

ⁿ Sedlacek and Townsend (1988b),

^o Koch and Pahs (2015)

segments of the antennae (i.e., segments four and five) are usually dark brown in *E. servus euschistoides* and yellowish-brown or reddish-brown in *E. servus servus* (Paiero et al. 2013). Finally, the edge of the abdomen is completely or nearly completely covered by the front wing in *E. servus euschistoides*, whereas the edge of the abdomen is more exposed in *E. servus servus* (Paiero et al. 2013). Hybrid adults present a combination of the characters of the two subspecies (McPherson 1982).

Euschistus variolarius (Onespotting Stink Bug)

Euschistus variolarius occurs throughout the midwestern United States and much of North America (McPherson 1982). This is the most common stink bug in northern states, but it is relatively uncommon in the southern United States, particularly below 37° latitude (Parish 1934, Slater and Baranowski 1978). Adults of *E. variolarius* are yellowish-brown and 11.0–15.0 mm long (McPherson 1982, Panizzi et al. 2000; Fig. 2). Males have a large

black spot on the underside near the tip of the abdomen (i.e., on the pygophore), hence the common name “onespotting stink bug” (Paiero et al. 2013). *Euschistus variolarius* and *E. servus* can be found in the same habitats at the same time (Koch and Pahs 2014, 2015) and look very similar to one another. However, *E. variolarius* can be fairly easily distinguished from *E. servus euschistoides* (i.e., the more common subspecies in much of the midwestern United States) by examination of the tip of the head and the “shoulders” (i.e., anterolateral margins of the pronotum; Fig. 2). The tip of the head of *E. servus euschistoides* appears notched, because the juga are longer than the tylus, whereas the tip of the head of *E. variolarius* does not appear notched, because the juga and tylus are equal or nearly equal in length (Paiero et al. 2013). The “shoulders” of *E. variolarius* are generally more pointed than those of *E. servus euschistoides* (McPherson 1982). Presence of the black spot on the pygophore of *E. variolarius* males is lacking in *E. servus* (McPherson 1982), but should not be confused with the black spots on the underside of the abdomen of a slightly smaller species, *Euschistus tristigmus* (Say).

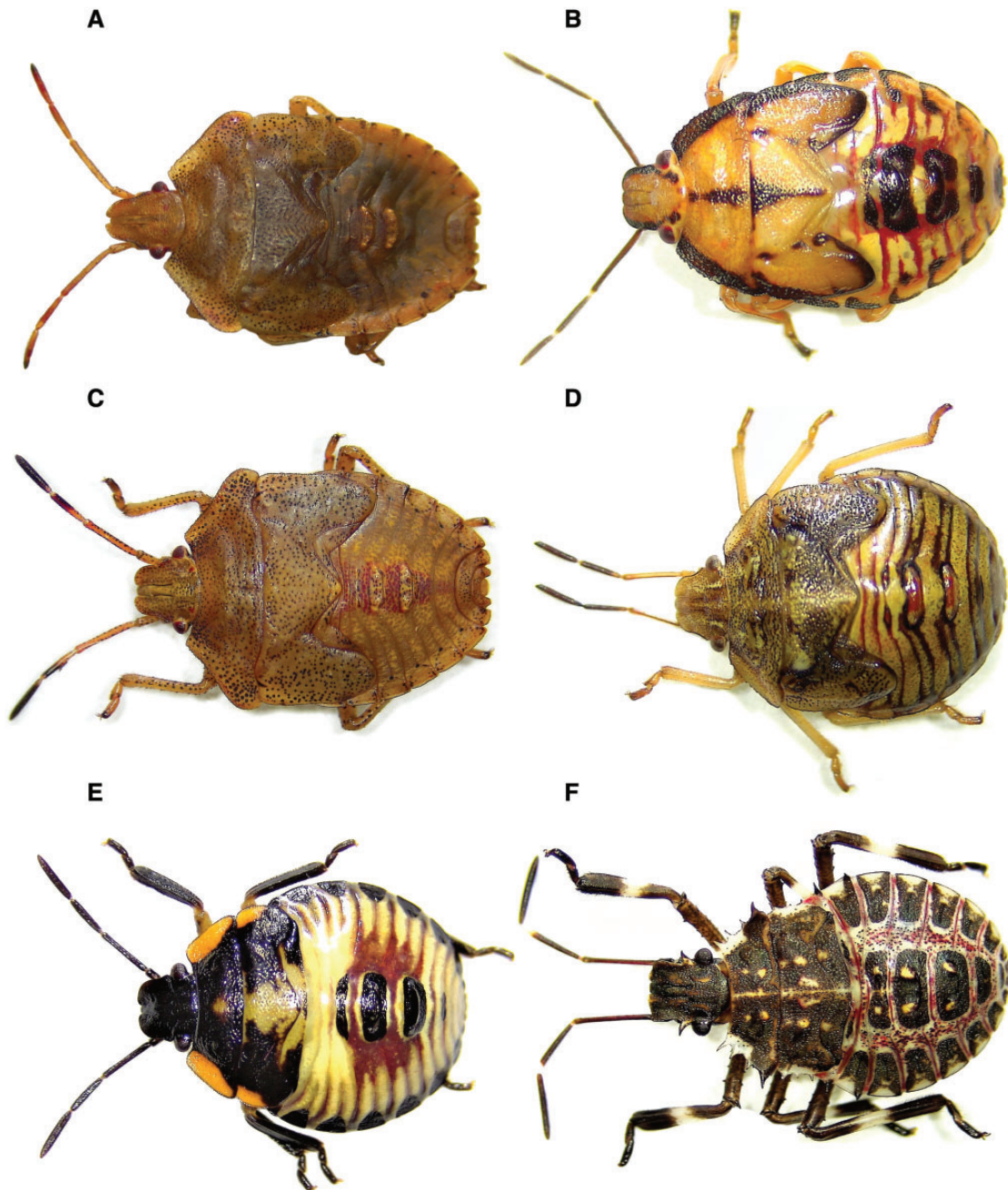


Fig. 3. Stink bug nymphs (late-instars) encountered in soybean and corn of the midwestern United States: (A) *Euschistus servus*, (B) *Podisus maculiventris*, (C) *Euschistus variolarius*, (D) *Thyanta custator*, (E) *Chinavia hiliaris*, and (F) *Halyomorpha halys* (photo credit: D. Pezzini).

A diagnostic key and descriptions of the nymphal instars of *E. servus* and *E. variolarius* are provided by Decoursey and Esselbaugh (1962) and Munyaneza and McPherson (1994). In general, nymphs of both species range from 1.5 to 10.4 mm long and are yellow brown in color (Fig. 3). First and second instars of the two species are nearly identical, but the later instars can be distinguished. Third to fifth instars *E. variolarius* have a white band on the under (i.e., ventral) side of the head that extends to or nearly to the eyes; however, this band is much reduced in *E. servus* (Munyaneza and McPherson 1994). In addition, the fourth and fifth instars differ in coloration of the last two (i.e., fourth and fifth) antennal segments, which are brownish-black in *E. variolarius* and red or reddish-brown in *E. servus* (Munyaneza and McPherson 1994; Fig. 3).

Chinavia hiliaris (Green Stink Bug)

Chinavia hiliaris occurs throughout the midwestern United States and much of North America (McPherson 1982). This species is often referred to *Acrosternum hilare* (Say) (Kamminga et al. 2012). Adults of *C. hiliaris* are green and 13.0–19.0 mm long (McPherson 1982, Panizzi et al. 2000; Fig. 2). However, an orange color form is infrequently encountered (Kamminga et al. 2012). In crops in the midwestern United States, *C. hiliaris* is generally larger than the other green-colored stink bugs, such as *Chlorochroa persimilis* (11–15 mm) or *T. custator accerra* (see below; McPherson 1982, Rider 2012). Nymphs of *C. hiliaris* are oval-shaped and range in size from 1.6 to 12.7 mm (DeCoursey and Esselbaugh 1962). Coloration of the nymphs transitions from mostly black with orange markings to

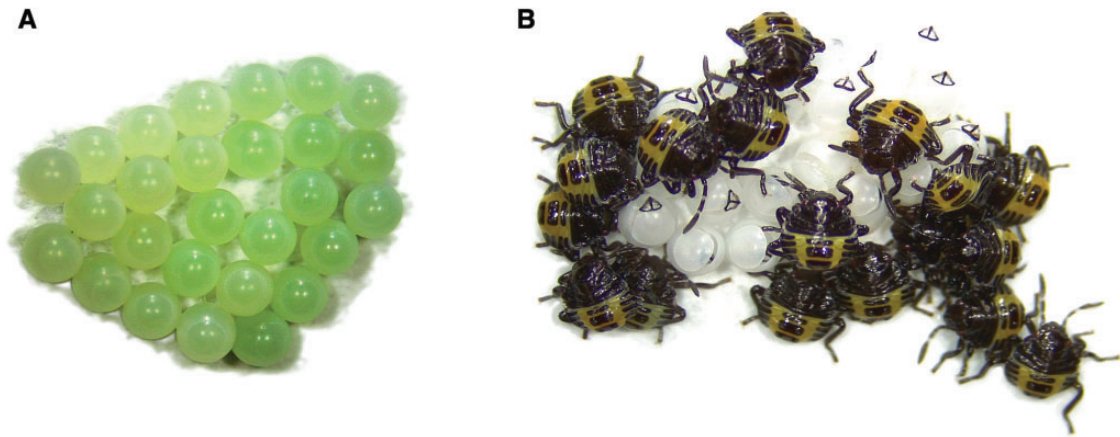


Fig. 4. Stink bug (*H. halys*) egg mass (A) and first-instar nymphs on hatched egg mass (B) (photo credit: D. Pezzini).

mostly green with black and orange markings as nymphs develop (DeCoursey and Esselbaugh 1962; Fig. 3). However, as with the adults, the nymphs of *C. hiliaris* present two different color forms (i.e., a light form and a dark form; Kamminga et al. 2012).

Thyanta custator accerra (Redshouldered Stink Bug)

Thyanta custator accerra occurs throughout the midwestern United States and large areas of North America (McPherson 1982, Rider and Chapin 1992). Adults of *T. custator accerra* are 9.0–13.0 mm long (McPherson 1982, Paiero et al. 2013; Fig. 2). Two color forms of this species exist; a green form in spring and summer, and a brown form in fall (McPherson 1982, Paiero et al. 2013). Some individuals of the green color form have a red- or pink-colored band across the pronotum, hence the common name “redshouldered stink bug” (Paiero et al. 2013). This species can be distinguished from *P. guildinii*, the redbanded stink bug, by the presence of a prominent spine extending from the base of the abdomen between the hind legs and pointing toward the head on *P. guildinii*, and the lack of such spine on *T. custator accerra* (Kamminga et al. 2009). A description of the nymphal instars of this species is provided by DeCoursey and Esselbaugh (1962). In general, nymphs range from 0.9 to 8.2 mm long (DeCoursey and Esselbaugh 1962). Coloration of the thorax and abdomen of nymphs transitions from brown with white and yellow markings to brown with white, amber, and yellow markings as nymphs progress from the first to fifth instars (DeCoursey and Esselbaugh 1962; Fig. 3). The third to fifth instars generally have a “T”-shaped mark on the pronotum (DeCoursey and Esselbaugh 1962).

Halyomorpha halys (Brown Marmorated Stink Bug)

Halyomorpha halys has rapidly spread throughout much of North America (Rice et al. 2014) and is becoming increasingly abundant in the midwestern United States. Adult *H. halys* are variable in color and size, but are generally 12.0–17.0 mm long with a marbled-brown coloration (Hoebeke and Carter 2003; Fig. 2), hence the common name the “brown marmorated stink bug.” *Halyomorpha halys* can be distinguished from other brown-colored stink bugs in the midwestern United States by its light colored underside, the presence of light-colored bands on dark antennae (i.e., the base and apex of the fourth antennal segment and the base of the fifth antennal segment are pale), alternating light–dark pattern on the exposed edges of abdomen (i.e., connexivum), and veins of the membranous parts of front wings are dark brown (Hoebeke and Carter 2003). A

diagnostic key and description of the nymphal instars of *H. halys* is provided by Hoebeke and Carter (2003). In general, nymphs range from 2.4 to 12.0 mm long, and body shape changes from elliptical to pear-shaped as they develop (Hoebeke and Carter 2003). Coloration of the abdomen of nymphs transitions from yellowish-orange with black markings to mostly brown as nymphs progress from the first to fifth instars (Hoebeke and Carter 2003, Rice et al. 2014; Figs. 3 and 4).

