

**Biology and Management of Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål), in Agricultural and Urban Environments**

John David Aigner Jr.

Dissertation submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Entomology

Thomas P. Kuhar, Chair

Donald E. Mullins

Douglas G. Pfeiffer

Tracy C. Leskey

March 24, 2016

Blacksburg, VA

Keywords: brown marmorated stink bug, *Halyomorpha halys*, cold tolerance, heat treatment, neonicotinoid, light trap, pheromone trap

# **Biology and Management of Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål), in Agricultural and Urban Environments**

**John D. Aigner Jr.**

## **ABSTRACT**

Brown marmorated stink bug, *Halyomorpha halys* (Stål), is a crossover pest impacting agriculture and invading urban environments. Studies were conducted to better understand the management of the bug through its physiology, behavior and susceptibility to insecticidal intervention.

*Halyomorpha halys* exhibit great variability in overwintering site selection with choices including man-made structures and tree bark. Because of these diverse sites, the bug must have the ability to withstand varying conditions throughout the overwintering cycle. We were able to determine that *H. halys* is chill intolerant and capable of adapting its tolerance to temperatures by season, sex, and location of acclimation. The mean supercooling point ( $\pm$  SEM) in the winter in Minnesota was  $-17.06^{\circ}\text{C} \pm 0.13^{\circ}$  and in Virginia was  $-13.90^{\circ}\text{C} \pm 0.09^{\circ}$ .

Laboratory experiments conducted in Blacksburg, VA were able to determine baseline lethal high temperatures over time against *H. halys* adults. To achieve 100% mortality, temperatures fell between  $45^{\circ}\text{C}$  and  $50^{\circ}\text{C}$ ,  $40^{\circ}\text{C}$  and  $45^{\circ}\text{C}$ , and  $42^{\circ}\text{C}$  and  $45^{\circ}\text{C}$ , over 15-min, 1-h, and 4-h, respectively. Moving forward, we were able to utilize this information to develop heat treatment guidelines for export shipping cargo infested with overwintering *H. halys*. In a controlled field experiment, we determined that exposing the coldest areas of an infested vehicle to temperatures greater than  $50^{\circ}\text{C}$  for a minimum of 15 minutes resulted in 100% mortality of overwintering BMSB adults.

In 2012 and 2013, citizen scientists were recruited through Virginia Cooperative Extension to assist in evaluating several in home light traps designed to help eradicate overwintering *H. halys* adults in homes. Over the course of the two year study, fourteen houses participated in the study with 72% of those houses having stink bug activity. It was found that the most effective trap was an aluminum foil pan trap. In 2013, the trap was 19 times more effective at catching stink bug adults than any other trap tested.

In September of 2014, a near-field experiment was conducted to determine the residual efficacy of several recommended and labeled insecticides for treatment of homes against invading *H. halys* adults. This study used constructed window screen bags that were dipped in insecticide solution. After the initial treatment, bugs were exposed to the bags for 24h weekly, up to 54 days after treatment (DAT). It was determined that 2 DAT all insecticides had activity except for indoxacarb. All insecticides lost efficacy after 29 DAT except for lambda-cyhalothrin, beta-cyfluthrin, beta-cyfluthrin + imidacloprid, lambda-cyhalothrin + thiamethoxam, and dinotefuran, which had some measureable activity even after 40 DAT. Each of these insecticides contained a pyrethroid alone or in combination with a neonicotinoid.

Laboratory bioassays were conducted to determine the  $\text{LC}_{50}$  values of clothianidin, dinotefuran, imidacloprid, and thiamethoxam against *H. halys* nymphs using a systemic application method. Those

LC<sub>50</sub> values were found to be 0.077, 0.013, 0.068, and 0.018 ppm, respectively. Field experiments conducted in Virginia in 2012 and 2013 showed a significant reduction of stink bug damage using two soil applications of neonicotinoid insecticides in pepper and tomato. In North Carolina, a single drip irrigation application significantly reduced stink bug damage in 2012 and 2014 using dinotefuran or imidacloprid.

## Acknowledgements

I would like to express my never-ending gratefulness and humble loyalty to my Ph.D. advisor Dr. Thomas P. Kuhar. Dr. Kuhar introduced me to the world of entomology over a decade ago as an undergraduate student, and I owe much of my success to his guidance and support. I will forever cherish my time working for him and look forward to a lifetime of friendship. I would also like to thank my Ph.D. committee members, Drs. Donald Mullins, Doug Pfeiffer and Tracy Leskey. Without your support and willingness to challenge me, I would not have become the scientist that I aspired to be.

I would also like to thank many of the Virginia Tech Entomology faculty. Drs. Troy Anderson, Carlyle Brewster, Scott Salom and Loke Kok have always made themselves available to offer assistance and their full support when I needed it during this process. I would like to extend a special thank you to Kathy Shelor and Sarah Kenley for their support in helping me conquer the heaps of administrative hurdles as well as Jon Wooge and the crew at Kentland Farm for help with my research.

I should also give my special thanks to the students of the Entomology Department. We have cultivated friendships that will last forever. The ups and downs of graduate school are not easy to navigate and all of you contributed to my success in this program. I would like to give special thanks to Sandra Gabbert, Jamie Hogue, James Wilson, Louie Nottingham, Adam Moorehead, Chris Philips, Anna Wallingford, Adam Wimer, James Mason, Ashly Lohr, Anthony Dimeglio, and Katlin Mooneyham. Your assistance and mentorship will always carry on with me in my career. Angel, Jake and James, we had a good run.

It's not often that you get to go to graduate school with your brother. Ben, I am so happy we had the opportunity to experience much of this together, and I am proud beyond words of your success. I look forward to watching you blossom in your own career, "Brother." I would also like to thank Shaun, Tanyua, Shannon, Joe, and Ashley for their continued encouragement throughout this long, arduous process.

Many thanks to my parents, Johnny and Shari, for giving me the strong foundation on which to build my success and never placing a limit on my dreams; your love and support were unwavering. I know it wasn't easy raising seven children, but I have always strived to make you proud. For my mother-in-law and father-in-law, Pat and Bobby, you have treated me like I was your own son from day one, and I hope I have also made you proud. You have been involved in my pursuit of this dream from the beginning, and I greatly appreciate everything you have done to aid in my success. To my dearest Gemma, you made everything better.

Lastly, but most importantly, I want to give my ever-long thanks to my wife, Mandy. I can't express how grateful I am for your sacrifices that allowed me to pursue my education. Your indiscriminant love and support has been more admirable than I can ever tell you. Without you, I could have never accomplished anything even close to this. You challenge me to be a better person, professional and husband every day. I love you.

**Table of Contents**

Acknowledgements.....i

List of Figures.....iii

List of Tables.....vi

*Chapter One*

    Literature Review: Brown Marmorated Stink Bug Biology and Management.....1

    Nomenclature.....1

    Invasion History and Distribution.....1

    Pest Significance.....2

    Life Cycle and Description.....3

    Injury.....4

    Management.....5

    References.....7

*Chapter Two*

    Cold Tolerance of *Halyomorpha halys* (Hemiptera: Pentatomidae) Across Geographic and Temporal Scales.....22

    Abstract.....22

    Introduction.....22

    Materials and Methods.....25

    Results and Discussion.....32

    Acknowledgements.....37

    References.....37

*Chapter Three*

    Lethal High Temperature Extremes of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) and Efficacy of Commercial Heat Treatments for Control in Export Shipping Cargo.....48

    Acknowledgements.....52

References.....52

*Chapter Four*

Using Citizen Scientists to Evaluate Light Traps for Catching Brown Marmorated Stink Bugs in Homes in Virginia.....59

Abstract.....59

Introduction.....59

Materials and Methods.....61

Results.....63

Discussion.....63

Acknowledgements.....64

References.....64

*Chapter Five*

Control of Brown Marmorated Stink Bug with Insecticide-Treated Window Screens.....74

*Chapter Six*

Toxicities of Neonicotinoid Insecticides for Systemic Control of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Fruiting Vegetables.....77

Abstract.....77

Introduction.....77

Materials and Methods.....79

Results.....82

Discussion.....83

Acknowledgements.....86

References.....86

*Chapter Seven*

Summary.....98

## List of Figures

<b>Figure 1.1:</b> Brown marmorated stink bug adult (Photo Credit: Stan Qilliam).....	16
<b>Figure 1.2:</b> Ventral view of the terminal abdominal segment in male BMSB adult (Photo Credit: Eric Day).....	17
<b>Figure 1.3:</b> Ventral view of the terminal abdominal segment in female BMSB adult (Photo Credit: Eric Day).....	18
<b>Figure 1.4:</b> Freshly laid BMSB egg mass (Photo Credit: Yurika Alexander).....	19
<b>Figure 1.5:</b> First instars surrounding a newly hatched BMSB egg mass (Photo Credit: Robert W. Bennett II).....	20
<b>Figure 1.6:</b> Brown marmorated stink bug nymph (Photo Credit: Fred Roe).....	21
<b>Figure 2.1:</b> Observed cumulative supercooling point distribution and mortality distribution for field-acclimated adult <i>Halyomorpha halys</i> in Minnesota in (A) December 2013 (B) December 2014. Extrapolation between observed data calculated with right and interval censored survival analysis.....	42
<b>Figure 2.2:</b> Predicted and observed cumulative supercooling point distribution for field-acclimated adult <i>H. halys</i> in Virginia in winter. Predicted curve calculated with a Weibull distribution.....	43
<b>Figure 2.3:</b> Mean supercooling points of adult <i>H. halys</i> field-acclimated either in Virginia or Minnesota. Individuals remained outdoors, experiencing temperature and photoperiodic cues, until the time of testing. Error bars indicate standard error of the mean. Statistics were run on transformed data and bars with the same letter are not statistically different ( $P > 0.05$ ; Tukey's HSD).....	44
<b>Figure 2.4:</b> Mean supercooling points of <i>H. halys</i> adults originating from either West Virginia or Virginia and field-acclimated as adults in Virginia (Top panel) Male, (Bottom panel) Female. Individuals remained outdoors, experiencing temperature and photoperiodic cues, until the time of testing. Error bars indicate standard error of the mean. Across both panels bars with the same letter are not statistically different ( $P > 0.05$ ; Tukey's HSD).....	45

**Figure 3.1:** Vehicle used for experiments parked inside a container heat treatment facility constructed by Willenius Wilhelmsen Logistics (WWL) a) and the 500,000 BTU propane heaters b) used to generate lethal temperature extremes >60°C for heat treatment of vehicles.....55

**Figure 3.2:** Dead brown marmorated stink bugs in a mesh bag after removal from under the seat of a vehicle exposed to heat treatments at the Port of Savannah, GA.....56

**Figure 4.1:** Aluminum foil pan trap with soapy water in action in a home in Loudoun County, Virginia...67

**Figure 4.2:** Commercially available 2011 Strube’s Stink Bug Trap.....68

**Figure 4.3:** Homemade 2-liter bottle trap with LED press-on light in the bottom.....69

**Figure 4.4:** Rescue® stink bug trap in a home in Loudoun County, Virginia in 2013 .....70

**Figure 4.5:** Mean ( $\pm$ SE) number of stink bugs caught by week in all light traps placed in houses in Virginia by citizen scientists in 2012 and 2013 .....71

**Figure 4.6:** Cumulative Mean ( $\pm$ SE) Number of BMSB Adults Captured per Trap per Week in 2012.....72

**Figure 4.7:** Cumulative Mean ( $\pm$ SE) Number of BMSB Adults Captured per Trap per Week in 2013.....73

**Figure 6.1:** Mean ( $\pm$ SE) cumulative percentage stink bug damage to pepper fruit from soil-drench insecticide field-efficacy experiments conducted near Blacksburg, Virginia. All neonicotinoids were applied twice as a soil drench 30 days apart. Fenprothrin was applied as four weekly foliar sprays. Bars within years with a letter in common are not significantly different according to Fisher’s Protected LSD ( $P > 0.05$ ).....94

**Figure 6.2:** Mean ( $\pm$ SE) cumulative percentage stink bug damage to tomato fruit from soil-drench insecticide field-efficacy experiments conducted near Blacksburg, Virginia, in 2013. All neonicotinoids were applied twice as a soil drench 30 days apart. Fenprothrin was applied as four weekly foliar



sprays. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ( $P > 0.05$ ).....95

**Figure 6.3:** Mean ( $\pm$ SE) cumulative percentage stink bug damage to tomato fruit resulting from drip-line application of soil insecticides conducted near Mills River, North Carolina. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ( $P > 0.05$ ).....96

## List of Tables

<b>Table 2.1:</b> Proportion mortality $\pm$ SEM of adult <i>H. halys</i> acclimated outdoors in Minnesota in 2013 and 2014 and exposed to one of five temperatures. Numbers in parentheses indicate the total number of adults that were either chilled (unfrozen) or frozen upon reaching the target temperature.....	46
<b>Table 2.2:</b> Observed and expected <i>H. halys</i> winter mortality. Minimum temperatures reached in Minnesota and Virginia in the winter of 2013-2014 and 2014-2015, expected mortality of <i>H. halys</i> adults for recorded temperatures, observed mortality.....	47
<b>Table 3.1:</b> Average mortality of <i>H. halys</i> adults* following exposure for different times and temperatures in an incubator located at Virginia Tech, Blacksburg, VA (USA).....	57
<b>Table 3.2:</b> Targeted temperatures, actual measured temperatures, and observed mortality of <i>H. halys</i> adults after 15 min exposures to heat treatments applied to a vehicles at the Port of Georgia, Savannah, GA, USA in 2015.....	58
<b>Table 5.1:</b> Residual efficacy of window screens treated with commercial insecticides for control of BMSB .....	76
<b>Table 6.1:</b> Observed LC <sub>50</sub> levels of four neonicotinoid insecticides on <i>Halyomorpha halys</i> nymphs 72 h after exposure on treated snap bean plants in an insecticide plant uptake bioassay.....	97

## Chapter One

### Literature Review: Brown Marmorated Stink Bug Biology and Management

Recent reviews of the biology and management of the brown marmorated stink bug, *Halyomorpha halys* (Stål) in the U.S. were written by Leskey et al. (2012) and Rice et al. (2014). In addition, Lee et al. (2013a) recently reviewed the biology and management of this insect in China, Japan, and Korea.