Podisus maculiventris (Spined Soldier Bug)

Podisus maculiventris occurs throughout the midwestern United States and much of North America (McPherson 1982), and is the most common predatory stink bug in much of the United States (Slater and Baranowski 1978, De Clerq 2000). Adults of *P. maculiventris* are brown and 8.5–13.0 mm long (Slater and Baranowski 1978, McPherson 1982; Fig. 2). The “shoulders” (i.e., anterolateral margins of the pronotum) of *P. maculiventris* are pointed (McPherson 1982), hence the common name “spined soldier bug.” These pointed “shoulders” may cause confusion between this species and some of the common herbivorous species such as *E. variolarius*. The thickness of the mouth parts (i.e., rostrum) can be used to distinguish *P. maculiventris* and other predatory stink bugs (Asopinae) from herbivorous stink bugs (Pentatominae; Paiero et al. 2013). The rostrum of the predatory species is thick (about twice the thickness of the antenna) and that of the herbivorous species is thin (similar to thickness of antenna; Fig. 5). Nymphs of *P. maculiventris* range from 1.15 to 8.6 mm long, and body shape changes from broadly oval to elongate oval as they develop (DeCoursey and Esselbaugh 1962). Coloration of the nymphs transitions from red with black markings to tan or orange with red and white markings as nymphs progress from the first to fifth instars (DeCoursey and Esselbaugh 1962, Evans 1985; Fig. 3). Nymphs of *P. maculiventris* can be distinguished from other *Podisus* spp. using a diagnostic key by Evans (1985).

Biology

General

Stink bugs are herbivorous, predaceous, or occasionally omnivorous; generalists or specialists in feeding preference; and occur in a diversity of habitats ranging from natural to cultivated, and grassy or herbaceous to arboreal (McPherson 1982, De Clerq 2000). With such diversity, it is no surprise that this group contains both

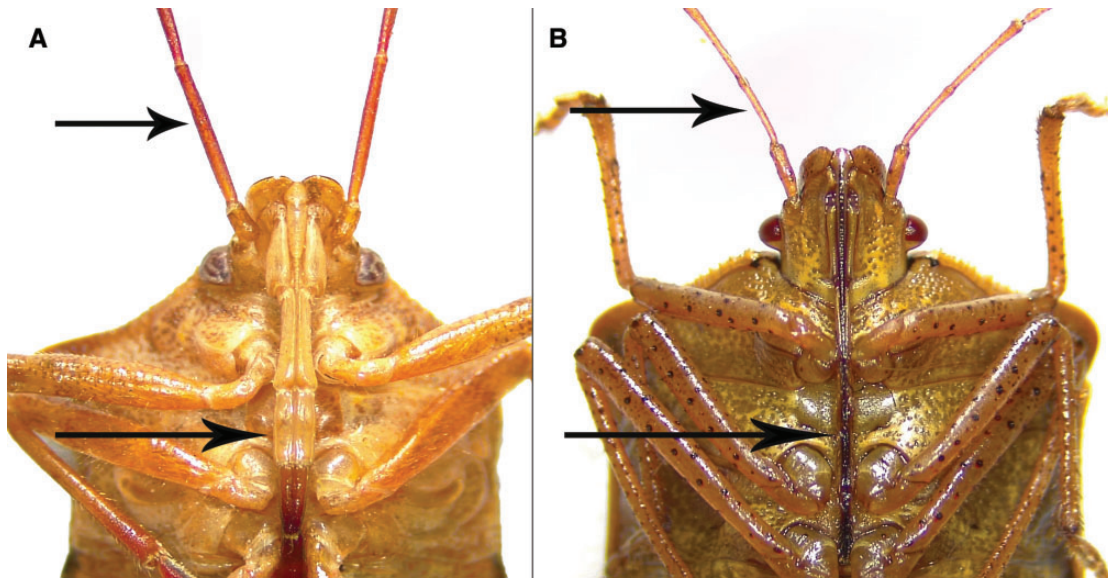


Fig. 5. Mouthparts (i.e., rostra) of predatory (A) and herbivorous (B) stink bugs. Rostrum of predator is thick (about twice the thickness of the antenna), and rostrum of herbivore is thin (similar to thickness of antenna) (arrows indicate rostra and antennae; photo credit: D. Pezzini).

beneficial and pest species (McPherson 1982, Panizzi et al. 2000, De Clerq 2000). Members of the subfamily Asopinae are predaceous and some (e.g., *Podisus maculiventris* and *Perillus bioculatus* (F.)) are well-known predators of significant pests (De Clerq 2000). Members of the subfamilies Pentatominae and Podopinae are primarily herbivorous, but some have been reported to occasionally prey on other insects (McPherson 1982). Most species, such as *E. servus*, *E. variolarius*, *C. hilaris*, and *H. halys*, are generalists and feed on many hosts across several plant families (McPherson 1982, Rice et al. 2014).

Stink bugs of temperate regions generally overwinter as adults in protected locations (e.g., under leaf litter or other debris; Saulish and Musolin 2012). Adults of *H. halys* will also overwinter under loose tree bark or in buildings (Rice et al. 2014). Some species overwinter in other life stages. For example, *Apoecilus cynicus* (Say), a predatory species in the region, overwinters as eggs (Saulish and Musolin 2012). The winter is passed in a physiological state called diapause, which is associated with suppressed sexual development and behavior, active growth of the fat body, reduced oxygen consumption, and increased cold hardiness (Saulish and Musolin 2012). In most species of temperate stink bugs with diapause in the adult stage, diapause is induced by short day lengths experienced during the nymphal stage; however, temperature and food quality can also play a role in diapause induction (Saulish and Musolin 2012). Development resumes in spring with longer day lengths, increasing temperatures, and availability of food resources (Saulish and Musolin 2012). The surrounding landscape can play a role in population buildup of stink bug species, particularly those that are agricultural pests (Panizzi 1997). The generalist feeding habits and high mobility of these species allow them to move throughout the landscape utilizing different plant species (wild and cultivated) at different times, often depending on timing of fruit and seed development of the plants (Panizzi 1997, McPherson and McPherson 2000, Reisig 2011, Pilkay et al. 2015). Mating occurs in an end-to-end position (McPherson 1982). Females generally lay barrel-shaped eggs in clusters on plant tissues, such as the undersides of leaves (Panizzi et al. 2000, McPherson and McPherson 2000; Fig. 4). After egg hatch, stink bugs develop through five instars prior to becoming

adults (Panizzi et al. 2000, McPherson and McPherson 2000). In the midwestern United States, stink bugs generally undergo one or two generations per year (i.e., univoltine or bivoltine, respectively; McPherson 1982).

For herbivorous stink bugs, adults and nymphs, except first instars, actively feed on plant tissues. The first instars are generally considered a nonfeeding stage and metabolize internal nutrient reserves, and acquire important microbial symbionts from the egg mass (McPherson and McPherson 2000, Panizzi et al. 2000; Fig. 4). The adults and fifth instars often cause more injury than the earlier stages (McPherson 1982). Stink bugs feed on all above-ground plant parts, including stems, petioles, leaves, flowers, fruits, and seeds, but they generally prefer developing shoots, fruits, and seeds (Todd and Herzog 1980, McPherson and McPherson 2000). Stink bugs feed by inserting their piercing-sucking mouthparts into plant tissues, injecting digestive enzymes, and sucking up nutrients from plant tissues (McPherson and McPherson 2000). The act of inserting the mouthparts into the tissue causes mechanical injury and tissues are chemically injured by the enzymes injected by the insects (Hori 2000). Feeding injury can result in reduced plant turgor pressure via removal of plant fluids, abnormal plant growth, deformation and discoloration of seeds and fruit, abortion of seeds and fruit, delayed plant maturity (e.g., stay-green syndrome in soybean), transmission of pathogens, or plant death (McPherson and McPherson 2000, Panizzi et al. 2000, Vyavhare et al. 2015b). Injury to fruit and seed is often greater when stink bugs feed earlier in the development of these plant structures (Panizzi et al. 2000). In crops, these various injuries can translate to reductions in quality and yield (McPherson and McPherson 2000, Panizzi et al. 2000).

Finally, as their common name implies, stink bugs produce odorous secretions from scent glands (Aldrich 1988). These secretions serve as defense against natural enemies or as aggregation-, sex-, or alarm pheromones (Aldrich 1988, McPherson and McPherson 2000).

Euschistus servus (Brown Stink Bug)

Euschistus servus can be found on a diversity of wild and cultivated plant species (McPherson 1982, McPherson and McPherson 2000,

Panizzi et al. 2000). For example, a combined list of crop plants from which *E. servus* subspecies or their hybrid have been collected includes corn, soybean, wheat, oats, sunflower, sugar beet, alfalfa, clover, tobacco, cotton, tomato, cabbage, bean, pepper, squash, pea, okra, cantaloupe, blueberry, raspberry, grape, cherry, blackberry, apple, pear, peach, citrus, and pecan (McPherson 1982, McPherson and McPherson 2000). This species is considered the most economically important *Euschistus* species in the United States and Canada (Panizzi et al. 2000). However, the broad host range of *E. servus* may contribute to it not being an even more significant pest of crops such as soybean (McPherson and McPherson 2000). Because its host range includes numerous cultivated and wild plants with temporally overlapping reproductive (fruiting) growth stages, populations of *E. servus* may spread across several plant species on the landscape and not necessarily be concentrated in any one crop (Jones and Sullivan 1982, McPherson and McPherson 2000). Adults of *E. servus* overwinter under objects such as crop debris, leaves, and grass, and prefer to overwinter in open fields rather than wooded areas (McPherson 1982). In Iowa and Illinois, *E. servus* has been reported as being bivoltine (McPherson 1982). For example, in southern Illinois, peaks of adult activity were observed in early April to mid-May (overwintered adults), early July to late August (first-generation adults), and mid-September to late October (second-generation adults; Munyaneza and McPherson 1994). This species may be univoltine farther north. Eggs are laid in masses of 8–41 eggs (mean = 17.6; Munyaneza and McPherson 1994). When reared on green beans in a growth chamber at 23 °C, mean development time of *E. servus* from egg to adult was 44.3 d (egg = 5.8 d, first instar = 5.0 d, second instar = 6.0 d, third instar = 6.7 d, fourth instar = 9.3 d, and fifth instar = 11.5 d; Munyaneza and McPherson 1994).

Euschistus variolarius (Onespotted Stink Bug)

Euschistus variolarius can be found on a diversity of wild and cultivated plant species, including crops such as corn, soybean, wheat, rye, oats, sugar beet, alfalfa, clover, cotton, tobacco, bean, asparagus, tomato, potato, onion, squash, cantaloupe, strawberry, grape, raspberry, cherry, peach, and pear (McPherson 1982). Adults overwinter in protected locations, such as under dry leaves, logs, and dead grass in fence rows (Parish 1934). This species has been reported as univoltine or bivoltine (McPherson 1982), with univoltine populations occurring north of 40° latitude (Panizzi et al. 2000). For example, *E. variolarius* was observed to be univoltine in southern Illinois, with peak adult activity in mid-April to mid-June (overwintered adults), followed by appearance of first-generation adults in late June to late August (Munyaneza and McPherson 1994). Eggs are laid in masses of 6–27 (mean = 16.2) eggs (Munyaneza and McPherson 1994). When reared on green beans in a growth chamber at 23 °C, mean development time of *E. variolarius* from egg to adult was 46.8 d (egg = 5.4 d, first instar = 4.9 d, second instar = 5.7 d, third instar = 7.8 d, fourth instar = 9.7 d, and fifth instar = 13.3 d; Munyaneza and McPherson 1994).

Chinavia hilaris (Green Stink Bug)

The biology of *C. hilaris* was recently reviewed by Kamminga et al. (2012). *Chinavia hilaris* prefers woody plants (McPherson 1982, Kamminga et al. 2012). However, this species can be found on a variety of wild and cultivated plants, including crops such as corn, soybean, sugar beet, cotton, alfalfa, clover, asparagus, cabbage, eggplant, green bean, lima bean, pea, pepper, tomato, turnip, mustard, okra, strawberry, raspberry, black berry, grape, apple, apricot, cherry, orange, peach, pear, plum, and pecan (McPherson 1982).

Adults of *C. hilaris* overwinter under leaf litter in deciduous wooded areas (McPherson 1982, Kamminga et al. 2012). This species is generally considered univoltine, particularly in the northern United States (McPherson 1982, Kamminga et al. 2012). Bivoltine populations of *C. hilaris* occur in the Gulf States and extend as far north as Kansas, Arkansas, and southern Illinois (McPherson 1982). Photoperiod (i.e., day length) is an important determinant of the number of generations for this species (Kamminga et al. 2012). This species is considered semimigratory (Panizzi et al. 2000), and migration from southern areas may contribute to populations in northern areas (<http://www.ent.iastate.edu/soybeaninsects/node/145>). Eggs are laid in masses of 1–72 eggs (Underhill 1934), with a mean of 32 eggs per cluster (Miner 1966). The lower developmental threshold for *C. hilaris* is 15 °C, and development from egg to adult requires the accumulation of 482.7 degree days (Simmons and Yeargan 1988). When reared on soybean seeds in a growth chamber at 24 °C, mean development time of *C. hilaris* from egg to adult was 48.3 d (egg = 9.9 d, first instar = 5.0 d, second instar = 8.9 d, third instar = 5.8 d, fourth instar = 7.2 d, and fifth instar = 11.5 d; Simmons and Yeargan 1988). However, Da Silva and Daane (2014) reported values considerably different (i.e., lower developmental threshold 12.3 °C and 588 degree days) and suggested the difference may be owing to genetics between populations in Kentucky and California or diet used in experiments.

Thyanta custator accerra (Redshouldered Stink Bug)

The biology of *T. custator accerra* has not been studied in detail compared with other common stink bugs. This species has been collected from numerous wild and cultivated plants, including crops such as corn, soybean, wheat, oats, sorghum, alfalfa, clover, bean, eggplant, lima bean, asparagus, and peach (McPherson 1982). This species overwinters as adults (McPherson 1982). *Thyanta custator accerra* is bivoltine in the southern United States (Panizzi et al. 2000), may be only partially bivoltine in north-central Illinois (McPherson 1982), and is likely univoltine in northern states.

Halyomorpha halys (Brown Marmorated Stink Bug)

The biology of *H. halys* in Asia and North America was recently reviewed by Lee et al. (2013) and Rice et al. (2014), respectively. In Asia, *H. halys* has been collected from 106 species of plants across 45 plant families (Lee et al. 2013). In the United States, *H. halys* has also been collected from numerous wild and cultivated plants, including crops such as corn, soybean, sunflower, cereal rye, wheat, garden cucumber, field pumpkin (summer squash), horseradish, Swiss chard, cabbage, collards, cayenne pepper, eggplant, garden tomato, filbert, hazelnut, common hop, bean, apricot, peach, raspberry, blackberry edible fig, highbush blueberry, wine grape, apple, cherry, pear, and pecan (Rice et al. 2014; http://www.stopbmsb.org/where-is-bmsb/host-plants/#host_plants_table). Adults overwinter under debris, in tree holes or under bark, in human-made structures, or in dry areas on mountains (Lee et al. 2013, 2014). This species is likely univoltine to bivoltine in the midwestern United States, as it is in the mid-Atlantic region (Rice et al. 2014). In southern Asia, it can have as many as four to six generations per year (Lee et al. 2013). Eggs are laid in masses of 20–30 eggs, and females lay about 244 egg clusters in a lifetime (Hoebeke and Carter 2003, Nielsen et al. 2008a). The lower and upper developmental thresholds for *H. halys* are 14 °C and 35 °C, respectively, and development from egg to adult requires the accumulation of 538 degree days (Nielsen et al. 2008a). When reared on green beans and Spanish peanuts in a growth chamber at 25 °C, mean development time of *H. halys* from egg to adult



Fig. 6. Injury to soybean resulting from stink bug feeding (increasing stink bug feeding from left to right; photo credit: A. Michel).

was 44.9 d (egg = 6.1 d, first instar = 4.8 d, second instar = 9.6 d, third instar = 7.1 d, fourth instar = 7.4 d, and fifth instar = 10.4 d; Nielsen et al. 2008a).

Podisus maculiventris (Spined Soldier Bug)

Unlike the previously described species, *P. maculiventris* is predatory. This predator shows a preference for lepidopteran larvae (i.e., caterpillars), but is known to feed on >90 species of insects spanning eight insect orders occurring on a diversity of wild and cultivated plants (McPherson 1982, De Clerq 2000). *Podisus maculiventris* and other predatory bugs often prefer prey that are large relative to their body size (Cohen 2000). Like the plant-feeding stink bugs, *P. maculiventris* feeds with piercing-sucking mouthparts. Cohen (2000) describes the feeding of *P. maculiventris* and other predatory bugs as solid-to-liquid feeding. The predators use their mouthparts to pierce the body wall of their prey and inject saliva (Cohen 2000). Enzymes and mechanical action of the mouthparts liquefy the tissues of the prey, and the predator then sucks up the liquefied nutrients from inside the prey (Cohen 2000). *Podisus maculiventris* can feed on plants to acquire moisture and additional nutrients when prey is scarce, but this feeding is not known to cause crop injury (De Clerq 2000, Lambert 2007). Adults overwinter in protected locations, such as in leaf litter or under stones or bark of trees (De Clerq 2000). In much of the midwestern United States and southern Canada, *P. maculiventris* is univoltine to trivoltine, but more generations are likely in the southern United States (McPherson 1982, De Clerq 2000). Eggs are laid in masses of 15–30 eggs (De Clerq 2000). When reared at 23 °C, development time of *P. maculiventris* from egg to adult was 33.5–36.5 d for a population for the northeastern United States (De Clerq 2000).

Injury to Crops

Stink bugs are pests of numerous crops, including soybean and corn (McPherson and McPherson 2000, Panizzi et al. 2000; Figs. 6 and 7). Though much of our knowledge of stink bug injury to soybean and corn is based on research from southern states, it serves to inform the reader about crop response to these pests until more research can be performed on the impact of stink bugs to these crops in the midwestern United States.

Soybean

The impact of stink bugs on soybean has been well studied and has been reviewed by Todd and Herzog (1980), Panizzi and Slansky (1985), and McPherson and McPherson (2000). Stink bugs can feed on all above-ground parts of soybean, but prefer pods and developing seeds (Todd and Herzog 1980, Lee et al. 2013). Fifth instars and adults cause more severe damage than early instars (Simmons and



Fig. 7. Injury to corn resulting from stink bug feeding (photo credit: P. Thomison).

Yeargan 1988, McPherson and McPherson 2000). Species may vary in feeding duration and depth of injury to seed, which can result in different levels of damage (Corrêa-Ferreira and De Azevedo 2002, Depieri and Panizzi 2011). Initial colonization of soybean in the midwestern United States typically occurs during flowering (Koch and Pahl 2014, Koch and Rich 2015, Hunt, personal observation), as in other regions (Pilkay et al. 2015). Populations of stink bugs in soybean then increase and peak during pod and seed development stages (McPherson and McPherson 2000, Koch and Pahl 2014, Koch and Rich 2015, Hunt, personal observation, Michel, personal observation). Stink bug abundance is affected by planting date and maturity group of soybean (Gore et al. 2006, Owens et al. 2013, Temple et al. 2013). In addition, stink bug populations may be affected by other pest management tactics. For example, Rich and Koch (2016) found that *H. halys* preferred and survived better on aphid-resistant soybean than on aphid-susceptible soybean.

Stink bug injury to soybean can impact yield, seed quality, and germination rates (Todd and Herzog 1980, Panizzi and Slansky 1985, McPherson and McPherson 2000, Mesquita et al. 2006). Although some studies report yield losses owing to stink bug injury (Boethel et al. 2000; McPherson and McPherson 2000; Vyavhare et al. 2015a,b), others show no difference in yield owing to stink bug feeding (Corrêa-Ferreira and De Azevedo 2002, Owens 2012, Owens et al. 2013). The variation of results may be explained by several factors. The severity of damage caused by stink bugs can depend on soybean developmental stage, density of bugs, and duration of infestation (Young et al. 2008, Owens 2012, Owens et al. 2013). Among these, soybean developmental stage is the main factor (Smith et al. 2009, Nielsen et al. 2011). In general, feeding during early pod and seed development can result in pod loss and seed abortion (flat pods); feeding during pod fill can result in shriveled, deformed, and smaller seeds; and feeding during seed maturation can result in slight deformation of seed and discolored puncture marks (Todd and Herzog 1980, Panizzi and Slansky 1985, McPherson and McPherson 2000, Mesquita et al. 2006, Owens 2012, Koch and Rich 2015, Vyavhare et al. 2015a; Fig. 6). For example, in a caged experiment, infestation of soybean with *E. servus* and *E. variolarius* at different timings resulted in decreased injury with increasing plant reproductive growth stage (McPherson and McPherson 2000). Soybean has been shown to compensate for stink bug feeding by increasing the weight of unaffected seeds (Todd and Turnipseed 1974, Russin et al. 1987, Boethel et al. 2000, McPherson and McPherson 2000, Koch and Rich 2015).

In addition to impacts on yield, stink bug feeding can affect the quality and maturity of soybean. When seed fed upon by stink bugs is sown, reductions in germination, emergence, and survival of seedlings can be observed (Jensen and Newsom 1972). Germination of seed is more affected by the location of feeding punctures (e.g., punctures near the radicle-hypocotyl axis) than the overall number of feeding punctures (McPherson and McPherson 2000). Locally, stink bug feeding punctures form small brown or black spots in the pod (Kogan and Herzog 1980). Stink bug feeding can increase protein and decrease oil content of soybean seeds and alter the fatty acid composition of soybean oil (Todd and Herzog 1980, Panizzi and Slansky 1985, McPherson and McPherson 2000). However, such impacts to quality were not detected for *H. halys* feeding on soybean in Minnesota (Koch and Rich 2015). In addition, stink bug feeding, particularly during pod-set and pod-filling stages, can cause delayed plant maturity (i.e., “stay-green” syndrome; Todd and Herzog 1980, Panizzi and Slansky 1985, McPherson and McPherson 2000, Musser et al. 2011, Vyavhare et al. 2015b), which can adversely affect harvest of the crop (Musser et al. 2011). In the midwestern United States, delayed maturity has been observed in Ohio soybean with mixed infestation of stink bugs (Michel, personal observation) and in a cage study with *H. halys* in Minnesota (Koch and Rich 2015). Although some studies try to explain the mechanisms of delayed maturity of soybeans (Boethel et al. 2000, Egli and Bruening 2006), questions remain about the specific mechanism (Vyavhare et al. 2015b). Finally, feeding by stink bugs can transmit pathogens to soybean. For example, stink bugs transmit *Nematospora coryli* Peglion, which causes yeast-spot disease (Daugherty 1967, Ragsdale et al. 1979). Stink bugs can also transmit bacteria with potential plant pathogenicity to soybean (Ragsdale et al. 1979, Husseneder et al. 2016).