**Nomenclature.** The brown marmorated stink bug (BMSB) is a member of the Pentatomidae family within the order Hemiptera. The species was first described as *Pentatoma halys* in 1855 by Stål and is a native of eastern China, Japan and Korea (Lee et al. 2013a). It has been described as *Poecilometis mistus* by Uhler in 1860, *Dalpada brevis* and *Dalpada remota* by Walker in 1867 and was synonymized by Josifov and Kerzhner (1978) as *Halyomorpha halys* (Hoebeke and Carter 2003). It has been misidentified as *Halyomorpha picus* Fabr.; however, *H. picus* is native to India and is not located in the native range of *H. halys* (Rider et al. 2002).

**Invasion History and Distribution.** BMSB was first detected in North America in Allentown, Pennsylvania in the 1990s (Hoebeke and Carter 2003), and was first reported in Virginia in 2004 (Day et al. 2011). The US population was recently found by Xu et al. (2014) to have originated from a single source in Beijing, China. The insect likely arrived in the U.S. as a hitchhiker on shipping freight (Hamilton 2009).

Since its introduction to the United States, BMSB has spread rapidly and, as of 2016, had been detected in 41 states in the continental U.S. as well as Ontario, Canada (Northeastern IPM Center 2016). It has become a serious nuisance and agricultural pest throughout the mid-Atlantic states from New Jersey to North Carolina as well as in parts of Oregon and Washington (Rice et al. 2014). Brown marmorated stink bug has also recently been detected in France, Hungary, Italy, and Switzerland (Vetek et al. 2014, Cesari

et al. 2015, Haye et al. 2015). Using climate matching models, Zhu et al. (2012) predicts that there are several areas in the United States, including the Pacific northwest, northern California, the eastern US, and parts of the Central Plains, that have favorable climatic conditions to support BMSB populations.

**Pest significance.** For over a century, Virginia's stink bug complex in most agricultural crops has been dominated by two native species, the green stink bug, *Chinavia hilaris* (Say), and the brown stink bug, *Euschistus servus* (Say) (Underhill 1934, McPherson and McPherson 2000, Day and Kuhar 2003, Kamminga 2008). However, Basnet et al. (2014) showed that over a 5-year span beginning in the late 2000s, BMSB went from being not present to the dominant stink bug species found in raspberries on a farm located in southwest Virginia. Other studies conducted in Virginia have also recently documented BMSB to be the dominant stink bug species occurring on tree fruit, unmanaged hardwood trees, grapes, soybeans, and vegetables in the western, northern, and central portions of Virginia (Acebes-Doria et al. 2015, Bakken et al. 2015, Basnet et al. 2015, Bergmann 2016). However, the aforementioned two native stink bug species still appear to dominate in the coastal and southeastern regions of Virginia and North Carolina, with very few BMSB found (Bakken et al. 2015).

Where it is established, BMSB is an annual nuisance pest in and around human dwellings and can be a serious agricultural pest in many commodities including tree fruit, fruiting vegetables, beans, and corn (Leskey et al. 2012b, Rice et al. 2014). Both adults and nymphs feed on plants by inserting their piercing-sucking mouthparts (stylets) into leaves, stems, and especially fruiting structures or pods. In 2010 alone, the pest caused millions of dollars of losses in the Mid-Atlantic Region with some Maryland growers experiencing up to 100% loss of their peach crop that year (Leskey et al. 2012a). Similar injury to sweet corn can occur if BMSB is not controlled (Kuhar et al. 2012f). Stink bug injury to tomato and pepper in Virginia commonly exceeds 30% when insecticides are not applied for stink bug control (Kuhar et al. 2012a-e, 2013a and b).

**Life Cycle and Description.** Adults are chevron-shaped, brownish in color, and are 12 – 17 mm long and up to 10 mm wide (Hoebecke and Carter 2003). They have distinctive white stripes on the legs and antennae of the insect, as well as a brown and white banding at the bottom of the abdomen (Fig. 1.1). Brown marmorated stink bug has been documented in its native range as having one to two generations per year (Zhang et al. 1993, Yu and Zhang 2009), although there has been a suggestion that an area in southern China has 4-6 generations per year (Hoffman 1931). It has been determined that BMSB has a consistent pattern in the U.S. and is likely univoltine in the upper mid-Atlantic region (Nielsen and Hamilton 2009) and bivoltine in areas of Virginia, West Virginia, and North Carolina (Leskey et al. 2012c, Bakken et al. 2015). Adult BMSB are clearly sexually dimorphic with males having a u-shaped indentation on the terminal abdominal segment (Fig. 1.2), whereas the females have what appears to be an enclosed terminal abdominal segment (Fig. 1.3).

During the winter months, adult bugs aggregate in tight areas of man-made structures, as well as protected areas in the natural landscape such as inside dead standing trees (Lee et al. 2014). BMSB adults begin to search for overwintering sites in early fall in man-made structures and other well protected areas in the natural landscape (Qin 1990, Hamilton 2008, Inkley 2012). The search for suitable overwintering habitat can continue into the late fall (Lee et al. 2014). The triggers determining the BMSB overwintering behavior have not been well characterized in the literature. Adults emerge from overwintering sites once temperatures exceed 10°C beginning in March (Qin 1990). Additionally, Funayama (2012) suggests that BMSB will start to abandon their overwintering sites regardless of temperature, when their nutritional reserves have become depleted.

Once adults emerge in the spring, females complete their ovarian development, mate, and begin depositing eggs on the undersides of leaves in trees. Some common ovipositional sites for BMSB include

Tree of Heaven (*Ailanthus altissima*), paulownia (*Paulownia tomentosa*), and catalpa (*Catalpa speciosa*), as well as many other fruit trees (Baaken et al. 2015). Egg masses are generally in clusters of 28 and are a light/mint green color when fresh (Fig. 1.4) and gradually become whiter in color as the chorion of the egg hardens and matures. Eggs can hatch in as little as four days ranging to twenty-two days, depending on temperature (Nielsen et al. 2008b).

Brown marmorated stink bug has a total of five instars before completing their development into adults. After hatching, first instar BMSB stay clustered on the egg mass, where they acquire essential endosymbionts critical for early development (Taylor et al. 2014). First instars are reddish orange and black in color (Fig. 1.5). After molting in 3-6 days, 2nd instars move from the egg mass and begin to feed on foliage and fruiting structures of the host plant. Third, fourth and fifth instars (Fig. 1.6) generally molt 12-13, 19-20, and 26-27 days after egg hatch, respectively (Nielsen et al. 2008b, Rice et al. 2014).

In addition to impacting development of BMSB, extreme temperatures can also impact survival of the bug. The effects of lethal high and lethal low temperatures on BMSB are discussed in Chapters 2 and 3 of this dissertation.

**Injury.** Visual symptoms of injury from BMSB can vary greatly depending on the crop in which the bug has been feeding upon. In many vegetables, including bean, pepper, tomato and eggplant, the injury is often described as a whitish mark where the tissue has sunken in and has a spongy feel (Kuhar et al. 2012f, 2015). On sweet corn and tree nuts, brown marmorated stink bug can feed directly through the husk, damaging the kernels, which can become shriveled and discolored once fed upon (Hedstrom et al. 2014, Cissel et al. 2015). Injury in apples is characterized by a discolored depression and can vary based on cultivar (Leskey et al. 2012b, Rice et al. 2014). Peaches that have been fed upon exhibit a puncture that sinks in leaving a depression in the fruit and can often be identified by gummosis (Nielsen and Hamilton 2009). In field crops, soybeans are a primary host where the damage can be shown in under-

or un-developed bean pods. Generally seen as being an edge effect, a large issue associated with soybeans is that BMSB feeding can cause “stay-green” syndrome, where the plant is unable to senesce, which inhibits a grower’s ability to harvest effectively (Owens et al. 2013, Koch and Rich 2015). Additionally, it has been shown that BMSB is capable of transmitting *Pauwlonia* witch’s broom, a phytoplasma disease in woody plants (Lee et al. 2013a).

## **Management.**

*Cultural.* Brown marmorated stink bug has a wide array of host species that support populations including unmanaged woodlots. There is the potential utilize cultural techniques to manipulate pest populations. For instance, knowing that BMSB is a border driven pest, control tactics can possibly be focused on borders alone (Basnet 2015). Indeed border-only applications of insecticides has resulted in effective control of BMSB in soybeans and peaches (Ames Herbert and Galen Dively, *unpublished data*, Venugopal et al. 2014, Blaauw et al. 2015). Use of trap crops has also been explored with plants such as sunflowers and sorghum representing attractive trap plants (Soergel et al. 2015).

*Chemical.* Applications of insecticides, especially pyrethroids, organophosphates, carbamates, and neonicotinoids has been the most effective and efficient strategy to manage most stink bug pest species (McPherson and McPherson 2000, Nielsen et al. 2008a, Kamminga et al. 2009, Wallingford 2012, Lee et al. 2013). Through a number of lab bioassay and field studies, it has been confirmed that many of the traditional insecticides including most pyrethroids, the organophosphate, acephate, the carbamates, methomyl and oxamyl, and the neonicotinoids dinotefuran, imidacloprid, thiamethoxam, clothianidin, and acetamiprid are also effective at controlling BMSB (Nielsen et al. 2008a, Lee et al. 2013b, Kuhar et al. 2012a-f, 2013a and b, Leskey 2012a and c, 2014). In Chapter 6, I examine the potential of using systemic applications of neonicotinoids for BMSB control on vegetables (see Chapter 6). Because of the

true nature of broad-spectrum chemistries, these products have become highly disruptive to IPM programs, risking secondary pest outbreaks, such as green peach aphid in peppers (Kuhar et al. 2012a).

*Biological.* Several species of predators and parasitoids have been identified as being active against all life stages of BMSB in both its native range in Asia (Yang et al. 2009) and in the US (Biddinger et al. 2012, Rice et al. 2014). Potential predators can include families such as Carabidae, Reduviidae, Cantharidae, Coccinellidae, Forficulidae, Tettigoniidae, and Tachinidae among others (Rice et al. 2014, Morrison et al. 2016). In addition, it has been noted that there are some Araneae that are exhibiting predacious behavior on BMSB egg masses (Leskey 2012b). Many of the native Hymenopteran egg parasitoids have fallen into families such as Eupelmidae (*Anastatus spp.*) and Platygasteridae (*Telonomus spp.* and *Trissolcus spp.*) (Jones et al. 2014, Rice et al. 2014). Several *Trissolcus spp.* have been under evaluation for potential release with the USDA. However, recent studies using sentinel egg masses have confirmed that there is a wild population of *Trissolcus japonicus* (Ashmead) in Beltsville, MD (Talamas et al. 2015).

*Monitoring and sampling.* A number of tactics can be used to sample stink bugs including beat sheeting, sweep netting, visual counts, blacklight traps, and pheromone traps (McPherson and McPherson 2000, Leskey and Hogmire 2005). Leskey et al. (2012b) found that a 4-ft tall black pyramid trap was an effective visual stimulus attracting BMSB adults and nymphs. Morrison et al. (2015) confirmed that this trap type constructed from black Coroplast™ caught the most BMSB. Since that time, a major effort has been underway to utilize these traps in combination with semiochemical attractants (Leskey et al. 2012c). It was discovered that BMSB males produce a two-component aggregation pheromone that has been identified as (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (Zhang et al. 2013, Khrimian et al. 2014). This pheromone has been found to be attractive to BMSB nymphs and adults season long (Leskey et al 2015a). (Rice et al. 2014). However, when used in combination with methyl (E,E,Z)-2,4,6-decatrienoate, BMSB has been shown to be



attractive season long (Weber et al. 2014). The combination of the two semiochemical lures has been shown to be an effective season-long trapping strategy in various agroecosystems (Leskey et al. 2015b).

In this dissertation I report on several studies that will help improve our knowledge of the biology and management of BMSB. My objectives are as follows:

1. Determine the supercooling cooling point of BMSB and impact of subfreezing temperatures on mortality.
2. Determine the lethal temperature exposure limits of BMSB and efficacy of commercial heat treatments for shipping cargo.
3. Determine the efficacy of several indoor-use light traps against BMSB.
4. Evaluate the efficacy of several labeled insecticides against BMSB using a novel application on window screen.
5. Evaluate the efficacy of neonicotinoid insecticides for systemic control of BMSB in fruiting vegetables.

## References

- Acebes-Doria, A. L., T. C. Leskey, and C. J. Bergh. 2016.** Host plant effects on *Halyomorpha halys* (Hemiptera: Pentatomidae) nymphal development and survivorship. *Environ. Entomol.* In Press.
- Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert Jr., D., T. P. Kuhar, and J. Walgenbach. 2015.** Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. *Environ. Entomol.* 44: 1011-1021.
- Basnet, S., L. M. Maxey, C. A. Laub, T. P. Kuhar, and D. G. Pfeiffer. 2014.** Stink bugs (Hemiptera: Pentatomidae) in primocane-bearing raspberries in southwestern Virginia. *J. Entomol. Sci.* 49: 304–312.