Corn

Stink bugs can colonize and feed on corn from emergence of the plants through maturity. The seedling and early reproductive stages of corn appear most susceptible to stink bug feeding. In the

midwestern United States, injury to early growth stage corn has been reported from Indiana and Illinois (Edwards et al. 1985), Minnesota (B. Potter, personal communication), and Nebraska (Hunt, personal observation). Fields with increased risk of injury from stink bugs on early growth stages of corn are those with no-till or reduced-tillage, cover crop prior to planting, or corn following wheat (Edwards et al. 1985, Townsend and Sedlacek 1986, Sedlacek and Townsend 1988a).

Stink bugs feed on early vegetative corn by inserting their mouthparts into the bases of plants while their bodies rest on the soil surface or on the plants with heads oriented downward (Townsend and Sedlacek 1986). Feeding at the plant base causes mechanical and chemical injury to the growing point of the plant (Sedlacek and Townsend 1988a). Injury to early vegetative growth stages of corn by stink bugs can result in yield reduction (Annan and Bergman 1988), and symptoms include elongate holes surrounded by chlorotic or necrotic tissue on the leaves, twisting and bending of terminal leaves, tightly rolled or severed whorl leaves, wilting, stunting, tillering, and plant death (Annan and Bergman 1988, Sedlacek and Townsend 1988a). Feeding by *E. servus* and *E. variolarius* on corn seedlings can cause immediate termination of or delay in plant growth and result in decreased above- and below-ground biomass (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988a). The most significant impacts from *E. servus* and *E. variolarius* feeding are tillering and plant mortality (Apriyanto et al. 1989a,b). Tillering of corn plants is caused by stink bugs feeding on lower portions of plants (Townsend and Sedlacek 1986). The type of tissue damaged and amount of tissue damaged are likely the most important factors contributing to injury, such as tillering (Apriyanto et al. 1989a). Plants that tiller in response to stink bug feeding are shorter, have delayed silking, and decreased yields compared with plants exposed to stink bugs that did not tiller and unexposed plants (Apriyanto et al. 1989b).

Susceptibility of early growth stages of corn to stink bug feeding varies with plant growth stage and stink bug life stage. In general, early corn growth stages (e.g., seedlings) are most susceptible and large nymphs and adults of stink bugs are most damaging (Sedlacek and Townsend 1988a).

In Minnesota, *E. variolarius* and *E. servus euschistoides* were the most abundant stink bug species found on corn during reproductive plant growth stages (Koch and Pahl 2015). During reproductive growth stages of corn, stink bugs will feed on developing ears and kernels and, depending on timing of infestation, can affect ear number, ear size, and kernel size and quality (Negrón and Riley 1987, Ni et al. 2010, Rice et al. 2014; Fig. 7). Corn plants appear most susceptible to stink bug feeding during early development of the corn ears, including late vegetative corn growth stages. Observations of ear abortion have been made for *H. halys* feeding on late vegetative stages of corn (Rice et al. 2014). Corn was more susceptible to *E. servus* feeding at the VT (tasseling) stage than the R1 (silking) or R2 (blister) stages (Ni et al. 2010). At the VT stage, three or more *E. servus* feeding for 9 d caused significant kernel damage and reduction in ear and kernel weight (Ni et al. 2010). As corn ear development progresses, feeding by stink bugs is more likely to affect grain quality. *Halyomorpha halys* will feed on developing kernels by piercing through corn husks and cause kernel shrinkage and discoloration (Rice et al. 2014, Cissel et al. 2015). *Euschistus servus* feeding at later reproductive growth stages caused greater effects on grain quality (kernel discoloration) than yield (Ni et al. 2010).

An additional concern related to stink bugs in corn production was the possibility that cattle fed *H. halys*-contaminated corn silage might produce milk tainted by odorous compounds from *H. halys*

(Baldwin et al. 2014). However, *H. halys* contamination of silage did not affect feed consumption by cattle or milk production, and odorous compounds (i.e., E-2-decenal and tridecane) from *H. halys* were not detected in milk after cattle were fed contaminated silage nor after odor compounds were infused directly into the rumen of the cattle (Baldwin et al. 2014). The process of ensiling and metabolism of the cattle appear to mitigate the risk of milk being tainted by stink bug contamination in corn silage (Baldwin et al. 2014).

Management

Much of the information provided here is derived from literature on management of stink bugs in southern states. This information serves to inform the reader about management of these pests until more research can be performed on the management of stink bugs on soybean and corn in the midwestern United States. As state- and region-specific management recommendations are developed and refined, check the recommendations from Extension in your state.

Scouting and Thresholds in Soybean

In general, scouting for stink bugs in soybean should start as pods begin to develop and continue through seed development. Scouting can be performed with a sweep net or drop cloth. A sweep net is more often used with narrow-row (76.2-cm [30-inch] spacing or less) soybean and a drop cloth with wide-row (>76.2-cm [30-inch] spacing) soybean. Although both methods are similar in efficiency of catching stink bugs, using a sweep net is more convenient owing to ease of use and being less time consuming (Rudd and Jensen 1977, Todd and Herzog 1980). Scouting should include edge and interior areas of fields, because the abundance of stink bugs within fields can be greater on field edges (i.e., an edge effect; Todd and Herzog 1980, Leskey et al. 2012a, Koch and Pahs 2014, Venugopal et al. 2014) and areas of soybean adjacent to wooded habitats or early maturing crops (Leskey et al. 2012a, Venugopal et al. 2014). Recent research in cotton and soybean show that *H. halys* has a “startle-response” and readily drop off the plants (Kamminga et al. 2014, Herbert et al. 2015). However, Owens et al. (2013) show that sweep-net sampling is still an efficient method for the stink bug complex containing *H. halys*.

For stink bugs in soybean, treatment decisions are based on the combined count of nymphs (>0.64 cm [1/4 inch]) and adults of all herbivorous stink bug species. Economic thresholds for stink bugs in soybean in the midwestern United States depend on the end use of the soybean. For soybean grown for seed production, the economic threshold is presently 5 stink bugs per 25 sweeps or 1 stink bug per 0.3 m (1 ft) of row (Kogan 1976). For soybean grown for grain, the economic threshold is presently 10 stink bugs per 25 sweeps or 3 stink bugs per 0.3 m (1 ft) of row (Kogan 1976). These thresholds will need validation and refinement as stink bug infestations increase in the region. Owens et al. (2013) show the economic threshold for the invasive *H. halys* not differing from those recommended for native stink bugs.

Scouting and Thresholds in Corn

Scouting during the first 2 wk after corn emergence is critical to managing infestations of stink bugs early in the season. Check at least 10 consecutive plants in five or more locations per field for stink bug injury and stink bugs. In these early vegetative growth stages, examine the entire corn plant from near the base to within the whorl. For corn <61 cm (2 ft) tall, consider treatment if stink bugs are present on 10% or more of the plants. When injured plants

are observed, consider treatment when 5% of the plants exhibit injury and stink bugs are present. Infestations of stink bugs on vegetative growth stages may be more likely to occur in late-planted fields and no-till fields. Fields planted during wet-field conditions may be particularly vulnerable if the seed furrow is not properly closed, allowing stink bug access to the below-ground growing point.

Corn is also vulnerable to injury from stink bugs during ear formation through ear fill. Scouting during this period is also performed by direct examination of plants, particularly in the ear zone. Action thresholds are based on counts of nymphs (>0.64 cm [1/4 inch]) and adults of herbivorous species. Check at least 10 consecutive plants in five or more locations in field for the presence of stink bugs. Insecticide sprays are recommended when stink bug density reaches one stink bug per four plants, from ear forming to beginning of pollen shed, and one stink bug per two plants, from end of pollen shed to the blister stage (Hunt et al. 2014).

Management Tactics

Panizzi and Slansky (1985) and McPherson and McPherson (2000) provide reviews of stink bug management, including tactics such as trap cropping, timing of planting, row spacing, resistant varieties, and biological control. Biological control of stink bugs is expounded upon by McPherson (1982), with a listing of natural enemies known to attack different species of stink bugs. A thorough review of management tactics is beyond the scope of this paper. However, we provide a brief review of more recent literature on chemical control for stink bugs, as this will be the most immediately implemented tactic against these emerging pests in soybean and corn in the midwestern United States.

Broad-spectrum insecticides are generally effective and commonly used for stink bug management (Willrich et al. 2003, Nielsen et al. 2008b, Kamminga et al. 2009, Leskey et al. 2012b, Rice et al. 2014). Stink bug susceptibility to insecticides varies by species, life stages, and sex. For example, *E. servus* has been shown to be less susceptible than *C. hiliaris* to pyrethroid and organophosphate insecticides (Willrich et al. 2003, Snodgrass et al. 2005). Kamminga et al. (2009) showed differences in susceptibility of *C. hiliaris* and *E. servus* to different neonicotinoid insecticides. The predator, *P. maculiventris*, is more susceptible than the herbivore, *E. servus*, to some insecticides (Tillman and Mullinix 2004). Nymphs of *C. hiliaris* are more susceptible to insecticides than adults of *C. hiliaris* (Kamminga et al. 2009). Organic insecticides can be more effective on early instars than older stages (Herbert et al. 2015). In addition, male stink bugs can be more susceptible to insecticides than females, owing to the smaller body size of males (Nielsen et al. 2008b). Residual activity of insecticides should be considered for mobile pests like *H. halys*, which can recolonize treated crops (Funayama 2012, Leskey et al. 2013). In addition to stink bugs, other insects can cause economic losses to these crops (e.g., soybean aphid in soybean); therefore, when economically significant infestations of multiple pests occur, products that can control multiple pests may be preferred.

Conclusion

In conclusion, the threat posed by new and emerging stink bug pests in corn and soybean in the midwestern United States is a challenge for growers and their crop advisors. Identification of these pests and knowledge of their biologies provides a foundation for management programs. Though much can be gained from review of literature, primarily from the southern states, on impacts of stink bugs to crops and management recommendations, further research on these topics

is needed in the midwestern United States. In addition, sampling methods, treatment thresholds, and management tactics for stink bugs require further validation in the midwestern United States. Furthermore, future studies should examine the interaction between stink bugs and other pests and pest management tactics in soybean and corn of the midwestern United States (Rich and Koch 2016).

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Cold tolerance of *Trissolcus japonicus* and *T. cultratus*, potential biological control agents of *Halyomorpha halys*, the brown marmorated stink bug



Erica Nystrom Santacruz^a, Robert Venette^b, Christine Dieckhoff^c, Kim Hoelmer^c, Robert L. Koch^{a,*}

^a Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108, USA

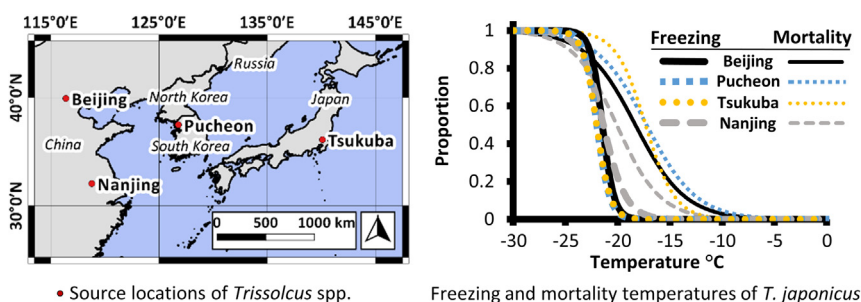
^b USDA-FS, Northern Research Station, 1990 Folwell Avenue, St. Paul, MN 55108, USA

^c USDA-ARS, Beneficial Insect Introduction Research Laboratory, 501 South Chapel St., Newark, DE 19713, USA

HIGHLIGHTS

- 50% of each of the populations of both species died between -17.4 and -20.0 °C.
- 50% of all four populations of *T. japonicus* froze between -21.4 and -22.0 °C.
- *Trissolcus japonicus* is chill-intolerant; populations die before freezing.
- Both *Trissolcus* spp. appeared more cold tolerant than the host, *Halyomorpha halys*.
- *Trissolcus japonicus* does not respond to the same overwintering cues as *H. halys*.

GRAPHICAL ABSTRACT



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ABSTRACT

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is native to Asia and has become a severe agricultural and nuisance pest in the U.S. Therefore, foreign exploration was conducted in Asia to identify potential classical biological control agents. Several *Trissolcus* spp. (Hymenoptera: Scelionidae) parasitize *H. halys* eggs in Asia and are being evaluated for potential release in the U.S. Since *H. halys* has invaded regions that experience sub-zero winter temperatures, cold tolerance is important for evaluation of *Trissolcus* spp. Our study compared the cold tolerance of populations of *T. japonicus* and *T. cultratus*, in order to assess relative suitability of the populations for release. We used thermocouple thermometry to determine the supercooling point and lower lethal temperature after brief exposure to cold temperature for each population. In addition, we subjected adult *T. japonicus* to a short photoperiod and low temperature regime, which increases cold tolerance in *H. halys*, to observe whether these conditions cause a change in cold tolerance in the parasitoid. We found that populations of both species froze and survived at colder temperatures than those reported for *H. halys*. In addition, there were no ecologically relevant differences in the temperature at which freezing or survival occurred among populations of either species, indicating that these populations are equally cold tolerant and suitable for introduction. Finally, *T. japonicus* does not acclimate by increasing cold tolerance in response to conditions that increase cold tolerance in *H. halys*, suggesting that the above-mentioned measures of cold tolerance are ecologically relevant.

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* Corresponding author.

E-mail address: koch0125@umn.edu (R.L. Koch).

1. Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug, is native to China, Japan, Korea, and Taiwan (Rider et al., 2002). In North America, this invasive species was first detected in Pennsylvania in 1996, and by June 2016 had spread to 43 states in the U.S., and two Canadian provinces (Hoebeker and Carter, 2003; Northeastern IPM Center, 2016). *Halyomorpha halys* has also invaded several European countries and has been intercepted in New Zealand (Harris, 2010; Haye et al., 2014). This highly polyphagous pest feeds on >100 species of plants in 45 families including fruits, vegetables, field crops, and ornamental plants in both native and invaded habitats (Haye et al., 2014; Hoebeker and Carter, 2003; Lee et al., 2013), and is considered a nuisance when aggregating en masse while overwintering inside man-made structures (Hoebeker and Carter, 2003; Inkley, 2012).

Some native North American insects have been identified as predators or parasitoids of *H. halys*, but are generally inconsistent in suppressing *H. halys* populations (Herlihy et al., 2016; Jones et al., 2014; Ogburn et al., 2016). As part of a classical biological control program for *H. halys*, foreign exploration has been conducted in Asia since 2007 and four species of egg parasitoids in the genus *Trissolcus* (Hymenoptera: Scelionidae) have been identified as potential biological control agents. These species are being evaluated for potential release against *H. halys* in the U.S. (Talamas et al., 2013). One of these species, *T. japonicus* (Ashmead), appears promising due to a short developmental time (10.5 d at 25 °C), multiple generations per year, highly female-biased sex ratio, and parasitism rates of 50–80% on *H. halys* in the native Asian range (Yang et al., 2009). A second candidate species, *T. cultratus* (Mayr), is also under consideration, though parasitism rates are lower than those of *T. japonicus* (Haye et al., 2015).

Low temperature is a limiting factor in the geographic distribution of many parasitoids (Hance et al., 2007); thus, understanding the cold tolerance of a potential biological control agent can help estimate its capacity to survive and establish in novel areas (Daane et al., 2012; Hanson et al., 2013). In fact, successful classical biological control programs ensure that potential biological control agents and the targeted pests share similar responses to temperature (Goolsby et al., 2005; Hoelmer and Kirk, 2005; Ulrichs and Hopper, 2008). Responses to temperature can vary among populations of the same species (Cira et al., 2016; Feng et al., 2014; Rochefort et al., 2011), so it is prudent to compare geographically-distinct populations to identify which population may be better suited for introduction (e.g., Mausel et al., 2011). Furthermore, because classical biological control programs depend on rearing individuals under laboratory settings for release, it is important to ensure that loss of overwintering potential over time due to exposure to long periods of non-diapause laboratory conditions does not occur (e.g., Garipey et al., 2015).

Commonly-used indices for quantifying cold tolerance in insects include the supercooling point (i.e., the lowest temperature reached prior to the release of latent heat of fusion, observed as an exotherm) and lower lethal temperature (i.e., the temperature at which brief exposure causes a desired level of mortality, often 50 or 90%) (Sinclair et al., 2015). The relationship between freezing and mortality in insects can be used to determine one of three basic cold tolerance categories: chill-intolerance (i.e., death from cold without internal ice formation), freeze intolerance (i.e., death coincides with internal ice formation), or freeze tolerance (i.e. survival of freezing) (Lee, 2010). However, during the onset of fall and through the winter, these strategies may change as insects acclimatize to decreasing temperatures and photoperiods (Hefty, 2016). Acclimatization can result in an increase in cold tolerance over the course of a season (Crosthwaite et al., 2011; Feng et al., 2016; Udaka and Sinclair, 2014). Therefore, it is important to try

to quantify indices of cold tolerance after prolonged exposure to cold temperatures and shorter photoperiods to see if there are any changes over time.

Halyomorpha halys has invaded northern temperate regions (Garipey et al., 2014; Koch, 2014) which experience winter temperatures below 0 °C. In fact, adult *H. halys* collected from Minnesota were found to be chill intolerant and to vary seasonally in their cold tolerance (Cira et al., 2016). In response to colder temperatures and shorter photoperiods, *H. halys* will enter a reproductive diapause (Niva and Takeda, 2002). In contrast, little is known about the cold tolerance or overwintering behavior of *T. japonicus* or *T. cultratus*, except that *T. japonicus* overwinters as an adult (Yang et al., 2009), presumably below bark (Hirose et al., personal communication). Related parasitoids are known to overwinter as adults (Fathi et al., 2011; Foerster and Nakama, 2002; James, 1988; Ryan et al., 1981) and below bark (Fathi et al., 2011; Safavi, 1968). No information exists on subzero temperature limits for *T. japonicus* and *T. cultratus*, nor the extent of cold tolerance variation among populations of each species.

Adventive populations of *T. japonicus* were recently found in the wild in North America (Milnes et al., 2016; Talamas et al., 2015), so there is a need to determine whether *T. japonicus* can survive cold winter temperatures. To assist in assessing the establishment potential of *T. japonicus* and *T. cultratus* in North America we measured supercooling points and lower lethal temperatures for unacclimated, geographically-distinct populations of these species (e.g., Hanson et al., 2013). In addition, we subjected adult *T. japonicus* to conditions that increase cold tolerance in *H. halys* in order to observe whether this induces a change in cold tolerance in the parasitoid.

2. Materials and methods

2.1. Parasitoid acquisition and rearing

Trissolcus japonicus and *T. cultratus* populations were collected from four locations in Asia (Beijing and Nanjing, China; Pucheon, South Korea; and Tsukuba, Japan) between 2007 and 2012 (Table 1). Populations were reared at 20 °C, 16:8 h L:D, and 60–65% RH, and provided honey for nutrition and laboratory-reared *H. halys* eggs for reproduction at the U.S. Department of Agriculture (USDA), Agricultural Research Service, Beneficial Insects Introduction Research Unit, in Newark, Delaware. Separate egg masses of *H. halys* were parasitized by four populations of *T. japonicus* or two populations of *T. cultratus* with four parasitized egg masses of each population shipped overnight under USDA PPQ permit P526-14-01139 to a Biosecurity-Level-2 containment facility in Saint Paul, Minnesota during July 2014, and February and May 2015 for experiments (Table 1). At the Saint Paul location, some individuals of each species were provided with laboratory-reared *H. halys* eggs masses and reared under the conditions described above unless otherwise specified.

2.2. Cold tolerance experiments

The cold tolerance of adult *T. japonicus* and *T. cultratus* was evaluated separately in August 2014, and March and July 2015. The supercooling points and lower lethal temperatures of *T. japonicus*, and the lower lethal temperatures of *T. cultratus* were examined. Due to the limited number of individuals, supercooling points for *T. cultratus* were not measured. In order to facilitate comparison to the host *H. halys*, the cold tolerance testing methods generally followed those employed by Cira et al. (2016) for *H. halys*, with the substitution of a smaller thermocouple (e.g., Stephens et al., 2015). Recently emerged (i.e., 12–36 h old)

Table 1Source information for populations of *T. japonicus* and *T. cultratus* used to measure lower lethal temperatures (LLTemp) and supercooling points (SCP).

Experiment	Population or generation	Experimental date	Latitude (°N)	Longitude (°E)	JALT [†] (°C)	Collection year	Shipment date	No. SCP tested	No. LLTemp tested (n/exposure temperature)
Supercooling point and lower lethal temperature	<i>T. japonicus</i>								
	Beijing, China	Aug.–Oct. 2014	39.91	116.39	–9.4	2009	Jul. 2014	22	88 (15–20)
	Pucheon, South Korea	Aug.–Oct. 2014	37.89	127.20	–6.1 [‡]	2009	Jul. 2014	22	111 (20–25)
	Tsukuba, Japan	Aug.–Oct. 2014	36.03	140.07	2.5 [‡]	2012	Jul. 2014	18	68 (12–15)
	Nanjing, China	Aug.–Oct. 2014	32.05	118.77	–1.6	2009	Jul. 2014	14	74 (14–15)
Lower lethal temperature	<i>T. cultratus</i>								
	Tsukuba 2007, Japan	Feb.–Mar. 2015	36.03	140.07	2.5 [‡]	2007	Feb. 2015	–	80 (16)
	Tsukuba 2012, Japan	Feb.–Mar. 2015	36.03	140.07	2.5 [‡]	2012	Feb. 2015	–	100 (20)
Cold acclimation	<i>T. japonicus</i> [*]								
	Summer, P	Jul.–Oct. 2015	39.91	116.39	–9.4	2009	May 2015	–	78 (6)
	Summer, F ₁	Jul.–Oct. 2015	39.91	116.39	–9.4	2009	May 2015	–	78 (6)
	Summer, F ₂	Jul.–Oct. 2015	39.91	116.39	–9.4	2009	May 2015	–	65 (5)
	Summer, F ₃	Jul.–Oct. 2015	39.91	116.39	–9.4	2009	May 2015	–	78 (6)
	Fall, F ₁	Jul.–Oct. 2015	39.91	116.39	–9.4	2009	May 2015	–	56 (4–5)
	Fall, F ₂	Jul.–Oct. 2015	39.91	116.39	–9.4	2009	May 2015	–	65 (5)
	Fall, F ₃	Jul.–Oct. 2015	39.91	116.39	–9.4	2009	May 2015	–	65 (5)

* Experiment included individuals from the Beijing, China population.

[†] JALT January average lowest temperature (30-year period). Data from the World Meteorological Organization, 2016.[‡] Due to the lack of climatological information available for Pucheon, South Korea, and Tsukuba, Japan, a substitution was made with information from the nearest cities: Seoul, South Korea (20 km) and Tokyo, Japan (70 km).

adult parasitoids were used in all experiments, and the sex of each individual was determined after death by examination of the antennae (Yang et al., 2009). All statistics were performed using R (R Core Development Team, 2015; RStudio Team, 2015), and all commands originate from the base package unless otherwise specified.

2.2.1. Supercooling point determination of *T. japonicus*

Methods for contact thermocouple thermometry followed Stephens et al. (2015). Adult *T. japonicus* were placed individually inside 1.5-ml microcentrifuge tubes. Each tube was sealed with a milled plastic dowel that had been fitted with an o-ring. A 0.127-mm copper-constantan thermocouple was threaded through a hole in the center of the dowel. Thermocouples with insects were placed inside 20 × 20 × 20 cm polystyrene cubes calibrated to cool at a rate of 1 °C per minute when placed inside an ultralow freezer at –80 °C (Carrillo et al., 2004). Cubes were cooled in groups (i.e., blocks) of 4–16 cubes and the temperatures of the insects were recorded every second by use of an analog data acquisition unit (USB-TC, Measurement Computing Core, Norton, MA), and visualized in real time on a computer using TracerDAQ Pro (Measurement Computing Core, Norton MA). For each individual, the supercooling point was recorded as the lowest temperature reached before the release of latent heat of fusion (i.e., an exotherm) (Carrillo et al., 2004). For each population, 14–22 exotherms were detected (Table 1).