- Basnet, S., T. P. Kuhar, C. A. Laub, and D. G. Pfeiffer. 2015.** Seasonality and distribution pattern of brown marmorated stink bug (Hemiptera: Pentatomidae) in Virginia vineyards. *J. Econ. Entomol.* 108: 1902 – 1909.
- Bergmann, E. J., P. D. Venugopal, H. M. Martinson, M. J. Raupp, P. M. Shrewsbury. 2016.** Host plant use by the invasive *Halyomorpha halys* (Stål) on woody ornamental trees and shrubs. *PLoS ONE* 11 (2): e0149975. doi:10.1371/journal.pone.0149975
- Biddinger, D., J. Tooker, A. Surcica, and G. Krawzyk. 2012.** Survey of native biocontrol agents of the brown marmorated stink bug in Pennsylvania fruit orchards and adjacent habitat, pp. 47-54, Pennsylvania Fruit News.
- Blaauw, B. R., D. Polk, and A. L. Nielsen. 2015.** IPM-CPR for peaches: Incorporating behaviorally-based methods to manage *Halyomorpha halys* and key pests in peach. *Pest Manag. Sci.* doi: 10.1002/ps.3955.
- Cesari, M., L. Maistrello, F. Ganzerli, P. Dioli, L. Rebecchi, and R. Guidetti. 2015.** A pest alien invasion in progress: potential pathways of origin of the brown marmorated stink bug *Halyomorpha halys* populations in Italy. *J. Pest Sci.* 88: 1-7.
- Cissel, W. J., C. E. Mason, J. Whalen, J. Hough-Goldstein, and C. R. R. Hooks. 2015.** Effects of brown marmorated stink bug (Hemiptera: Pentatomidae) feeding injury on sweet corn yield and quality. *J. Econ. Entomol.* 108(3): 1 – 7.
- Day, E. R. and T. P. Kuhar. 2003.** Stink Bugs Fact Sheet, Revised 2003. Virginia Coop. Ext. Publ. No. 444-621.
- Day, E. R., T. McCoy, D. Miller, T. P. Kuhar, and D. G. Pfeiffer. 2011.** Brown marmorated stink bug. 2902-1100. ([http://www.pubs.ext.vt.edu/2902/2902-1100/2902-1100\\_pdf.pdf](http://www.pubs.ext.vt.edu/2902/2902-1100/2902-1100_pdf.pdf))
- Funayama, K. 2012.** Nutritional states of post-overwintering adult brown-marmorated stink bugs, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae). *Jpn. J. Appl. Entomol. Zool.* 56: 12 - 15.

- Hamilton, G. C. 2009.** Brown marmorated stink bug. *Am. Entomol.* 55: 19.
- Haye, T., T. Garipey, K. Hoelmer, J.-P. Rossi, J.-C. Streito, X. Tassus, and N. Desneux. 2015.** Range expansion of the invasive brown marmorated stinkbug, *Halyomorpha halys*: an increasing threat to field, fruit and vegetable crops worldwide. *J. Pest Sci.* 88: 665-673.
- Hedstrom, C., P. Shearer, J. Miller, and V. Walton. 2014.** The effects of kernel feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) on commercial hazelnuts. *J. Econ. Entomol.* 107: 1858-1865.
- Hoffmann W.E. 1931.** A pentatomid pest of growing beans in South China. *Peking Nat. Hist. Bull.* 5: 25–26.
- Hoebeke, E. R., and M. E. Carter. 2003.** *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. *Proc. Entomol. Soc. Washington* 105: 225-237.
- Inkley, D. B. 2012.** Characteristics of home invasion by the brown marmoratedstink bug (Hemiptera: Pentatomidae). *J. Entomol. Sci.* 47: 125–130.
- Jones, A. L., D. E. Jennings, C. R. Hooks, and P. M. Shrewsbury. 2014.** Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. *Biol. Contr.* 78: 61-66.
- Josifov, M., and I. Kerzhner. 1978.** Heteroptera aus Korea. II. Teil (Aradidae, Berytidae, Lygaeidae, Pyrrhocoridae, Rhopalidae, Alydidae, Coreidae, Urostylidae, Acanthosomatidae, Scutelleridae, Pentatomidae, Cydnidae, Plataspidae). *Fragm. Faun.* 23: 137-196.
- Kamminga, K.L. 2008.** Species survey, monitoring and management of economically important stink bug species in eastern Virginia. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA.
- Khrimian, A., A. Zhang, D. C. Weber, H.-Y. Ho, J. R. Aldrich, K. E. Vermillion, M. A. Siegler, S. Shirali, F. Guzman, and T. C. Leskey. 2014.** Discovery of the aggregation pheromone of the brown

marmorated stink bug (*Halyomorpha halys*) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols. J. Nat. Prod. 77: 1708-1717.

**Koch, R. L., and W. A. Rich. 2015.** Stink bug (Hemiptera: Heteroptera: Pentatomidae) feeding and phenology on early-maturing soybean in Minnesota. J. Econ. Entomol. 108: 2335-2343.

**Kuhar, T., H. Doughty, K. Kamminga, and L. Lilliston. 2012a.** Evaluation of insecticides using bean dip bioassay for control of brown marmorated stink bug, 2011. Arthropod Manag. Tests 37: L1.

**Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips & J. Aigner. 2012b.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 1. Arthropod Mgt. Tests 38: E37. online publ. DOI: 10.4182/amt.2012.E37.

**Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips & J. Aigner. 2012c.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 2. Arthropod Mgt. Tests 38: E38. online publ. DOI: 10.4182/amt.2012.E38.

**Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips & J. Aigner. 2012d.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 3. Arthropod Mgt. Tests 38: E39. online publ. DOI: 10.4182/amt.2012.E39.

**Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips & J. Aigner. 2012e.** Evaluation of insecticides for the control of brown marmorated stink bug in tomatoes in Virginia, 2011. Arthropod Mgt. Tests 37: E72. online publ. DOI: 10.4182/amt.2012.E72.

**Kuhar, T. P., K. L. Kamminga, J. Whalen, G. P. Dively, G. Brust, C. R. R. Hooks, G. Hamilton, and D. A. Herbert. 2012f.** The pest potential of brown marmorated stink bug on vegetable crops. Online. Plant Health Prog. doi:10.1094/PHP-2012-0523-01-BR

**Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips & J. Aigner. 2013a.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 1. Arthropod Mgt. Tests 38: E39. online publ. DOI: 10.4182/amt.2013.E39.

- Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips & J. Aigner. 2013b.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 2. *Arthropod Mgt. Tests* 38: E40. online publ. DOI: 10.4182/amt.2013.E40.
- Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips & J. Aigner. 2013c.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 3. *Arthropod Mgt. Tests* 38: E41. online publ. DOI: 10.4182/amt.2013.E41.
- Kuhar, T.P., J.A. Morehead, and T. Dimeglio. 2015.** Diagnosing stink bug injury to vegetables. Virginia Coop. Ext. Pub. No. ENTO-173NP. <https://pubs.ext.vt.edu/ENTO/ENTO-173/ENTO-173.html>
- Lee, D.-H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. 2013a.** Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environ. Entomol.* 42: 627-641.
- Lee, D.-H., S. E. Wright, and T. C. Leskey. 2013b.** Impact of insecticide residue exposure on the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae): Analysis of adult mobility. *J. Econ. Entomol.* 106: 150-158.
- Lee, D.-H., J. P. Cullum, J. L. Anderson, J. L. Daugherty, L. M. Beckett, and T. C. Leskey. 2014.** Characterization of overwintering sites of the invasive brown marmorated stink bug in natural landscapes using human surveyors and detector canines. *PLoS One* 9: e91575.
- Leskey, T. C., and H. W. Hogmire. 2005.** Monitoring stink bugs (Hemiptera: Pentatomidae) in mid-Atlantic apple and peach orchards. *J. Econ. Entomol.* 98: 143-153.
- Leskey, T. C., D.-H. Lee, B. D. Short, and S. E. Wright. 2012a.** Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): analysis of insecticide lethality. *J. Econ. Entomol.* 105: 1726-1735.
- Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk,**

- P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. A. Hoelmer, D.-H. Lee, and S. E. Wright. 2012b.** Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. *Outlooks Pest Manag.* 23: 218-226.
- Leskey, T. C., D.-H. Lee, B. D. Short & S. E. Wright. 2012c.** Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): Analysis of insecticide lethality. *J. Econ. Entomol.* 105: 1726–1735.
- Leskey, T. C., S. E. Wright, B. D. Short, and A. Khimian. 2012d.** Development of behaviorally based monitoring tools for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) in commercial tree fruit orchards. *J. Entomol. Sci.* 47: 76-85.
- Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. 2012e.** Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-Atlantic tree fruit orchards in the United States: Case studies of commercial management. *Psyche: A J. Entomol.* 2012: 1-14.
- Leskey, T. C., B. D. Short, and D. H. Lee. 2014.** Efficacy of insecticide residues on adult *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards. *Pest. Manag. Sci.* 70: 1097-1104.
- Leskey, T. C., A. Khimian, D. C. Weber, J. C. Aldrich, B. D. Short, D. Lee, D., and W. R. Morrison. 2015.** Behavioral responses of the invasive *Halyomorpha halys* (Stal) to traps baited with stereoisomeric mixtures of 10,11-Epoxy-1-bisabolen-3-OL. *J. Chem. Ecol.* 41:418-429.
- Leskey, T. C., A. Agnello, J. C. Bergh, G. P. Dively, G. C. Hamilton, P. Jentsch, A. Khimian, G. Krawczyk, T. P. Kuhar, and D.-H. Lee. 2015b.** Attraction of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) to traps baited with semiochemical stimuli across the United States. *Environ. Entomol.* 44: 746-756.
- McPherson, J. E., and R. M. McPherson. 2000.** Stink Bugs of Economic Importance in America North of Mexico. CRC Press, Boca Raton, FL. 253 pp.

- Morrison III, W. R., J. P. Cullum, and T. C. Leskey. 2015.** Evaluation of trap designs and deployment strategies for capturing *Halyomorpha halys* (Hemiptera: Pentatomidae). *J. Econ. Entomol.*: 1683-1692.
- Morrison III, W. R., C. R. Mathews, and T. C. Leskey. 2016.** Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. *Biol. Cont.* Vol. 97, June 2016. 120-130.
- Nielsen, A. L., P. W. Shearer, and G. C. Hamilton. 2008a.** Toxicity of insecticides to *Halyomorpha halys* (Hemiptera: Pentatomidae) using glass-vial bioassays. *J. Econ. Entomol.* 101: 1439-1442.
- Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008b.** Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* 37: 348-355.
- Nielsen, A. L., and G. C. Hamilton. 2009.** Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. *J. Econ. Entomol.* 102: 1133-1140.
- Northeastern IPM Center. 2016.** State-by-State. (<http://www.stopbmsb.org/where-is-bmsb/state-by-state>). Accessed 2-20-2016.
- Owens, D., J. Herbert, DA, G. Dively, D. Reisig, and T. Kuhar. 2013.** Does feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) reduce soybean seed quality and yield? *J. Econ. Entomol.* 106: 1317-1323.
- Qin, W. 1990.** Occurrence rule and control techniques of *Halyomorpha picus*. *Plant Prot.* 16: 22–23.
- Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Garipey, G. Hamilton, T. Haye, A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G. Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, and J. F. Tooker. 2014.** Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *J. Integr. Pest Manag.* 5: A1-A13.

- Rider, D., L. Zheng, and I. Kerzhner. 2002.** Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera). II. Pentatominae. Zoosyst. Rossica 11: 135-153.
- Soergel, D. C., N. Ostiguy, S. J. fleischer, R. R. Troyer, E. G. Rajotte, and G. Krawczyk. 2015.** Sunflower as a potential trap crop of *Halyomorpha halys* (Hemiptera: Pentatomidae) in pepper fields. Environ. Entomol. 44(6): 1581–1589 (2015); DOI: 10.1093/ee/nvv136
- Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. L. Buffington, M.-C. Bon, and D. C. Weber. 2015.** *Trissolcus japonicus* (Ashmead)(Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119-128.
- Taylor, C. M., P. L. Coffey, B. D. DeLay, and G. P. Dively. 2014.** The importance of gut symbionts in the development of the brown marmorated stink bug, *Halyomorpha halys* (Stål). PLoS One 9: e90312.
- Venugopal, P. D., P. L. Coffey, G. P. Dively, and W. O. Lamp. 2014.** Adjacent habitat influence on stink bug (Hemiptera: Pentatomidae) densities and the associated damage at field corn and soybean edges. PlosOne 9(10): e109917. doi:10.1371/journal.pone.0109917.
- Vetek, G., V. Papp, A. Haltrich, and D. Redei. 2014.** First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa 3780: 194-200.
- Underhill, G.W. 1934.** The green stink bug. Virginia Agric. Exp. Stn. Bull. 294: 1-26.
- Watanabe, M., R. Arakawa, Y. Shinagawa, and T. Okazawa. 1994.** Anti-invading methods against the brown marmorated stink bug, *Halyomorpha mista*, in houses. Jpn. J. Sanit. Zool. 45: 311–317.
- Wallingford, A.K. 2012.** Investigating host plant selection of harlequin bug, *Murgantia histrionica* (Hahn), in order to improve trap-cropping system for its management. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA.



- Weber, D. C., T. C. Leskey, G. C. Walsh, and A. Khrimian. 2014.** Synergy of aggregation pheromone with methyl (E, E, Z)-2, 4, 6-decatrienoate in attraction of *Halyomorpha halys* (Hemiptera: Pentatomidae). *J. Econ. Entomol.* 107: 1061-1068.
- Xu, J., D. M. Fonseca, G. C. Hamilton, K. A. Hoelmer, and A. L. Nielsen. 2014.** Tracing the origin of US brown marmorated stink bugs, *Halyomorpha halys*. *Biol. Invasions* 16: 153-166.
- Yang, Z.-Q., Y.-X. Yao, L.-F. Qiu, and Z.-X. Li. 2009.** A new species of *Trissolcus* (Hymenoptera: Scelionidae) parasitizing eggs of *Halyomorpha halys* (Heteroptera: Pentatomidae) in China with comments on its biology. *Ann. Entomol. Soc. Am.* 102: 39-47.
- Yu, G., and J. Zhang. 2009.** The brown-marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae) in P. R. China. *In* Y. Wu and X.-Y. Wang (eds.), "International workshop on biological control of invasive species of forests", 20–25 September 2007, Beijing, China.
- Zhang, C. T., X. Y. Yao, L. F. Qiu, and Z. F. Li. 1993.** A study on the biological characteristics of *Halyomorpha picus* and *Erthesina fullo*. *For.Res.* 6: 271–275.
- Zhang, A., A. Khrimian, J. R. Aldrich, T. C. Leskey, and D. C. Weber. 2013.** Compositions and methods to attract the brown marmorated stinkbug (BMSB) *Halyomorpha halys*. US Patent WO2013090703 A1.
- Zhu, G., W. Bu, Y. Gao, and G. Liu. 2012.** Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). *PLoS One* 7: e31246.