Regression analysis was used to compare the distributions of supercooling points among populations of *T. japonicus* because such distributions are often not normal (Sinclair et al., 2015). Logistic regression was used to compare the cumulative proportion of individuals that began to freeze (i.e., an event) by a particular temperature among populations of *T. japonicus* (e.g., Stephens et al., 2015). For this analysis a generalized linear model was created using the glm() command with the probability of observing freezing as the response variable, and population, temperature, sex, and their interaction as explanatory variables. There was no effect of sex ($Z = -0.15, P = 0.88$), so this variable was dropped, and the data were pooled in the reduced model. To test for differences among populations in the temperature at which freezing occurred, intercepts (b_0) and slopes (b_1) among the four populations of

T. japonicus were compared by changing the model reference population, and a Bonferroni adjustment (critical $\alpha = 0.008$ per comparison) was used to maintain an overall $\alpha = 0.05$. The dose. p() command from the MASS package (Ripley, 2015) followed the general equation: $P(\text{Insect freezes}) = 1/[1 + e^{-(b_0 + b_1 \cdot \text{temperature})}]$, to identify the 50th percentile freezing temperature (i.e., $P(\text{Insect freezes}) = 0.5$) (\pm SE) for each population.

2.2.2. Lower lethal temperature determination of *T. japonicus* and *T. cultratus*

To determine the lower lethal temperature of *T. japonicus* and *T. cultratus*, two experiments were performed (one for each species), and both experiments followed a randomized block design. For each population of a given species, individual adults were cooled to an assigned exposure temperature following the same protocol as the supercooling point experiments, except that individuals were removed from the freezer and cubes upon reaching the assigned exposure temperatures. Individuals were assigned randomly to exposure temperatures of –23, –21, –19, –17 °C, or a control of 25 °C. Previously-collected data suggested that mortality varied from 0 to 100% in this range. The number of individuals randomly assigned to each exposure temperature ranged from 12 to 22 individuals for each population of both species (Table 1). Up to 20 individuals were cooled in a block, for a total of 18 blocks for *T. japonicus* and 12 blocks for *T. cultratus*. Due to the limited supply of individuals and the inability to synchronize emergence, it was not possible to represent each population and exposure temperature equally within a block; however, each population represented in a block included one control. Survivorship of each individual was visually determined 30 min after returning to room temperature and survival was defined as the parasitoid walking within the centrifuge tube. To determine the effect of low temperature exposure on longevity, individuals from the lower lethal temperature experiments were transferred in their centrifuge tubes to a growth chamber at 23 °C, 16:8 h L:D, and 60–65% RH, and were maintained without food and monitored every 1–3 d until death.

To compare cumulative mortality as a function of exposure temperature among populations of each species, generalized linear models were prepared as described for the supercooling point analysis. However, for lower lethal temperature, an event was

defined as whether an insect died by the exposure temperature. As before, sex was dropped from both models because the effect was not significant (*T. japonicus*: $Z = -1.21$, $P = 0.22$, and *T. cultratus*: $Z = -0.74$, $P = 0.46$), and the data were pooled in the reduced models. Testing for differences in the temperature at which mortality occurs among populations and identification of the 50th percentile mortality temperature (\pm SE) for each population were determined as outlined for the supercooling point analysis.

To compare the effect of low temperature exposure on longevity among populations of each species, a parametric survival regression model was created for each species by using the `survreg()` command from the Survival package (Therneau, 2015). For these models, the response variable was the number of days an individual was alive, and individuals that died between monitored dates were censored at the last day that they were seen alive. The explanatory variables for each model consisted of population, temperature, and their interaction. Models were reduced with a maximum likelihood ratio test (χ^2) through use of the `anova()` command. The `predict()` command was used to identify the median number of days until death ($\pm 95\%$ CI) (i.e. longevity) for each combination of population and temperature. Tukey pairwise comparisons within a population were made using the `glht()` command from the `multcomp` package (Hothorn et al., 2015). All reported p-values were adjusted at $\alpha = 0.05$.

2.2.3. Cold-tolerance strategy analysis of *T. japonicus*

To determine cold-tolerance strategies for the four populations of *T. japonicus*, cumulative mortality and cumulative freezing as a function of temperature were compared within a population as per Stephens et al. (2015). The cumulative mortality curve describes the total proportion of individuals that would be expected to die after brief exposure to a specified temperature; death may have occurred at a warmer temperature than the specified temperature. Similarly, the cumulative supercooling point curve describes the total proportion of individuals that would be expected to start freezing at a temperature greater than or equal to the specified temperature. This comparison was performed by creating generalized linear models as described for the supercooling point analysis for each population, but in this case the probability of observing an event (i.e., freezing or dying) as the response variable, and treatment (i.e., supercooling point or lower lethal temperature), temperature, and their interaction, as explanatory variables.

2.2.4. Cold acclimation experiment for *T. japonicus*

An experiment was performed to determine if temperature and photoperiod conditions cooler than those known to increase cold tolerance in *H. halys* (e.g., 20 °C, 12:12 h L:D, T. Cira, personal communication) could enhance the cold tolerance of *T. japonicus*. Adult parasitoids from the Beijing, China population were received from Newark in May 2015 and maintained at summer conditions (23 °C, 16:8 h L:D, and 60–65% RH), for two generations. Recently emerged (i.e., 12–36 h old) adult males and females were transferred into eight separate (9 dram) plastic vials (1–3 per sex, per vial), provisioned with honey, and allowed to mate for 24 h in the summer conditions. After 24 h, males were removed and the vials were evenly split between two sets of rearing conditions: summer or fall (18 °C, 12:12 h L:D, and 60–65% RH). Each vial was provisioned with a thawed *H. halys* egg mass (~25–28 eggs per egg mass) prepared following Haye et al. (2015). *Halyomorpha halys* egg masses less than 24 h old were collected from a University of Minnesota (St. Paul, MN) laboratory colony (maintained at 23 °C, 16:8 h L:D, and 60–65% RH), and stored in a –80 °C freezer for up to four weeks until needed. The egg masses were thawed for 30 min at room temperature before use. After 48 h, females were removed from the vials and the egg masses (containing first

generation, or F_1) were maintained in their respective rearing conditions until emergence. Upon emergence, the mating and oviposition process outlined above was repeated for two additional generations (F_2 and F_3), though individuals remained in their respective rearing conditions.

Each generation of adult female parasitoids produced under the two rearing conditions were used in lower lethal temperature experiments similar to those described for the lower lethal temperature determination. However, exposure temperatures for these lower lethal temperature measurements spanned every degree between –25 and –14 °C, and a control of 25 °C, and the number of individuals randomly assigned to each exposure temperature ranged from 4 to 6 individuals for each generation of each rearing condition (Table 1). Again, up to 20 individuals were cooled in a block, for a total of 40 blocks.

Like the lower lethal temperature analyses, generalized linear models were created to compare cumulative mortality as a function of exposure temperatures between rearing conditions of the same generation, and among generations of each rearing condition. Three models were created to compare rearing conditions (summer or fall) within a generation (F_1 , F_2 , or F_3), where the explanatory variables included temperature, rearing condition, and their interaction. Two models were created to compare generations (P , F_1 , F_2 , and F_3) within each rearing condition (summer or fall), where the explanatory variables included temperature, generation, and the interaction. For the latter two models, testing for differences in the temperature at which mortality occurs among generations and identification of the 50th percentile mortality temperature (\pm SE) for each generation was determined as outlined for the supercooling point analysis.

3. Results

3.1. Supercooling point of *T. japonicus*

Among all populations of *T. japonicus*, supercooling points ranged from –23.67 to –16.73 °C. The temperatures that caused 50% of the population to begin to freeze (\pm SE) ranged from -22.04 ± 0.07 (Pucheon) to -21.43 ± 0.19 °C (Nanjing) (Table 2). The distributions of supercooling points were not the same for each population. From the logistic regressions of cumulative percent of individuals freezing (i.e., supercooling) as a function of temperature for *T. japonicus*, the intercept (b_0) and slope (b_1) for Nanjing were significantly higher than the intercepts and slopes of Beijing and Pucheon, but not significantly different from those of Tsukuba (critical $\alpha = 0.008$) (Table 2, Supplemental Table 1). In general, more individuals from Nanjing began to freeze at warmer temperatures than individuals from Beijing or Pucheon, but not Tsukuba, and the change in the rate at which individuals began to freeze as temperatures declined was significantly greater for individuals from Nanjing than from Beijing or Pucheon, but not Tsukuba (Fig. 1A–D).

3.2. Lower lethal temperature of *T. japonicus* and *T. cultratus*

For *T. japonicus*, the 50th percentile mortality temperature (\pm SE) was lowest for the southernmost population of Nanjing (-20.03 ± 0.61 °C) and highest for the second northernmost population of Pucheon (-17.36 ± 0.84 °C) (Table 2). The 50th percentile mortality temperature (\pm SE) for Tsukuba 2007 and Tsukuba 2012 of *T. cultratus* was -19.88 ± 0.46 °C and -19.86 ± 0.45 °C, respectively. In general, while mortality increased as individuals were exposed to decreasing temperature, the likelihood of mortality did not differ significantly among populations within either species (Fig. 1A–F). Logistic regressions describing mortality as a function

Table 2

Coefficients (\pm SE) for logistic regression models to describe the cumulative frequency of freezing (from supercooling point measures) and the cumulative extent of mortality from brief cold exposure (from lower lethal temperature treatments) for populations of *Trissolcus japonicus*.

Population	df	Intercept (b_0)				Slope (b_1)				50th percentile	
		Freezing	Mortality	Z	P	Freezing	Mortality	Z	P	Freezing	Mortality
Beijing	106	$-37.88 \pm 3.85_a$	$-7.71 \pm 2.64_a$	-6.46	<0.001	$-1.76 \pm 0.18_a$	$-0.42 \pm 0.13_a$	-6.02	<0.001	-21.50 ± 0.06	-18.56 ± 0.72
Pucheon	129	$-35.71 \pm 3.41_a$	$-7.26 \pm 2.50_a$	-6.74	<0.001	$-1.62 \pm 0.15_a$	$-0.42 \pm 0.13_a$	-5.99	<0.001	-22.04 ± 0.07	-17.36 ± 0.84
Tsukuba	82	$-33.54 \pm 4.40_{ab}$	$-12.34 \pm 4.32_a$	-3.44	<0.001	$-1.52 \pm 0.20_{ab}$	$-0.71 \pm 0.23_a$	-2.66	0.01	-22.00 ± 0.09	-17.41 ± 0.64
Nanjing	84	$-19.71 \pm 3.27_b$	$-9.67 \pm 2.92_a$	-2.60	0.02	$-0.92 \pm 0.15_b$	$-0.48 \pm 0.15_a$	-2.09	0.04	-21.43 ± 0.19	-20.03 ± 0.61

Models follow the general equation: $P(\text{Insect freezes or dies}) = 1/[1 + e^{-(b_0 + b_1 \cdot \text{temperature})}]$.

Within rows (i.e., within populations), intercepts and slopes from the models for freezing were all significantly different from the models for mortality at $\alpha = 0.05$.

Within a column, different letters indicate significant differences for intercepts or slopes among population freezing (df = 68) or mortality (df = 333) treatments at an adjusted $\alpha = 0.008$ to maintain an overall $\alpha = 0.05$.