Figure 1.1. Brown marmorated stink bug adult (Photo Credit: Stan Qilliam).



Fig. 1.2. Ventral view of the terminal abdominal segment in male BMSB adult (Photo Credit: Eric Day).



Fig. 1.3. Ventral view of the terminal abdominal segment in female BMSB adult (Photo Credit: Eric Day).



Figure 1.4. Freshly laid BMSB egg mass (Photo Credit: Yurika Alexander).



Figure 1.5. First instars surrounding a newly hatched egg mass (Photo Credit: Robert W. Bennett II).



Figure 1.6. Brown marmorated stink bug nymph (Photo Credit: Fred Roe).

## Chapter Two

### Cold Tolerance of *Halyomorpha halys* (Hemiptera: Pentatomidae) Across Geographic and Temporal Scales

(As Published: T.M. Cira, R.C. Venette, J. Aigner, T. Kuhar, D.E. Mullins, S.E. Gabbert, and W.D. Hutchison. 2016. Environmental Entomology. 1 – 8. doi: 10.1093/ee/nvv220)

#### Abstract

The brown marmorated stink bug, *Halyomorpha halys* (Stål), is native to eastern Asia and is presently invading North America. Little is known about the exposure to and effects of winter temperatures in newly invaded regions on *H. halys*. The overwintering habitats that this species utilizes vary greatly in their thermal buffering capacity. They naturally overwinter in aggregations beneath loose bark on trees and in cliff-outcroppings, but will also commonly aggregate in buildings. Effects of cold temperatures such as mortality and freezing have yet to be quantified in the invading population. We report that *H. halys* is chill intolerant (i.e., dies before reaching its freezing point), and that the degree of cold tolerance of populations in North America differs by season, sex, and acclimation location. The mean winter supercooling point ( $\pm$  SEM) of individuals acclimated in Minnesota was  $-17.06^{\circ}\text{C} \pm 0.13$  and in Virginia was  $-13.90^{\circ}\text{C} \pm 0.09$ . By using laboratory assays of lower lethal temperatures and ambient air temperature records we accurately forecasted mortality for field experiments in Minnesota and Virginia. Temperature refugia provided by human-built structures are likely crucial for overwintering survival during atypically cold winters and possibly contribute to the northern geographic range expansion of this economically damaging insect in the temperate climates of North America.

#### Introduction

*Halyomorpha halys* (Stål), the brown marmorated stink bug, is native to East Asia and has become a severe invasive agricultural pest in the United States (US) on over 33 crop host plants (Bergmann et al. 2015). Damage to Mid-Atlantic apples alone exceeded \$37 million in 2010 (U.S. Apple Association News



Release 2011). This invading insect has been found in 42 US states since it was first detected in the US in the mid-1990's (Northeastern IPM Center 2015) and is predicted to establish across the entire contiguous United States based on the geographic distribution of hosts and degree day models (Holtz and Kamminga 2010) and ecological niche models (Zhu et al. 2012). These inductive approaches to modeling however, do not predict fluctuations in population levels, nor do they define the impacts of environmental stresses on populations for ecological and economic risk assessment (Venette et al. 2010). Insects are poikilotherms; ambient temperature strongly affects their development and mortality. Cold often dictates the poleward limits of a species' distribution. To clarify the relationship between cold-stress and mortality, we studied the overwintering strategies and lower lethal temperatures of this invasive species.

The capacity of *H. halys* to survive cold exposure (i.e., cold tolerance) is dependent on the temperatures individuals are *exposed to* and the *effects* of those cold temperatures on an insects' physiology. An observational study in Japan found that winter mortality was reduced by 13.5% for every 1°C rise in mean January and February temperature above 4°C (Kiritani 2007). Our work expands on these observations by experimentally testing the effects of cold and assessing mortality and cold exposure of *H. halys* that are invading North America. As winter approaches, *H. halys* enters a facultative reproductive diapause (Watanabe et al. 1978) and begins to aggregate in protected areas (Watanabe et al. 1994). In natural settings this species seeks shelter beneath loose bark on trees, but aggregations can also occur in human-made structures (Lee et al. 2014), which may offer greater thermal protection. We do not know the extent to which the North American population utilizes these overwintering habitats.

In order to examine the effects of cold temperatures on *H. halys* we first determined *H. halys*' cold tolerance strategy. Three broad insect cold tolerance categories exist to describe the relationship

between freezing and mortality. Freeze tolerant insects are able to live after the formation of ice within their bodies, freeze intolerant insects live up until the point at which they freeze, and chill intolerant insects die before freezing occurs (Lee 2010). Most temperate insects in the Northern Hemisphere are freeze intolerant (Bale 1991). To avoid freezing, various mechanisms are used, such as the production of cryoprotectants to depress the insect's supercooling point (i.e., the temperature at which body fluids begin to freeze). Overlaying supercooling points and mortality elucidates the relationship between freezing and mortality and thus an insects' cold tolerance strategy (Hanson et al. 2013).

To further examine the effects of cold temperatures on *H. halys* we compared supercooling points across season, sex, acclimation location, and geographic origin. Acclimation, on both short (Lee et al. 1987, Lee and Denlinger 2010) and long (Salt 1961) time scales, can affect the cold tolerance of an insect. Short-term acclimation, such as rapid cold hardening, can quickly confer an increased cold tolerance (Lee et al. 1987) while long-term acclimation (i.e., acclimatization), such as from changing environmental temperature and photoperiod cues, leads to seasonal differences in cold tolerance (Salt 1961). The climate to which an individual is acclimated can also alter the degree of cold tolerance an insect can achieve (Bradshaw 2010). Extreme weather events have the potential to cause high mortality when insects are inadequately acclimated. During the winter of 2013–2014 much of North America experienced what was commonly referred to as the “polar vortex,” i.e., low pressure and cold arctic air which escaped the typical circulation pattern that generally revolves around a low pressure system over the North Pole. The uncommonly low temperatures experienced during the polar vortex provided a rare opportunity to measure field mortality of *H. halys* under extreme conditions.

Field-acclimated *H. halys* from Minnesota were used to determine the species' cold tolerance strategy and field-acclimated *H. halys* from Minnesota, Virginia, and West Virginia were used to investigate

factors affecting supercooling points. As *H. halys* occurs in temperate climates we hypothesized that *H. halys* would be freeze intolerant, that the temperatures at which *H. halys* freezes would vary according to season, sex, and acclimation location, but not geographic origin. We also assessed the degree to which our laboratory measurements could deductively forecast the winter field mortality in Minnesota and Virginia in two common types of overwintering habitats.

## Materials and Methods

### Insects

#### *Virginia-sourced & Minnesota-acclimated insects*

*Halyomorpha halys* eggs were shipped overnight from Blacksburg, VA to St. Paul, MN on July 2, 2013. Eggs were maintained at 25°C 16L:8D in a growth chamber until the insects molted into second instars. Second instar nymphs were placed in mesh cages 38cm x 38cm x 61cm (BioQuip, Rancho Dominguez, CA) within a larger wire screen enclosure and provisioned with potted snap bean plants (*Phaseolus vulgaris* L. cv 'Romano Bush #14'), dried raw organic sunflower (*Helianthus annuus* L.) seeds and soybean (*Glycine max* (L.) Merr.) seeds as needed. These cages were maintained outdoors on the St. Paul campus of the University of Minnesota (44°59'20.7"N 93°11'10.6"W, elev. 300m). On October 18, 2013 insects were transferred into circular plastic dishes (18.5cm diameter x 8cm high) (Pioneer Plastics, Inc., North Dixon, KY) with a 25 x 89cm piece of cotton canvas provisioned with dry organic soybean seeds and placed into an unheated shed on the St. Paul campus of the University of Minnesota to mimic where and how the insects might otherwise aggregate.

#### *Minnesota-sourced & Minnesota-acclimated insects*

Eggs, nymphs, and adults were maintained as above; however, eggs originated from a laboratory colony at the University of Minnesota which had been maintained since 2012. Additionally, adults were transferred from outdoors to either an unheated shed or a walk-in cooler on October 30, 2014. Mean

walk-in cooler temperature  $\pm$  standard error of the mean was  $4.52^{\circ}\text{C} \pm 0.001$  with constant darkness as a constant temperature control.

#### *Virginia-sourced & Virginia-acclimated insects*

From June to October in 2012 and 2013, adult *H. halys* were collected from sweet corn and unmanaged trees located on a private farm, Garrett Farms in Glenvar, VA. In October of each year, a sample of >2000 adults was collected and placed in artificial overwintering habitats that consisted of eight plastic 18.9 liter buckets (Encore Plastics Corp., Byesville, OH) packed tightly with 12.7 mm thick foam pipe insulation (Thermwell Products, Inc. Mahwah, NJ). A 10 cm diameter ventilation hole was made into the side of each bucket, and covered with screen to prevent insect escape. Buckets were maintained outdoors in Blacksburg, VA ( $37^{\circ} 12.417' \text{ N}$ ,  $80^{\circ}35.513' \text{ W}$ , 616 m elev.) throughout the winter. This approach allowed for access to a population of insects that were exposed to ambient winter temperatures.

#### *West Virginia-sourced & Virginia-acclimated insects*

On November 1, 2012 overwintering adult *H. halys* were collected from a private facility in the panhandle of West Virginia and shipped overnight to Blacksburg, VA. They were maintained in the same artificial overwintering habitat as described above.

### **Cold tolerance strategy and supercooling point testing methods**

#### *Minnesota*

Supercooling points and lower lethal temperatures were measured by using contact thermocouple thermometry where individual adults were placed in close proximity to coiled copper-constantan thermocouples (e.g., Hanson and Venette 2013) that were attached to a multichannel data logger (USB-TC, Measurement Computing, Norton, MA). Temperatures were recorded once per second and logged by using Tracer-DAQ software (Measurement Computing, Norton, MA). We identified the lowest

temperature reached before the exotherm, or spontaneous release of heat indicative of a phase change from liquid to solid, to denote an individual's supercooling point.

In 2013 the insect and thermocouple were confined in a 20 or 35ml syringe (Monoject syringes with leur lock tip) that was placed at the center of a 20 x 20 x 20cm polystyrene cube and then into a -80°C freezer where the insects cooled at a realized rate of  $-0.82^{\circ}\text{C} (\pm 0.008^{\circ}\text{C})$  per minute according to Carrillo et al. (2004). Supercooling points from 14 males and 14 females in fall (October 9-10, 2013), and 10 males and 9 females in winter (December 7-9, 2013) were measured. Additionally on December 7-9, 2013, for the determination of cold tolerance strategy, 85 Virginia-sourced, Minnesota-acclimated adult *H. halys* were randomly assigned to one of five temperature treatments (-20, -15, -10, -5°C, or 25°C), so n=17 adults per temperature. Insects were cooled until they reached the desired temperature as tracked through the above thermocouple method, removed immediately, and allowed to warm to room temperature. After warming, insects were transferred to individual plastic cups provisioned with water and dry organic soybean seeds and monitored daily for mortality for four days. One day after treatment, all insects which were going to recover from chilling had done so and mortality measurements from one day after treatment were used for statistical analysis. Mortality of the insect was defined as a lack of any movement after being gently prodded with a small paintbrush; moribund insects, defined as having the inability to right itself after ~10 s or the inability to walk, were considered dead for the purposes of analysis.

In 2014 the insect and thermocouple were confined in an 18x150mm (ODxL) Kimax glass test tube, stabilized with one sheet (11.18 x 21.34cm) of Kimtech delicate task wipers, and a rubber test tube stopper with a 5mm hole. This apparatus was placed in a refrigerated bath of silicon 180 oil (Thermo Fischer Scientific A40, Waltham MA) at room temperature and chilled at a rate of  $-0.95^{\circ}\text{C} (\pm 0.003^{\circ}\text{C})$  per

minute. Eighty five Minnesota-sourced, Minnesota-acclimated insects were chilled according to the above methods for cold tolerance strategy on December 10, 2014.

### *Virginia*

Starting in June of 2012 samples of 10 male and 10 female *H. halys* adults were tested at approximately two month intervals throughout the year to determine supercooling point. Supercooling points were determined by placing adult insects on an apparatus where a copper-constantan thermocouple (Omega Technologies, Stamford, CT) was placed on the ventral and dorsal side of each insect. Weighted aluminum blocks were used to apply pressure and ensure contact between the insect and the thermocouple. Additionally, a small amount (<0.1 mL) of thermal grease (zinc oxide) was placed on the tip of the thermocouple to assist with any gaps in contact. DaqView (MC Measurement Computing, Norton, MA) measured temperatures generated from a 50/50 water and ethanol mixture in a refrigerated water bath (Fisher Scientific Isotemp, Waltham, MA) in circulation with a cold plate (Stir-Kool Cold Plate [SK-31], Thermoelectrics Unlimited, Wilmington, DE). This setup was able to cool the plate down to -28°C. The arena with insects was placed on the cold plate with a series of foam and aluminum blocks to reduce the temperature around 0.3°C per minute until the exotherm occurred (Bentz and Mullins 1999).

### **Field temperature observations**

In Minnesota, air temperatures were recorded at 15 minute intervals from October 18, 2013 to March 5, 2014 with an Hobo U12 4-External channel outdoor/industrial data logger (Onset Computing, Bourne, MA) and October 30, 2014 to February 26, 2015 via an U12 Temp/RH/2 External Channel Logger (Onset Computing, Bourne, MA). Temperature probes or loggers were placed next to insect cages in an unheated shed in Minnesota.

In Virginia, minimum daily air temperatures were collected from a NOAA weather station that was 3.75 km from where the insects were stored.

## **Statistics**

### *Cold tolerance strategy*

All statistics were run using R version 3.2.0 (R Core Team 2014) in RStudio version 0.98.1102 (RStudio 2014) and for all analyses an  $\alpha$  value of 0.05 was used. Modified “survival curves,” where temperature substituted for time, were created to describe the probability that *H. halys* acclimated in Minnesota and tested in winter would freeze or die at a particular temperature. Our analyses formally considered censoring (i.e., incomplete information about supercooling points or lethal temperatures) of individuals in the study. Insects that died were interval censored because death occurred between room temperature and the temperature at which the insects were removed. Insects that remained alive were considered right censored (i.e., they could survive the coldest temperature to which they were exposed but would likely die if exposed to a colder temperature). Insects which did not freeze were right censored as they would be expected to freeze at a temperature colder than when they were removed from chilling. The following R packages were used to estimate survival functions with censored data: *survival* (Therneau 2015) to create a survival object, and *interval* (Fay and Shaw 2010) to calculate the non-parametric maximum likelihood estimate for the distribution from interval censored data. Curves were fitted to the binomial data for mortality and cumulative supercooling points. A Kaplan-Meier-Turnbull non-parametric model was used for both years. Curves for freezing and mortality were compared within years via the *icfit* command from the *interval* package.

### *Supercooling point comparisons*

Some of our supercooling points seemed unusually warm, which could occur if a water-bearing substance (e.g., feces) triggered exogenous ice formation as the insect was cooled. We removed any supercooling point which was more than two standard deviations warmer than the overall mean

observed for the entire data set. Seven observations ranging from -3.27 to -2.16 °C were removed, and the remaining data (n= 188 adults) were used for subsequent analyses.

Supercooling points for *H. halys* that were collected from West Virginia and Virginia and acclimated in Virginia were compared. A Shapiro-Wilk test for normality of residuals ( $W = 0.99$ ;  $df = 11, 86$ ;  $P = 0.90$ ) and a Levene test for homogeneity of variance across groups ( $F = 0.99$ ;  $df = 11, 86$ ;  $P = 0.46$ ) confirmed no violations of ANOVA assumptions so a fully crossed three-factor analysis of variance was performed on untransformed data with location, season, and sex as main effects. This ANOVA revealed no significant main effects of location ( $F = 2.40$ ;  $df = 1, 86$ ;  $P = 0.13$ ) nor interaction effects between season and location ( $F = 2.32$ ;  $df = 2, 86$ ;  $P = 0.10$ ), sex and location ( $F = 1.61$ ;  $df = 1, 86$ ;  $P = 0.21$ ), or the three-way interaction between season, sex, and location ( $F = 0.41$ ;  $df = 2, 86$ ;  $P = 0.66$ ); therefore, data for individuals originally from West Virginia and Virginia and acclimated in Virginia were combined for analysis of season, sex, and acclimation location.

In all analyses, season was defined by the month the insect was tested and followed standard climatological definitions: Spring = April or May; summer = June, July, or August; fall = September, October, November; winter = December or February. Acclimation location was either Blacksburg, VA (37° 12.417' N, 80°35.513' W, 616 m elev.) or St. Paul MN (44° 59' 18.9672" N, -93° 10' 51.06" W, 299 m elev.). Where geographic origin was considered, location was determined by where eggs were laid in the field, either Blacksburg, VA (37° 12.417' N, 80°35.513' W, 616 m elev.) or Harper's Ferry, WV (39° 19.31' N, 77° 44.37' W, 489 m elev.). Sex was determined by visual inspection of the genitalia on the posterior ventral surface of the insect's abdomen.

*Season, sex, and acclimation location effect on supercooling points*



To test the hypotheses that season, sex, and acclimation location affect cold hardiness we ran a fully crossed three-factor ANOVA (season × sex × location) of supercooling points from Minnesota and Virginia adults from fall and winter. The data met assumptions of normality (Shapiro-Wilk  $W = 0.98$ ;  $df = 9, 111$ ;  $P = 0.12$ ) but not homoscedasticity (Levene's test:  $F = 2.03$ ;  $df = 9, 111$ ;  $P = 0.04$ ). A Box-Cox transformation ( $y_\lambda = (y^\lambda - 1)/\lambda$ ) where  $\lambda = 2.0$  was used and all analyses were done on transformed data. Sex and all interactions were not significant and were pooled for this analysis. Despite the interaction of season and acclimation location being non-significant we were still interested in comparing mean supercooling points between states and seasons to test the hypothesis that season matters to acclimation, and acclimation location matters to mean supercooling points. Tukey's HSD was used to determine the significance of each pairing of interest.

#### *Geographic origin effect on supercooling points*

A third hypothesis, that geographic origin matters to cold hardiness, was tested with a fully crossed three-factor analysis of variance (origin × season × sex) for adults that came from eggs laid either in West Virginia or Virginia but were all acclimated as adults in Virginia then tested in fall, winter, and spring. The data satisfied assumptions of homoscedasticity (Levene's test ( $F = 1.35$ ;  $df = 11, 81$ ;  $P = 0.21$ )) and normality (Shapiro-Wilk ( $W = 0.99$ ;  $df = 11, 81$ ;  $P = 0.50$ )). The interaction of season and sex was significant ( $F = 3.37$ ;  $df = 2, 81$ ;  $P = 0.04$ ) so sexes were not pooled. Tukey's HSD was used to determine the significance of season by sex by acclimation location.

#### *Predicted field mortality*

Predicted field mortality in Minnesota was determined through the survival analysis as described above. Where only supercooling points have been measured and not mortality, an estimate of mortality can still be predicted from the cumulative distribution of supercooling points. With a chill intolerant species we can be confident that individuals that had frozen would be dead, even though mortality likely occurred before freezing began. Consequently, mortality estimates that are based on the cumulative

relative frequency of supercooling points (i.e., the cumulative proportion of individuals that gave an exotherm when cooled to a specific temperature) would predict a species to be able to survive colder temperatures than in reality. This risk averse estimate is still useful however, especially in cases when only supercooling points are known, because observed mortality is unlikely to be less than predicted mortality (unless insects are in a microhabitat which is not exposed to the recorded air temperatures). Based on the cumulative relative frequency of supercooling points for adult *H. halys* acclimated as adults in Virginia and tested in winter, we fit a three-factor Weibull curve which can be used to forecast field mortality in Virginia.

## **Results and Discussion**

### **Cold tolerance strategy**

Until now, the specific effects of cold temperatures on *H. halys* were unknown. Contrary to our original hypothesis that *H. halys* would be freeze intolerant, able to survive all temperatures up until the point of freezing, we found that *H. halys* is a chill intolerant species; adults died at significantly warmer temperatures than they froze in 2013 ( $Z = 2.50$ ,  $P = 0.01$ ) and 2014 ( $Z = 3.99$ ,  $P < 0.001$ ) (Figure 2.1). In the course of our lower lethal temperature experiments, we found that no individual survived if it froze (Table 2.1), ruling out freeze tolerance as a strategy. In the groups of insects that were cooled to -5 or -10°C, no individuals froze but a portion of the insects died (Table 2.1), further supporting our assessment that *H. halys* is chill intolerant. Although the temperatures that caused *H. halys* to freeze or die in Minnesota varied between years, the cold tolerance strategy, i.e., chill intolerance, remained the same.

### **Season, sex, and acclimation location effects on supercooling points**

*Halyomorpha halys* mediates exposure to cold in a variety of ways. Previous research demonstrated that *H. halys* enters diapause, aggregates, and seeks shelter. Our work highlights an additional means by which *H. halys* reduces exposure to lethal temperatures, by acclimating seasonally and thus lowering the

temperatures which would result in mortality. Seasonal collections of adults in Minnesota and Virginia showed that supercooling points changed with season (Two-way ANOVA,  $F = 63.03$ ;  $df = 1, 111$ ;  $P < 0.001$ ). Mean supercooling points ( $\pm$  SEM) were relatively high in even in winter. Pooling Minnesota and Virginia supercooling points, the means were  $-9.43^{\circ}\text{C} \pm 0.42$  in summer,  $-15.40^{\circ}\text{C} \pm 0.43$  in fall, and  $-16.11^{\circ}\text{C} \pm 0.37$  in winter. Supercooling points of adults acclimated in Minnesota and Virginia were significantly lower in fall and winter than summer (Figure 2.2) which supports a seasonal acclimation to cold temperatures that is typical of many temperate insects (Bale and Hayward 2010).

Other effects of cold changed based on acclimation location. For example, supercooling points significantly changed according to acclimation location (Two-way ANOVA,  $F = 28.74$ ;  $df = 1, 111$ ;  $P < 0.001$ ). Mean supercooling point ( $\pm$ SEM) across seasons in Virginia was  $-10.86^{\circ}\text{C} \pm 0.40$ , and in Minnesota  $-16.93^{\circ}\text{C} \pm 0.23$ . We found that *H. halys* acclimated in Minnesota became more cold tolerant earlier in the year as compared to those acclimated in Virginia, likely due to photoperiodic and temperature differences between latitudes (Figure 2.3).

Our simultaneous comparison of the effects of season and acclimation location show that beginning in summer we saw a steady reduction in supercooling points by season in Virginia (Figure 3). In Minnesota we saw no change in supercooling points from fall to winter. In fall, individuals acclimated as nymphs and adults in Virginia ( $-13.06^{\circ}\text{C} \pm 0.14$ ) had warmer mean supercooling points than in Minnesota ( $-16.85^{\circ}\text{C} \pm 0.08$ ). No statistical difference was found in winter between Virginia ( $-13.90^{\circ}\text{C} \pm 0.09$ ) and Minnesota ( $-17.06^{\circ}\text{C} \pm 0.13$ ). Sex did not significantly impact the supercooling point (Two-way ANOVA,  $F = 0.11$ ;  $df = 1, 111$ ;  $P = 0.73$ ) and no significant two or three way interactions occurred between location, season, and sex (Figure 2.3).

#### **Geographic origin effect on supercooling points**

The supercooling points of adult *H. halys* collected in West Virginia and Virginia and held outside in Virginia were not different (Two-way ANOVA,  $F = 1.21$ ;  $df = 1, 81$ ;  $P = 0.27$ ). This supports our hypothesis that acclimation location, not geographic origin, has a stronger effect on the cold tolerance of *H. halys*. Supercooling points for individuals from both locations changed seasonally (Two-way ANOVA,  $F = 3.87$ ;  $df = 2, 81$ ;  $P = 0.02$ ), though this effect was not the same for both sexes (Two-way ANOVA, interaction of sex and season:  $F = 3.37$ ;  $df = 2, 81$ ;  $P = 0.04$ ). The sex of the insect did not have a consistent effect on supercooling point (Two-way ANOVA,  $F = 3.83$ ;  $df = 1, 81$ ;  $P = 0.05$ ) (Figure 2.4).

Females from the eastern United States exhibited less seasonal change than males, which suggests that once acclimated in fall, females do not continue to acclimate, but males do. West Virginia males in the fall had the warmest mean supercooling points ( $-7.93^{\circ}\text{C} \pm 1.93$ ) and Virginia males in winter had the coldest ( $-15.72^{\circ}\text{C} \pm 0.56$ ). Mean supercooling points for all other season, sex, and geographic location combinations were not significantly different from each other (Figure 2.4).

No interactions occurred between geographic origin and season ( $F = 1.12$ ;  $df = 2, 81$ ;  $P = 0.33$ ), origin and sex ( $F = 1.84$ ;  $df = 1, 81$ ;  $P = 0.16$ ), or between origin, season, and sex ( $F = 0.37$ ;  $df = 2, 81$ ;  $P = 0.69$ ). In each season, the mean SCPs between West Virginia and Virginia were statistically equivalent (Figure 2.4).

#### **Predicted and observed field mortality of *H. halys***

Supercooling points are relatively easy to measure and give clear results, so they are often a starting point for cold hardiness experiments when specimens are limited (Morey et al. 2012). The cumulative frequency distribution of supercooling points can also provide estimates of mortality for a chill-intolerant or freeze-intolerant species after acute exposure to a specified temperature. It should be noted, however, that these forecasts do not apply to chronic exposures to cold nor do they account for

other causes of winter mortality such as starvation or desiccation. For chill-intolerant species, we know that the estimate will be biased, consistently forecasting less mortality (i.e., greater survivorship) than will be observed (Figure 2.2). Despite this, forecasts from a cumulative supercooling point curve can still prove to be useful when making conservative estimates of winter mortality (Table 2.2).

Using field temperature data, laboratory determined cumulative frequency distribution of supercooling points from *H. halys* in Virginia (Figure 2.1), and laboratory mortality measurements from *H. halys* in Minnesota (Figure 2.2), we accurately predicted field mortality in Minnesota and Virginia before and after low temperature events (Table 2.2). In December 2014, the expected mortality for overwintering *H. halys* in an unheated shed in Minnesota was greater numerically than our observed mortality; however, observed mortality at this time falls within the 95% confidence interval for expected mortality (0 to 44%). Our laboratory measurements provided an accurate way to forecast winter field mortality of unheated *H. halys* aggregations in Minnesota and Virginia; however, *H. halys* utilizes an array of overwintering habitats. The tendency of *H. halys* to aggregate in thermally-protected areas, such as human-made structures, means the unheated microclimates into which our experimental insects were placed are not representative of the whole population. Following the extreme low temperatures of the polar vortex of 2014, *H. halys* were still present in the summer of 2014 (Herbert 2014). This suggests that while lethal temperatures were reached across much of the United States, exposure to those temperatures did not occur across the population. Refugia from cold temperatures for *H. halys* are likely contributing to the northern geographic range expansion and ability to survive winter temperatures in North America. Further data should be gathered on a range of overwintering microclimate environments to determine the exposure of *H. halys* to cold temperatures and to better forecast overall overwintering mortality.

In 2014 a subset of insects were maintained in a walk-in cooler as a control group. The temperature in the walk-in cooler was below *H. halys* lower developmental threshold (14.17°C) (Nielsen et al. 2008) and never reached a point at which we expected mortality; however, in December and January we observed 5% and 15% mortality, which was greater than the 0% (0 – 4%; 95% CI) that was expected when the coldest temperatures these insects experienced was 4.3°C (Table 2.2). This indicates that other factors, possibly desiccation or starvation, are contributing to overwintering mortality in addition to cold temperatures. Future directions could include questions about multiple stressors and their effects on overwintering mortality. Additional factors such as nutrition (Gash and Bale 1985), rate of cooling (Baust and Rojas 1985), life stage (Lee 1991), and temperature fluctuations (Salt 1961) can also contribute to cold tolerance and could be investigated in future studies.