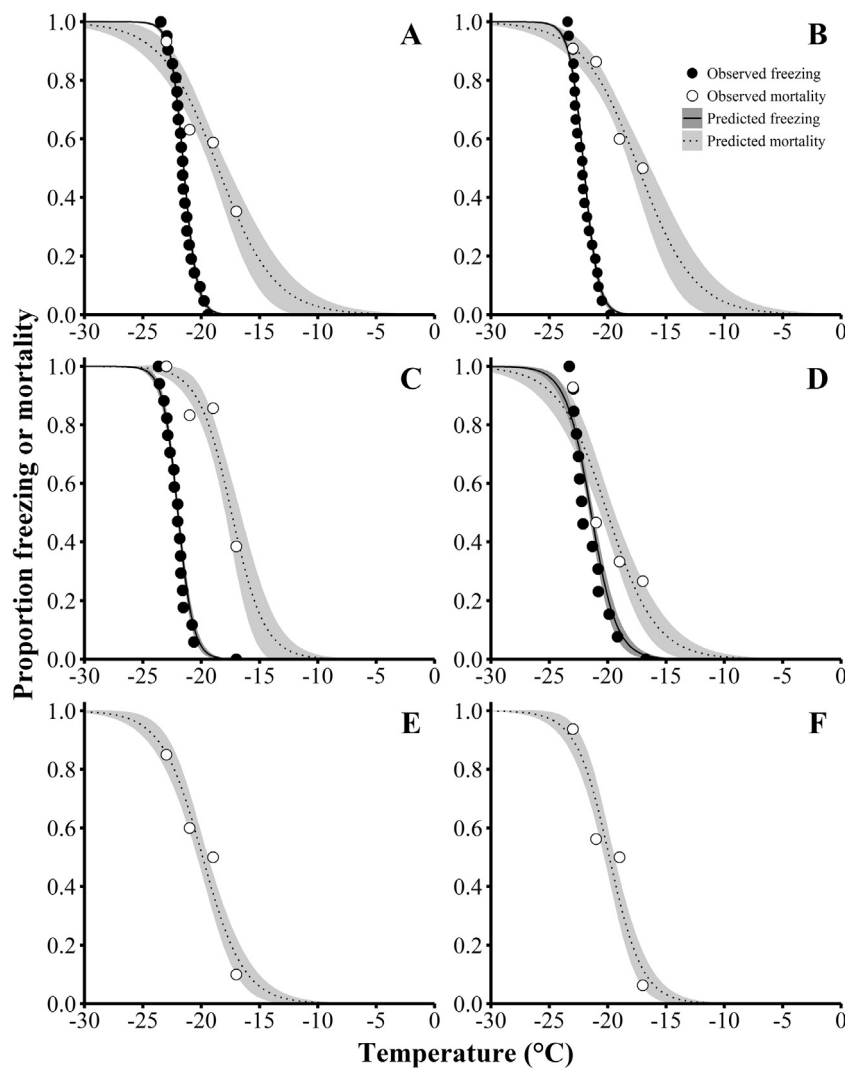


Fig. 1. Observed and predicted (\pm SE band) cumulative freezing and/or mortality for populations of *Trissolcus japonicus* (A: Beijing, China; B: Pucheon, South Korea; C: Tsukuba, Japan; D: Nanjing, China) and *T. cultratus* (E: Tsukuba 2007, Japan; F: Tsukuba 2012, Japan).

of temperature for *T. japonicus* did not have significantly different intercepts or slopes among populations (Table 2, Supplemental Table 1). Similarly, the intercepts (-11.28 ± 2.67 and -13.98 ± 3.39) and the slopes (-0.57 ± 0.13 , and -0.70 ± 0.17) for the Tsukuba 2007 and Tsukuba 2012 populations of *T. cultratus* did not differ significantly between collection years ($Z = -0.63$, $P = 0.53$ and $Z = -0.63$, $P = 0.53$, respectively).

For *T. japonicus* and *T. cultratus*, longevity for each population was generally lower after exposure to low temperature (i.e., -23 and -21 °C), than to the control temperature (i.e., 25 °C) (Fig. 2). Among all populations of *T. japonicus* and *T. cultratus*, median longevity after exposure to -23 and 25 °C ranged from 0.78 to 1.33, and 4.28 to 6.44 d, respectively (Fig. 2). Maximum likelihood ratio tests revealed that the interaction of population and temperature was

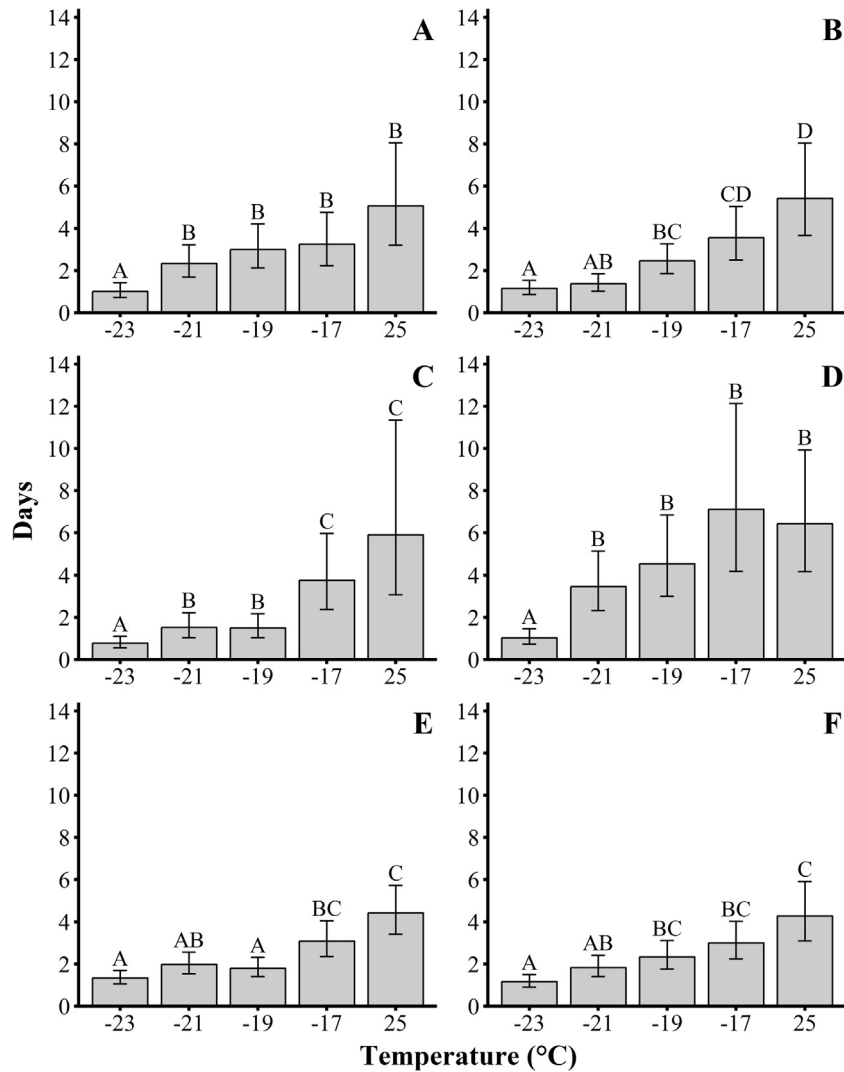


Fig. 2. Median longevity ($\pm 95\%$ CI), following brief exposure to low temperatures for populations of *Trissolcus japonicus* (A: Beijing, China; B: Pucheon, South Korea; C: Tsukuba, Japan; D: Nanjing, China) and *T. cultratus* (E: Tsukuba 2007, Japan; F: Tsukuba 2012, Japan).

not significant in either model for *T. japonicus* ($\chi^2_1 = 3.32$, $P = 0.34$) nor *T. cultratus* ($\chi^2_1 < 0.002$, $P = 0.97$). Therefore, among populations within a species, the relative differences in longevity were constant across all exposure temperatures. In the reduced model for *T. japonicus* population was significant ($\chi^2_3 = 23.5$, $P < 0.001$), and the median longevity ($\pm 95\%$ CI) for Nanjing was 1.50 (1.12, 2.00) days longer than Beijing ($Z = 2.75$, $P = 0.03$), 1.73 (1.32, 2.27) days longer than Pucheon ($Z = 3.92$, $P < 0.001$), and 1.98 (1.46, 2.69) days longer than Tsukuba ($Z = 4.37$, $P < 0.001$). Population was not significant in the reduced model for *T. cultratus* ($\chi^2_1 < 0.001$, $P = 0.97$), indicating there were no differences in longevity between the two populations. In the final model for each species, temperature was significant for both *T. japonicus* ($Z = 6.98$, $P < 0.001$) and *T. cultratus* ($Z = 6.48$, $P < 0.001$), indicating that longevity within a population increased with increasing (i.e., warmer) exposure temperatures (Fig. 2).

3.3. Cold-tolerance strategy of *T. japonicus*

From the comparisons of curves that described cumulative freezing and cumulative mortality within each population of *T. japonicus*, mortality appeared to precede freezing as temperature

declined (Fig. 1A–D). For each population, the slope and intercept of the logistic regression model characterized the cumulative frequency of supercooling points. In the logistic regression models, supercooling points intercepts and slopes were all significantly lower than the lower lethal temperature intercepts and slopes (Table 2).

3.4. Cold acclimation of *T. japonicus*

From the logistic regressions of cumulative percent of individuals dying as a function of temperature, there were no significant differences among the intercepts and slopes of generations within rearing conditions or rearing conditions within a generation (Table 3, Supplemental Table 2). The 50th percentile mortality temperature (\pm SE) for the summer and fall conditions were similar, spanning from -17.95 ± 0.50 to -15.09 ± 0.76 °C (Table 3). As temperature decreased, mortality increased at the same rate among all generations within a rearing condition, as well as between rearing conditions for a given generation (Fig. 3A–D). Therefore, exposure over three generations to conditions known to induce reproductive diapause in *H. halys* did not affect the likelihood of survival of *T. japonicus*.

Table 3

Coefficients (\pm SE) for logistic regression models to describe the extent of mortality after brief cold exposure among *Trissolcus japonicus* adults that had been reared under summer or fall conditions for up to three generations.

Generation	df	Intercept (b_0)				Slope (b_1)				50th percentile ($^{\circ}$ C)	
		Summer	Fall	Z	P	Summer	Fall	Z	P	Summer	Fall
P*	76	$-12.60 \pm 2.89_a$	–	–	–	$-0.70 \pm 0.16_a$	–	–	–	-17.95 ± 0.50	–
F ₁	130	$-8.19 \pm 2.29_a$	$-7.68 \pm 2.79_a$	0.06	0.95	$-0.50 \pm 0.13_a$	$-0.50 \pm 0.16_a$	0.14	0.89	-16.24 ± 0.71	-15.44 ± 0.91
F ₂	126	$-7.65 \pm 2.25_a$	$-8.52 \pm 2.51_a$	0.26	0.80	$-0.45 \pm 0.12_a$	$-0.51 \pm 0.14_a$	0.33	0.74	-16.92 ± 0.79	-16.56 ± 0.74
F ₃	139	$-8.73 \pm 2.84_a$	$-14.05 \pm 4.31_a$	1.03	0.30	$-0.58 \pm 0.17_a$	$-0.88 \pm 0.26_a$	0.96	0.33	-15.09 ± 0.76	-15.98 ± 0.52

Models followed the general equation: $P(\text{Insect dies}) = 1/[1 + e^{-(b_0 + b_1 \cdot \text{temperature})}]$.

Within rows (i.e. within generations), intercepts and slopes from the summer conditions were not significantly different from those of the fall conditions at $\alpha = 0.05$.

Within columns, different letters indicate significant differences for intercepts or slopes among generations for summer ($df = 291$) or fall ($df = 256$) conditions at an adjusted $\alpha = 0.008$. The summer P generation was also included in comparison among those reared under fall conditions.

* The P generation was only reared under summer conditions, hence no comparison for this generation to a fall counterpart, nor a predicted fall 50th percentile mortality temperature.