Diapause can enhance insect cold tolerance but does not always necessarily do so (Denlinger 1991). *Halyomorpha halys* is known to go into a reproductive diapause before overwintering (Niva and Takeda 2003, Nielsen and Hamilton 2009). However, nothing is known about the relationship between diapause and cold hardiness in *H. halys*. A limitation of our studies was that no dissections were done to positively determine if individuals were in diapause when being tested. Nevertheless, because *H. halys* is predicted to be univoltine in Mid-Atlantic states we believe that, by the time winter testing occurred, insects would have received the cues needed to enter diapause (Nielsen and Hamilton 2009). More work is needed to understand how and when *H. halys* enters diapause and what effects, if any, diapause has on *H. halys* overwintering capabilities.

Our work provides new insight into the exposure and effects of cold temperatures on *H. halys*. Studying cold stress on this species has the potential to illuminate new modes of management. *Halyomorpha halys* has become a severe pest in parts of North America. This exotic invasive species causes severe

economic damage to many crops and seeks shelter, sometimes in homes, to overwinter, making it an agricultural pest as well as a structural and nuisance pest. A lack of exposure to lethal temperatures when *H. halys* overwinters in thermally buffered areas not only affects the potential geographic range of *H. halys*, it enlarges management problems and solutions beyond typical agricultural settings. More studies are needed to: Quantify the proportion of *H. halys* in specific types of overwintering sites, model the relationship between those microhabitats and the reported air temperature, and investigate other stressors leading up to and through winter that may contribute to mortality.

### Acknowledgements

This project would not have been possible without the dedication of several people, especially Eric Burkness, Lindsey Christianson, Amy Morey, Robert Koch, Katherine Kamminga, Tracy Leskey and Doo-Hyung Lee, as well as, Jaana Iverson, Benjamin Aigner, and Ashley Lohr. We thank Nathaniel Cira for reviewing this paper prior to submission. We also appreciate the use of the laboratory facilities of the USDA Forest Service Northern Research Station. This project was funded in part by a MnDRIVE Global Food Ventures graduate fellowship at the University of Minnesota, USDA-NIFA-SCRI Grant # 2011-51181-30937, and the Virginia Department of Agriculture and Consumer Services USDA Specialty Crop Block Grant for the 2011 fiscal year.

### References

- Bale, J. S. 1991.** Insects at low temperature: A predictable relationship? *Funct. Ecol.* 5: 291–298.
- Bale, J. S., and S. A. L. Hayward. 2010.** Insect overwintering in a changing climate. *J. Exp. Biol.* 213: 980–94.
- Baust, J. G., and R. R. Rojas. 1985.** Review—Insect cold hardiness: Facts and fancy. *J. Insect Physiol.* 31: 755–759.

- Bentz, B. J., and D. E. Mullins. 1999.** Ecology of mountain pine beetle (Coleoptera: Scolytidae) cold hardening in the Intermountain West. *Environ. Entomol.* 28: 577–587.
- Bergmann, E., K. M. Bernhard, G. Bernon, M. Bickerton, S. Gill, C. Gonzales, G. C. Hamilton, C. Hedstrom, K. Kamminga, C. Koplinka-Loehr, G. Krawczyk, T. P. Kuhar, B. Kunkel, J. Lee, T. C. Leskey, H. Martinson, A. L. Nielsen, M. Raupp, P. Shearer, P. Shrewsbury, J. Walgenbach, Jim Whalen, and N. Wiman. 2015.** Host plants of the brown marmorated stink bug. (<http://www.stopbmsb.org/where-is-bmsb/host-plants>).
- Bradshaw, W. E. 2010.** Insects at not so low temperatures: Climate change in the temperate zone and its biotic consequences, pp. 242–275. *In* Denlinger, D.L., Lee, R.E. (eds.), *Low temperature biology of insects*. Cambridge University Press, New York.
- Carrillo, M. A., N. Kaliyan, C. A. Cannon, R. V. Morey, and W. F. Wilcke. 2004.** A simple method to adjust cooling rates for supercooling point determination. *CryoLetters*. 25: 155–160.
- Denlinger, D. L. 1991.** Relationship between cold hardiness and diapause, pp. 174–198. *In* Lee, R.E., Denlinger, D.L. (eds.), *Insects at low temperature*. New York.
- Fay, M. P., and P. A. Shaw. 2010.** Exact and asymptotic weighted logrank tests for interval censored data: the interval R package. *J. Stat. Softw.* 36: 1–34.
- Gash, A. F., and J. S. Bale. 1985.** Host plant influences on supercooling ability of the black-bean aphid. *CryoLetters*. 6: 297–304.
- Hanson, A. A., and R. C. Venette. 2013.** Thermocouple design for measuring temperatures of small insects. *CryoLetters*. 34: 261–266.
- Hanson, A. A., R. C. Venette, and J. P. Lelito. 2013.** Cold tolerance of Chinese emerald ash borer parasitoids: *Spathius agrili* Yang (Hymenoptera: Braconidae), *Tetrastichus planipennis* Yang



(Hymenoptera: Eulophidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae).  
Biol. Control. 67: 516–529.

**Herbert, D. A. 2014.** Virginia Ag Pest and Crop Advisory. (<https://blogs.ext.vt.edu/ag-pest-advisory/bmsb-and-kudzu-bug-update-sept-4-2014/>).

**Holtz, T., and K. Kamminga. 2010.** Qualitative analysis of the pest risk potential of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), in the United States. Plant Epidemiology and Risk Analysis Laboratory of the Center for Plant Health Science and Technology (USDA-APHIS).  
([https://www.michigan.gov/documents/mda/BMSB\\_Pest\\_Risk\\_Potential\\_-\\_USDA\\_APHIS\\_Nov\\_2011\\_344862\\_7.pdf](https://www.michigan.gov/documents/mda/BMSB_Pest_Risk_Potential_-_USDA_APHIS_Nov_2011_344862_7.pdf)).

**Kiritani, K. 2007.** The impact of global warming and land-use change on the pest status of rice and fruit bugs (Heteroptera) in Japan. Glob. Chang. Biol. 13: 1586–1595.

**Lee, D.-H., J. P. Cullum, J. L. Anderson, J. L. Daugherty, L. M. Beckett, and T. C. Leskey. 2014.** Characterization of overwintering sites of the invasive brown marmorated stink bug in natural landscapes using human surveyors and detector canines. PLoS One. 9: e91575.

**Lee, R. E. 1991.** Principles of insect low temperature tolerance, pp. 17–46. *In* Lee, R.E., Denlinger, D.L. (eds.), Insects at low temperature. Chapman and Hall, New York.

**Lee, R. E. 2010.** A primer on insect cold-tolerance, pp. 3–34. *In* Denlinger, D.L., Lee, R.E. (eds.), Low temperature biology of insects. Cambridge University Press, New York.

**Lee, R. E., C.-P. Chen, and D. L. Denlinger. 1987.** A rapid cold-hardening process in insects. Science. 238: 1415–1417.

**Lee, R. E., and D. L. Denlinger. 2010.** Rapid cold-hardening: Ecological significance and underpinning

mechanisms, pp. 35–58. *In* Denlinger, D.L., Lee, R.E. (eds.), *Low temperature biology of insects*. Cambridge University Press, New York.

**Morey, A. C., W. D. Hutchison, R. C. Venette, and E. C. Burkness. 2012.** Cold hardiness of *Helicoverpa zea* (Lepidoptera: Noctuidae) pupae. *Environ. Entomol.* 41: 172–179.

**Nielsen, A. L., and G. C. Hamilton. 2009.** Life history of the invasive species *Halymorpha halys* (Hemiptera: Pentatomidae) in northeastern United States. *Ann. Entomol. Soc. Am.* 102: 608–616.

**Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008.** Developmental rate estimation and life table analysis for *Halymorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* 37: 348–355.

**Niva, C. C., and M. Takeda. 2003.** Effects of photoperiod, temperature and melatonin on nymphal development, polyphenism and reproduction in *Halymorpha halys* (Heteroptera: Pentatomidae). *Zoolog. Sci.* 20: 963–970.

**Northeastern IPM Center. 2015.** State-by-State. (<http://www.stopbmsb.org/where-is-bmsb/state-by-state>).

**R Core Team. 2014.** R: A language and environment for statistical computing. Vienna, Austria. (<http://www.r-project.org/>).

**RStudio. 2014.** Integrated development environment for R, Version 0.98.1102. (<http://www.rstudio.org/>).

**Salt, R. W. 1961.** Principles of insect cold-hardiness. *Annu. Rev. Entomol.* 6: 55–74.

**Therneau, T. M. 2015.** A package for survival analysis in S, Version 2.38. (<http://cran.r-project.org/package=survival>).

**U.S. Apple Association News Release. 2011.** Losses to mid-Atlantic apple growers at \$37 million from

brown marmorated stink bug.

([http://www.usapple.org/index.php?option=com\\_content&view=article&id=160:bmsb-loss-midatlantic&catid=8:media-category](http://www.usapple.org/index.php?option=com_content&view=article&id=160:bmsb-loss-midatlantic&catid=8:media-category)).

**Venette, R. C., D. J. Kriticos, R. D. Magarey, F. H. Koch, R. H. A. Baker, S. P. Worner, N. N. Gómez**

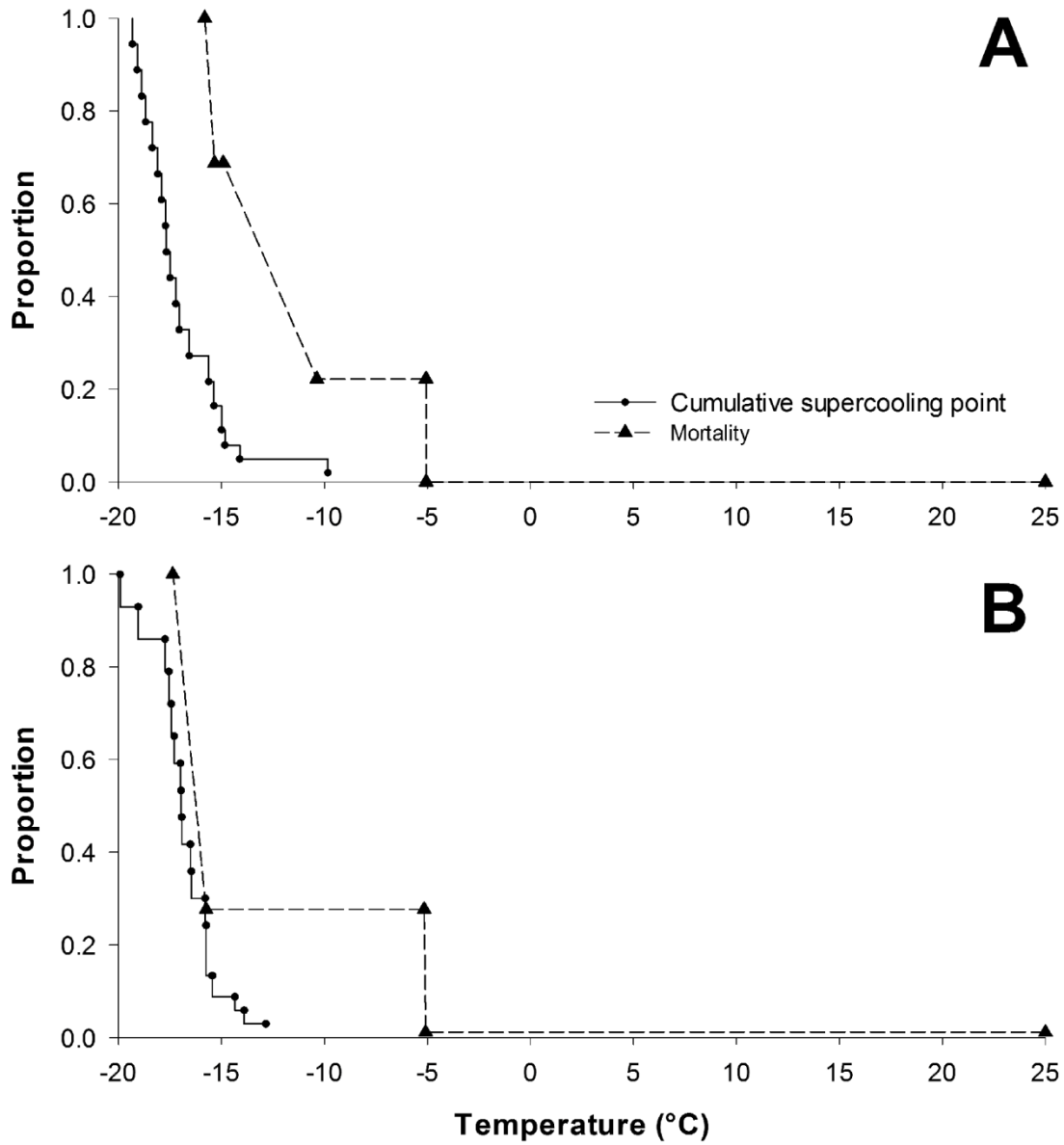
**Raboteaux, D. W. McKenney, E. J. Dobesberger, D. Yemshanov, P. J. De Barro, W. D.**

**Hutchison, G. Fowler, T. M. Kalaris, and J. Pedlar. 2010.** Pest risk maps for invasive alien species: A roadmap for improvement. *Bioscience*. 60: 349–362.

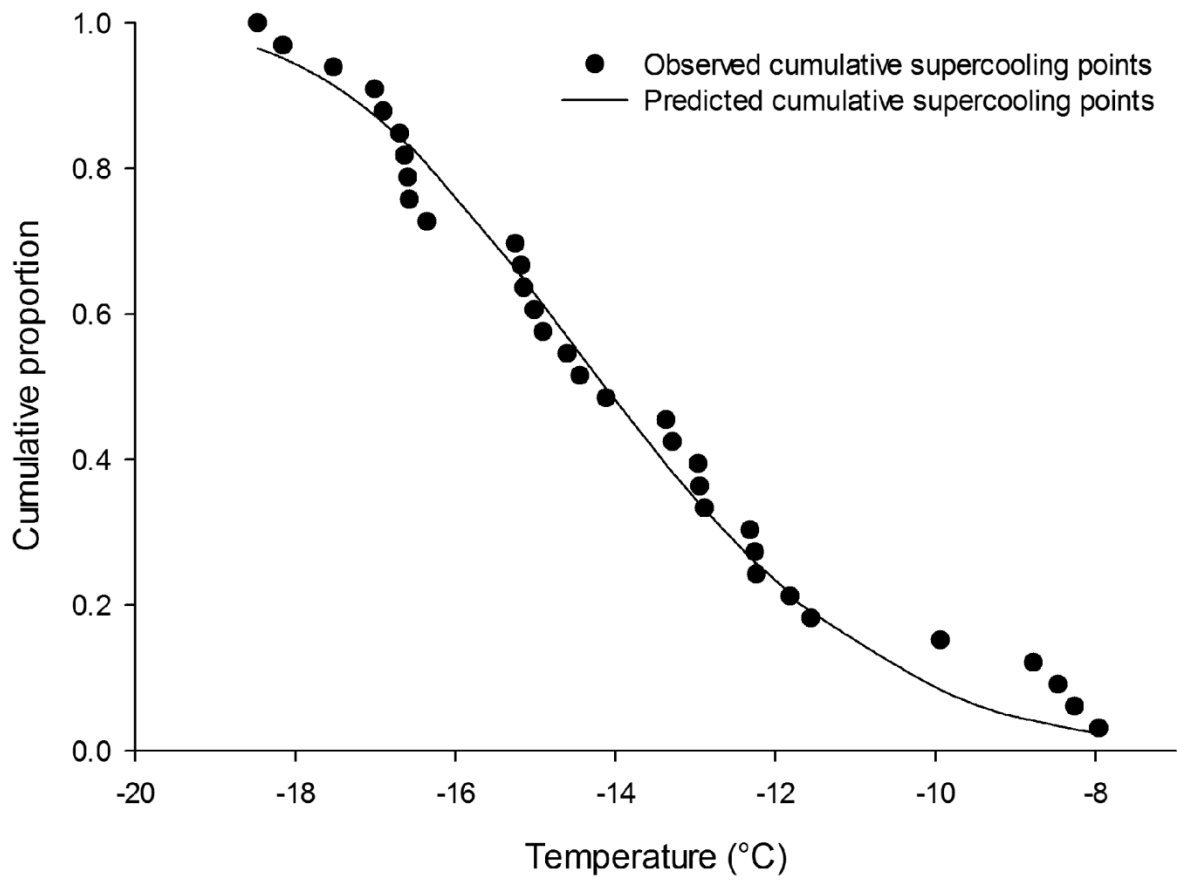
**Watanabe, M., R. Arakawa, Y. Shinagawa, and T. Okazawa. 1994.** Anti-invading methods against brown marmorated stink bug, *Halyomorpha mista*, in houses. *Anim. Heal.* 45: 25–31.

**Watanabe, M., K. Kamimura, and Y. Koizumi. 1978.** The annual life cycle of *Halyomorpha mista* and ovarian development process. *Toyama J. Rur. Med.* 9: 95–99.

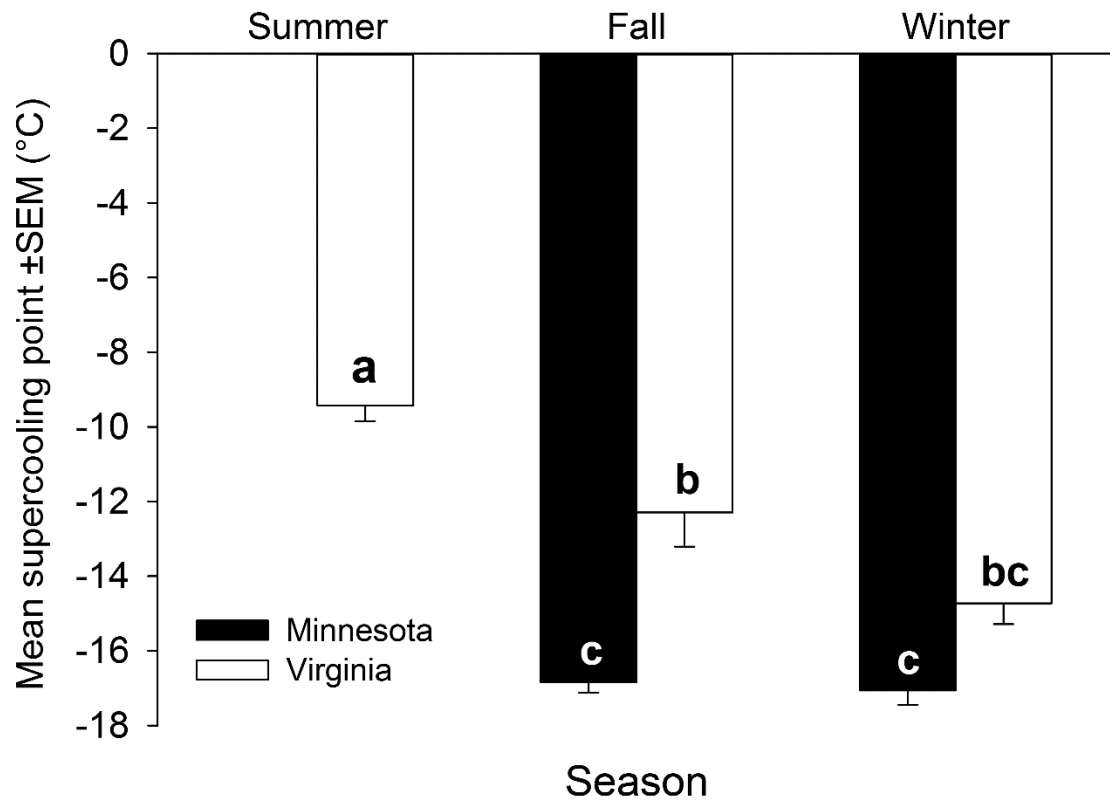
**Zhu, G., W. Bu, Y. Gao, and G. Liu. 2012.** Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). *PLoS One*. 7: e31246.



**Figure 2.1.** Observed cumulative supercooling point distribution and mortality distribution for field-acclimated adult *Halyomorpha halys* in Minnesota in (A) December 2013 (B) December 2014. Extrapolation between observed data calculated with right and interval censored survival analysis.

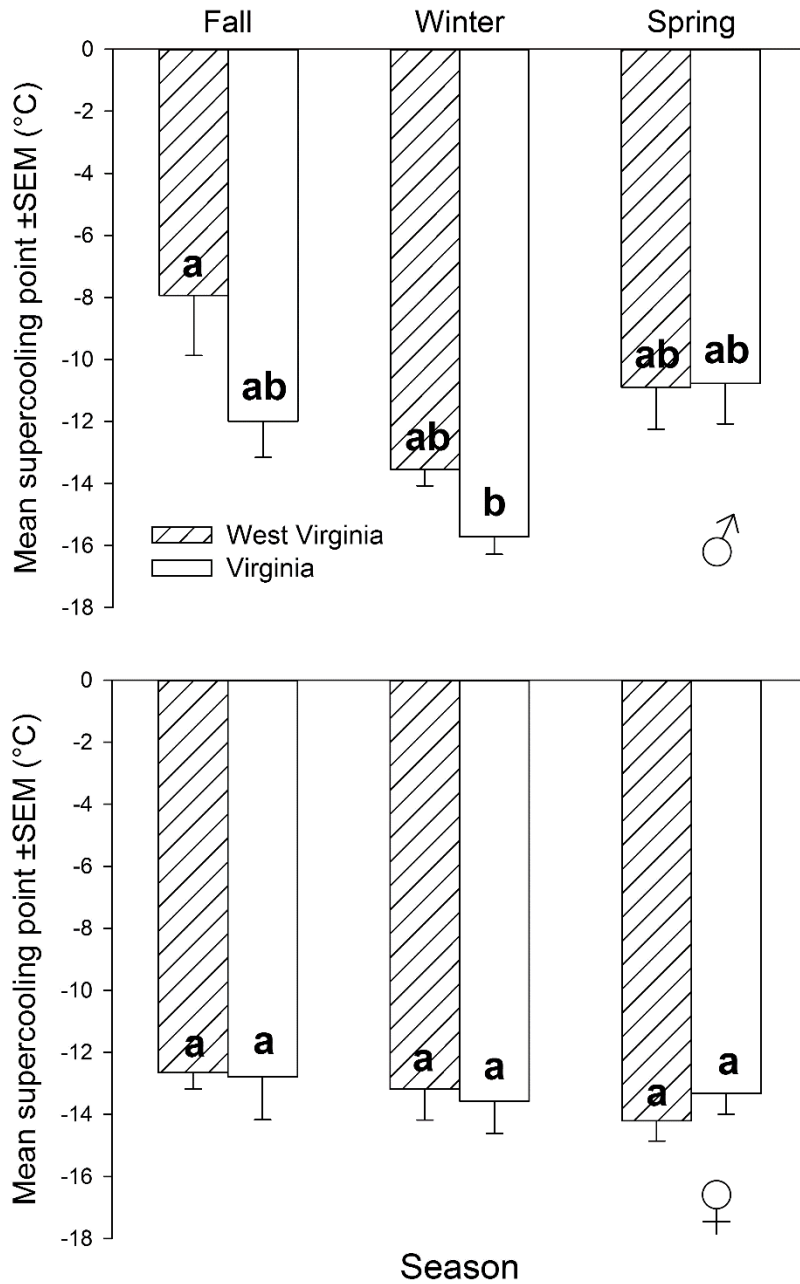


**Figure 2.2.** Predicted and observed cumulative supercooling point distribution for field-acclimated adult *H. halys* in Virginia in winter. Predicted curve calculated with a Weibull distribution.



**Figure 2.3.** Mean supercooling points of adult *H. halys* field-acclimated either in Virginia or Minnesota.

Individuals remained outdoors, experiencing temperature and photoperiodic cues, until the time of testing. Error bars indicate standard error of the mean. Statistics were run on transformed data and bars with the same letter are not statistically different ( $P > 0.05$ ; Tukey's HSD).



**Figure 2.4.** Mean supercooling points of *H. halys* adults originating from either West Virginia or Virginia and field-acclimated as adults in Virginia (Top panel) Male, (Bottom panel) Female. Individuals remained outdoors, experiencing temperature and photoperiodic cues, until the time of testing. Error bars indicate standard error of the mean. Across both panels bars with the same letter are not statistically different ( $P > 0.05$ ; Tukey's HSD).

**Table 2.1.** Proportion mortality  $\pm$  SEM of adult *H. halys* acclimated outdoors in Minnesota in 2013 and 2014 and exposed to one of five temperatures. Numbers in parentheses indicate the total number of adults that were either chilled (unfrozen) or frozen upon reaching the target temperature.

Temperature (°C)	2013		2014	
	Chilled	Frozen	Chilled	Frozen
-20	-- (0)	1.00 $\pm$ 0.00 (17)	1.00 $\pm$ 0.00 (1)	1.00 $\pm$ 0.00 (16)
-15	0.64 $\pm$ 0.12 (14)	1.00 $\pm$ 0.00 (3)	0.13 $\pm$ 0.08 (16)	1.00 $\pm$ 0.00 (1)
-10	0.18 $\pm$ 0.09 (17)	-- (0)	0.29 $\pm$ 0.11 (17)	-- (0)
-5	0.18 $\pm$ 0.09 (17)	-- (0)	0.24 $\pm$ 0.10 (17)	-- (0)
25 (control)	0.00 $\pm$ 0.00 (17)	-- (0)	0.00 $\pm$ 0.00 (17)	-- (0)



**Table 2.2.** Observed and expected *H. halys* winter mortality. Minimum temperatures reached in Minnesota and Virginia in the winter of 2013-2014 and 2014-2015, expected mortality of *H. halys* adults for recorded temperatures, observed mortality.

		2013		2014	
		MN unheated	VA unheated	MN unheated	MN walk-in
		shed	19 liter bucket	shed	cooler
Dec.	Min. temp. reached (°C)	5.5	15.0	-12.09	4.30
	Expected mort. (95% CI) <sup>a</sup>	0.0% (0-4%)	0.0%	27.7% (0-44%)	0.0% (0-4%)
	Observed mort. <sup>b</sup>	0.01 %	N/A	0.0%	5.0%
	Sample size	86	N/A	85	20
Jan.	Min. temp. reached (°C)	-22.6	-20.7	-17.86	4.35
	Expected mort. (95% CI) <sup>a</sup>	99% (53-100%)	99%	99% (17-100%)	0.0% (0-4%)
	Observed mort. <sup>c</sup>	100%	97.6%	100%	15.0%
	Sample size	92	2654 <sup>d</sup>	20	20

<sup>a</sup> Based on the minimum temperature reached before testing dates from each location and the mortality curves from Figure 1 for Minnesota (MN) and Figure 2 for Virginia (VA).

<sup>b</sup> Mortality determined on December 6, 2013 and December 10, 2014 in Minnesota.

<sup>c</sup> Mortality determined on January 6, 2014 and January 10, 2015 in Minnesota and February 12, 2014 in Virginia.

<sup>d</sup> This large sample size was possible in Virginia where *H. halys* is abundant.

## Chapter Three

### **Lethal High Temperature Extremes of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) and Efficacy of Commercial Heat Treatments for Control in Export Shipping Cargo**

(As Published: Aigner, J.D. and T.P. Kuhar. 2016. Journal of Agricultural and Urban Entomology January 2016 : Vol. 32, Issue 1 (Jan 2016), pg(s) 1-6)

Since its accidental introduction into the U.S. in the mid-1990s, the brown marmorated stink bug, *Halyomorpha halys* (Stål), has spread rapidly across North America and become an economically significant pest of tree fruits, vegetables, tree nuts, and field crops (Leskey et al. 2012, Rice et al. 2014). Consequently, there has been tremendous interest in better understanding aspects of its biology. Knowledge on the effects of temperature on the bug's biology is critical to developing ecological models (Nielsen et al. 2008), predicting range expansion (Zhu et al. 2012), and for potentially developing pest control tactics using controlled temperature (Hammond 2015). In two laboratory experiments, we determine the lethal high temperature extremes of *H. halys* and efficacy of commercial heat treatments for control of the bug in export shipping cargo.