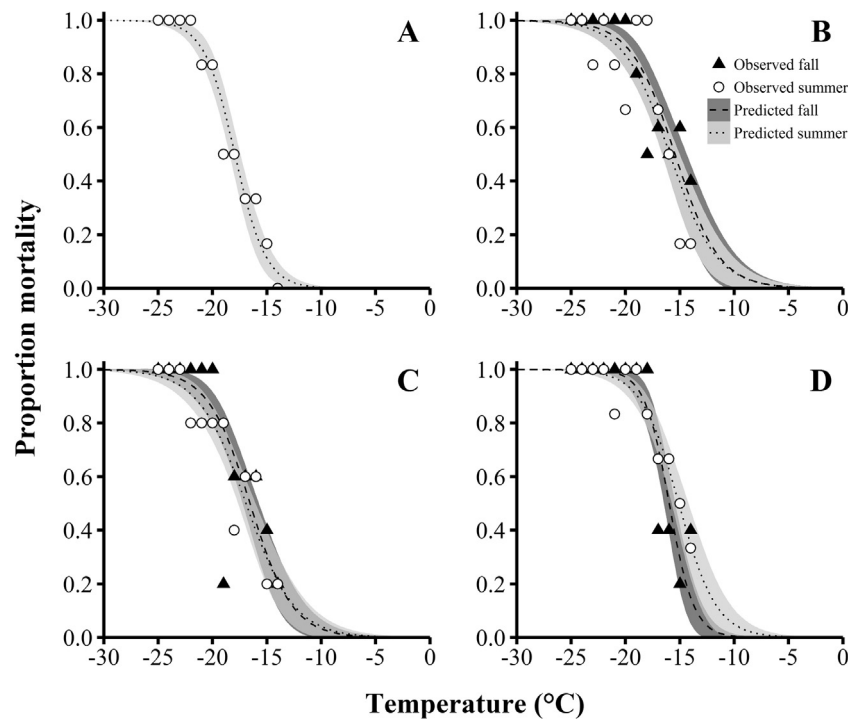


Fig. 3. Observed and predicted mortality (\pm SE band) of *Trissolcus japonicus* reared over multiple generations (A: parental (P) generation; B: first (F₁) generation; C: second (F₂) generation; D: third (F₃) generation) under summer and fall rearing conditions.

4. Discussion

Classical biological control by Asian *Trissolcus* spp. may provide an additional tool for managing *H. halys* beyond North American natural enemies (Herlihy et al., 2016; Ogburn et al., 2016) and the current use of pesticides (Leskey et al., 2012). Recently, populations of *T. japonicus* were detected in both the eastern and western U.S. (Milnes et al., 2016; Talamas et al., 2015). However, these areas currently represent a fraction of the range of *H. halys* in North America (Northeastern IPM Center, 2016), and do not reflect the northern extent of the invaded range of this pest, which seasonally experiences cold winter temperatures (USDA, 2012). The present research is the first assessment of cold tolerance for *T. japonicus* and *T. cultratus*, and will facilitate evaluation of classical biological control for *H. halys*.

Many insects (Crostwaite et al., 2011; Kim and Song, 2000; Régnière and Bentz, 2007), including parasitic Hymenoptera (Hanson et al., 2013), are known to seasonally acclimatize to cold conditions by increasing cold tolerance as a response to environmental conditions such as photoperiod or temperature

(Denlinger, 1991). Such seasonal acclimatization could affect ecological relevance of cold tolerance estimates attained from unacclimated, laboratory populations. Whether *T. japonicus* seasonally acclimatizes to colder temperatures or shorter photoperiod was unknown to date. Therefore, we began our investigation with unacclimated individuals that were reared under standard conditions (23 ± 1.5 °C, 16:8 h L: D, 65% RH) to determine a reference point for comparing populations reared under cooler temperature or shorter photoperiod conditions.

Interestingly, median supercooling point and lower lethal temperature results for unacclimated populations of *T. japonicus* and *T. cultratus* were all below the winter mean supercooling point (-17.06 °C) of *H. halys* from Minnesota, an area that experiences severe winter temperatures (Cira et al., 2016). In addition, prolonged exposure to laboratory conditions for *T. cultratus* (e.g., Tsukuba 2007) did not appear to affect the baseline cold tolerance when compared to a population collected five years later (e.g., Tsukuba 2012). This suggests that both parasitoid species, when unacclimated, are capable of surviving colder temperatures than acclimated *H. halys*, and that this unacclimated cold tolerance is

retained during laboratory rearing. Such results are promising for potential long-term laboratory populations reared for classical biological control. Nevertheless, we evaluated lower lethal temperature of *T. japonicus* under more relevant conditions by performing an experiment that investigated the ability of *T. japonicus* to modify its cold tolerance in response to conditions known to increase cold tolerance in *H. halys*. Our results suggest that unlike *H. halys*, exposure to conditions cooler than 20 °C and a photoperiod of 12:12 h does not increase the cold tolerance *T. japonicus*. Parasitic wasps are dependent on their hosts and for most species, synchrony in behavior to that of their host is crucial to their survivorship (Hance et al., 2007). *Trissolcus japonicus* has been recorded from several other Asian pentatomid species (Matsuo et al., 2016; Yang et al., 2009), and it overwinters as an adult (versus inside of the host) (Yang et al., 2009). This lack of dependency on a single host species and behavior of overwintering outside of the host may in part explain why *T. japonicus* did not respond to the same photoperiod cues as *H. halys*. However, our study does not rule out the possibility that *T. japonicus* can further adjust its cold tolerance if exposed to other conditions. Further work should explore how *T. japonicus* responds to temperature or photoperiod regimes beyond those used in this study.

Supercooling points of *T. japonicus* were not the same for all source populations. Populations originating from the southernmost population (i.e., Nanjing) began to freeze at significantly higher temperatures than the northernmost populations (i.e., Beijing and Pucheon). This pattern aligns with the latitude and 30-year average lowest temperature from January for the source population areas in Asia (Table 1). Similar correlations have also been found with other studies that demonstrate that the mean supercooling points of geographically-distinct populations of a single insect species increase in temperature as latitude decreases (Jing and Kang, 2003; Zhou et al., 2011). However, the small magnitude of difference in median freezing values among the populations tested here is not likely to confer an ecologically-relevant advantage for the populations that supercooled at statistically lower temperatures. Therefore, from the standpoint of using supercooling point as an index of cold tolerance, these four populations of *T. japonicus* should have similar likelihoods for freezing at cold winter temperatures.

We did not find significant differences in mortality as a function of exposure temperature among any of the populations of *T. japonicus* or *T. cultratus*. This suggests that all populations should have similar likelihoods for surviving brief exposure to cold and that all of the populations are equally suitable for introduction with respect to winter survival. Because freezing and mortality can occur at significantly different temperatures (Sømme, 1982), it is important to assess the relationship between these two phenomena in order to determine whether supercooling point and lower lethal temperature are interchangeable indices for mortality (Renault et al., 2002). Our results demonstrate that each of the populations of *T. japonicus* appeared to be dying before freezing. Thus, lower lethal temperature is a more ecologically-relevant source of information than the supercooling point for assessing the cold tolerance of *T. japonicus* (e.g., Baust and Rojas 1985), and should be used for more accurate comparisons of *T. japonicus* cold tolerance to that of *H. halys*. Although informative, our study is limited in that we investigated survivorship after brief exposure to low temperatures (e.g., lower lethal temperature). This deliberate measurement was used to decouple low temperature and exposure time, as duration of exposure to a low temperature can differentially affect survivorship (Renault et al., 2002). Further cold tolerance studies with *T. japonicus* should expand on these initial measures of lower lethal temperature by incorporating the element of time (e.g., lower lethal time). This could help assess how

survivorship of this species is affected under conditions that more closely simulate what the insects may encounter in nature.

Comparison of the temperatures at which insects begin to freeze or die also can be used to infer cold tolerance strategy (e.g., Hanson and Venette 2013), and those that die from cold temperatures before body fluids begin to freeze are considered to be chill-intolerant (Lee, 2010). Our results demonstrate that *T. japonicus* is chill-intolerant, which is a cold-tolerance strategy shared by other parasitoids (Carrillo et al., 2005; Hanson et al., 2013; Rivers et al., 2000), as well as by *H. halys* (Cira et al., 2016). Therefore, similar to *H. halys*, populations of *T. japonicus* are freezing at temperatures significantly lower than what they could survive.

Interestingly, while we did not find differences among populations of either parasitoid in lower lethal temperature, we did find that the southernmost population, Nanjing, lived longer without food after brief exposure to all low temperatures in comparison with the other *T. japonicus* populations. This increase of approximately two days in longevity was relatively small, but it could provide the individuals from this population with additional time to seek nutrition during the spring, when food is not yet available or scarce. Little is known about the natural feeding habits of *T. japonicus* except that it does not host-feed; however, if it is similar to that of other scelionids, this species likely also feeds on flower nectar and insect honeydew (Orr, 1988; Safavi, 1968). Other studies have documented that longevity decreases as a result of prolonged exposure to decreasing temperatures (Colinet et al., 2006; Langer and Hance, 2000; Lysyk, 2004). Our results provide evidence of an effect on longevity after brief cold exposure, where individuals of *T. japonicus* and *T. cultratus* that are able to survive brief exposure to subzero temperatures do so at the expense of their longevity. Such sublethal trade-offs could have negative implications for establishment success of *T. japonicus* or *T. cultratus* in areas that experience even brief subzero temperatures (e.g., Bale 2002).

Localized detections of *T. japonicus* in the U.S. over consecutive summers provide evidence that the parasitoid is able to successfully overwinter or re-colonize. In Winchester, Virginia, the coldest area in the U.S. where *T. japonicus* was found for multiple summers (2015 and 2016, Hoelmer, unpublished data), the average annual extreme minimum temperature ranges from −20.6 to −17.8 °C (USDA, 2012). Based on our laboratory experiments, in which the insects were in direct contact with low temperature, this range of temperatures caused mortality in approximately 25–91% of the laboratory populations of *T. japonicus*. However, some insects seek overwintering habitats that buffer extreme cold as measured by ambient temperature (Inkley, 2012; Koch et al., 2004; Vermunt et al., 2012). Therefore, it is possible that our predictions underestimate survivorship in nature because our methodology does not account for potential thermal buffering provided by an overwintering habitat. *Trissolcus japonicus* may overwinter as an adult below *Zelkova* spp. tree bark (Hirose et al., personal communication), which is a similar behavior to that of its host, *H. halys* (Lee et al., 2014), and other *Trissolcus* spp. (Fathi et al., 2011; Safavi, 1968). It is likely that during this overwintering period *T. japonicus* adults experience some degree of thermal buffering to ambient air temperatures, which could improve their ability to survive in these areas (e.g., Turnock and Fields 2005). Field studies targeting the adventive populations of *T. japonicus* in the U.S. could help investigate the overwintering behavior of the wasps and the degree of thermal buffering provided by bark. In addition, the adventive populations provide a unique opportunity to compare cold tolerance measurements between laboratory and field populations.

As *H. halys* has established in areas that experience cold temperatures, effective biological control agents must be able to withstand similar conditions. Based on our comparison of cold

tolerance indices for geographically-distinct populations of two *Trissolcus* spp., it appears that the populations are equally suited for survival in these areas. In regards to assessing the potential for classical biological control of *H. halys*, these *Trissolcus* spp. appear more cold tolerant than their host and therefore may not be limited by cold in areas that *H. halys* has invaded. However, if microclimate of specific overwintering habitat is not accounted for, resulting estimates of potential geographic range will be conservative. Therefore, to refine estimates of the geographic range of these species, further work is needed to determine the overwintering habitats of these *Trissolcus* spp. and to quantify cold exposure in such habitats.

Finally, arthropod overwintering success is contingent on a number of factors beyond temperature stress. Arthropods employ a number of mechanisms to survive cold exposure including (but not limited to), cryoprotectant and antifreeze protein synthesis, membrane restructuring, adjustments to ion transports, upregulation of heat shock proteins, etc. (Teets and Denlinger, 2013). Further studies with *T. japonicus* or *T. cultratus* should examine additional factors affecting overwintering potential (e.g., Colinet et al., 2007), or the mechanisms that drive that success (e.g., Foray et al., 2013).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocontrol.2017.01.004>.

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