Experiments were conducted in 2014 and 2015 in a laboratory at Virginia Tech in Blacksburg, VA, USA to determine the lethal high temperature of the brown marmorated stink bug. All insects used in experiments were obtained from a laboratory colony maintained in mesh cages (30.48 cm x 30.48 cm x 30.48 cm) (BioQuip, Rancho Dominguez, CA, USA) at 23°C, 12-h photoperiod, and 50% relative humidity. Bugs were fed fresh green beans (*Phaseolus vulgaris* L.) and sunflower (*Helianthus annuus* L.) seeds, and supplied with water through a wick. The colony was continuously supplemented with field-collected *H. halys* from trees and crops in Virginia during the summer months or from manmade structures in the fall and winter of each year. Adults of both sexes were haphazardly selected from the colony and placed in groups of ten in 9-cm glass Petri dishes (Corning, Inc., Corning, NY). These groups were placed in an incubator (Fisher Scientific Thermo Incubator 537D, Fisher Scientific, Waltham, MA) at different temperature-time intervals. Bugs were exposed for 4 h at the following temperatures: 35, 38, 40, 42,

and 45°C. Additionally, bugs were exposed for 15-min and 1-h intervals at 40, 45, and 50°C. Mortality was assessed at 1 h and 24 h after exposure. Bugs unable to walk properly and right themselves after being turned over on their backs were considered lethally injured from the heat exposure and were recorded as dead. Each time and temperature combination was replicated at least four times.

Percentage mortality at 24 h after exposure were arcsine-square-root transformed to normalize the variances (Sokal & Rohlf 1995) then analyzed using analysis of variance (ANOVA) (JMP version 10.0; SAS Institute 2012). Means were separated using Fisher's Protected Least Significant Difference (LSD) at  $\alpha = 0.05$ .

Temperature had a significant effect on bug mortality for the 15-min ( $F = 10.88$ ; d.f. = 2, 9;  $P < 0.004$ ), 1-h ( $F = 80,000$ ; d.f. = 2, 9;  $P < 0.0001$ ), and 4-h exposure times ( $F = 50.57$ ; d.f. = 4, 25;  $P < 0.0001$ ). All *H. halys* adults that were exposed to 50°C for  $\geq 15$  min or to 45°C for  $\geq 1$  h were killed (Table 1). The *H. halys* adults exposed to 35°, 38°, 40°, 42°, and 45°C for 4 h had an observed mean mortality of 5, 12, 38, 91, and 100%, respectively. Mortality at 42 and 45°C was significantly higher than the other temperatures, and 40°C was higher than either 38°C or 35°C.

A second study was conducted to evaluate the potential of heat treatments for control of live *H. halys* in export cargo. In 2015, a heat treatment facility at the Port of Savannah, GA, USA was constructed by Willenius Wilhelmsen Logistics, Lysaker, Norway (WWL) to create a usable heated space approximately 6.1 m  $\times$  12.2 m  $\times$  3 m (Figure 1a). The interior of the facility was coated with a series of spray foam insulation and 5 cm wide foam board insulation to reduce heat loss. One end of the facility allowed for vehicles to enter through a standard exterior grade metal garage door, while the opposite end housed three 500,000 BTU propane heaters (PEST-HEAT, Aston, PA; Figure 1b) that allowed the heat to move throughout the space. These heaters were supplied by a 3785-liter propane tank located in the rear of

the facility. Current capabilities of this space allow for treatment of up to four standard-sized passenger vehicles at one time.

For our studies at the Port of Savannah, *H. halys* field mortality was assessed using a 7-passenger 2003 Chevrolet Astro Extended Wagon. This vehicle had a 4.6-liter V6 CPI engine. The same vehicle was used for all experiments. Previous testing by WWL identified the engine compartment, under the driver or passenger seats and interior areas that house the spare tire to be the coldest spots during the heat treatment. Our test vehicle does not have an interior spare tire compartment; therefore, we were only able to test for bug survival in the engine compartment and under the seat.

The vehicle was parked in the heat treatment facility and subjected to targeted heat treatments of 40°, 50°, and 60°C. Ten adults (collected near Sharpsburg, MD the week prior to testing) were placed in 1-liter mesh bags (Figure 2) and then placed in the engine compartment and under the driver's seat for 15 min once the minimum temperatures in these areas reached the target temperatures. Temperatures were monitored at each location every 60 sec for 15 min once reaching the minimum targeted temperature every 60 seconds using a hardwired thermocouple integrated to a BlueTherm Duo system (Thermoworks, Lindon, UT) integrated with the BlueTherm Pro software (Thermoworks, Lindon, UT) for tablets for ease of monitoring. Stink bugs were removed and assessed for mortality at 0, 1, and 24 h after the heat treatment. Each temperature was replicated eight times and each replication had a control group of ten *H. halys* that were held at ambient temperature with a mean of 16°C. As with the previous experiment, percentage mortality at 24 h after exposure were arcsine-square-root transformed and analyzed using ANOVA (JMP version 10.0; SAS Institute 2012). Means were separated using Fisher's Protected LSD.

In the second study, temperatures recorded from under the driver's seat were about 2 to 3°C cooler than those recorded in the engine compartment, but both were at least at the minimal targeted

temperature generated from the heat treatment (Table 2). Temperature had a significant effect on bug mortality ( $F = 21,600$ ; d.f. = 2, 21;  $P < 0.0001$ ). Targeted heat treatment exposures for 15 min at either 50 or 60°C resulted in 100% mortality of the adults tested, regardless of location in the vehicle. Heat treatments at 40°C resulted in very low mortality of adults. All *H. halys* held under ambient conditions during this study resulted in 0% mortality.

Most insects cannot survive exposure to extreme heat (>50°C) for even brief durations (Hammond 2015). High temperatures disrupt the function of proteins, metabolic enzymes and the respiratory and endocrine systems, all of which can lead to insect mortality (Neven 2000). Our results showed that *H. halys* adults are killed after exposure for 15 min to 50°C or 1 h or more exposure to 45°C. These lethal high temperature levels are consistent with many other insects (Hammond 2015). According to Burkes et al. (2000) and Fields & White (2002), most stored-product insect pests (coleopterans and lepidopterans) are effectively controlled under the following time-temperature combinations: 24 h at 40°C, 12 h at 45°C, 5 min at 50°C, 1 min at 55°C, and 30 sec at 60°C. Thus, *H. halys* appear to follow a similar pattern of mortality.

These results may have implications on heat treatment control guidelines (restrictions) for shipping cargo. For instance, recent restrictions were placed on the shipping of potential *H. halys*-infested cargo from the U.S. to other countries. During periods of restriction, if vehicles cannot be fumigated prior to transport, they are currently required to be heat-treated at 60°C for 20 min (restrictions set by New Zealand) (Thompson 2014) or 30 min (restrictions set by Australia) (Australian Government 2015) prior to boarding cargo ships. This heat treatment regime has been found to be quite difficult to achieve in an efficient manner for treating large cargo such as multiple vehicles because of the difficulty in constantly maintaining an enclosure at 60°C. Consequently, fumigants have been the primary choice for treatment of stink bugs in export goods mostly because of efficiency (Thompson 2014). However, heat treatments

are often less expensive, and do not involve the use of toxic gases, such as phosphine (PH<sub>3</sub>) or methyl bromide, which have numerous environmental and human safety issues (Hammond 2015). Based on our research, heat treating export cargo at 50°C rather than 60°C would dramatically improve the efficiency of the process without losing insect control efficacy. Based on our estimates and field testing with WWL at the shipping port in Savannah, GA, such a switch would basically cut the time of heat treatment in half, and thus double the amount of vehicles that could be treated in a day. More efficient heat treatment controls could help to reduce our reliance on fumigation as a means of insect control in exports.

### **Acknowledgements**

The authors would like to thank Ashley Lohr and Jamie Hogue for their invaluable assistance with the implementation of this study. We would like to extend our thanks to Craig Kessler of the Georgia Port Authority and Phil Hansen and Sean Lilly of Wallenius Wilhelmsen Logistics for allowing the use of their facility and their hospitality. This project was funded in part by the USDA-NIFA-SCRI Grant # 2011-51181-30937.

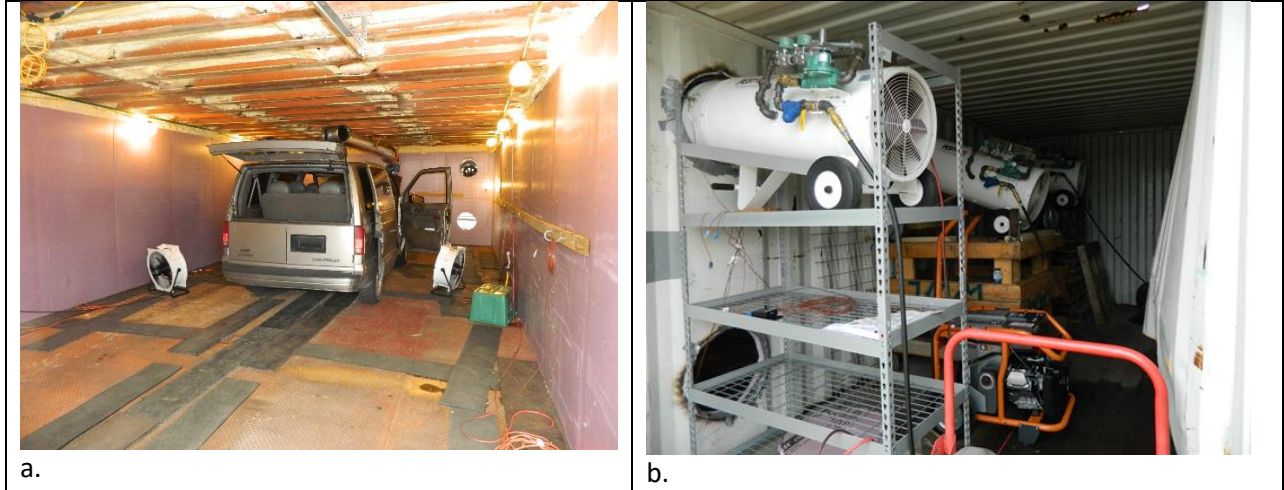
### **References**

- Australian Government. Department of Agriculture. 2015.** Brown Marmorated Stink Bug: emergency measures for break bulk and containerised vehicles, machinery, automotive parts and tyres. Canberra City, Australia.
- Burkes, C., J. Johnson, D. Maier and J. Heaps. 2000.** Temperature, 437 pp. In Subramanyam, B. H. and D. W. Hagstrum (Eds.), Alternatives to pesticides in stored-product IPM. Boston: Kluwer Academic, Boston, MA.

- Fields, P. G. and N. D. G. White. 2002.** Alternatives to methyl bromide treatments for stored-product and quarantine insects. *Annu. Rev. Entomol.* 47: 331-59.
- Hammond, D. 2015.** Heat treatment for insect control: developments and applications. Woodhead Publishing, Amsterdam, Netherlands.
- Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. A. Hoelmer, D.-H. Lee and S. E. Wright. 2012.** Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. *Outlooks Pest Manage.* 23: 218-226.
- Neven, L. G. 2000.** Physiological responses of insects to heat. *Postharvest Biol. Tec.* 21: 103 – 111.
- Nielsen, A. L., G. C. Hamilton and D. Matadha. 2008.** Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* 27: 348–355.
- Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Garipey, G. Hamilton, T. Haye, A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G. Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey and J. F. Tooker. 2014.** Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *J. Integr. Pest Manag.* 5: 1-13.
- SAS Institute. 2012.** JMP version 10.0. SAS Institute, Cary, NC.
- Sokal, R. R. and F. J. Rohlf. 1995.** *Biometry* (3rd Ed.). W. H. Freeman and Company, New York, NY. 419 pp.
- Thompson, P. 2014.** Import health standard for vehicles, machinery and tyres. Ministry for Primary Industries. Issued 23 Dec 2014. Wellington, NZ.

**Zhu, G., W. Bu, Y. Gao and G. Liu. 2012.** Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). PLoS ONE 7: e31246.





**Figure 3.1.** Vehicle used for experiments parked inside a container heat treatment facility constructed by Willenius Wilhelmsen Logistics (WWL) a) and the 500,000 BTU propane heaters b) used to generate lethal temperature extremes  $>60^{\circ}\text{C}$  for heat treatment of vehicles.



**Figure 3.2.** Dead brown marmorated stink bugs in a mesh bag after removal from under the seat of a vehicle exposed to heat treatments at the Port of Savannah, GA.

**Table 3.1.** Average mortality of *H. halys* adults\* following exposure for different times and temperatures in an incubator located at Virginia Tech, Blacksburg, VA (USA).

% mortality (Mean ± SE)				
Temp. °C	Replications	15-min exposure	1-h exposure	4-h exposure
35	4	-	-	5.0 ± 1.4 c
38	4	-	-	11.7 ± 1.9 c
40	4	0.0 ± 0.0 b	0.0 ± 0.0 b	38.3 ± 3.7 b
42	4	-	-	91.3 ± 2.2 a
45	4	22.5 ± 19.3 b	100.0 ± 0.0 a	100.0 ± 0.0 a
50	4	100.0 ± 0.0 a	100.0 ± 0.0 a	-

\* >50 bugs per temperature-time treatment.

Means within columns followed by the same letter are not significantly different (ANOVA, LSD test,  $\alpha = 0.05$ ).

**Table 3.2.** Targeted temperatures, actual measured temperatures, and observed mortality of *H. halys* adults after 15 min exposures to heat treatments applied to a vehicles at the Port of Georgia, Savannah, GA, USA in

Target Temp. °C	Actual Measured Temp. (Mean °C ± SE)		% Mortality (Mean ± SE)*	
	Under Driver's Seat	Engine Compartment	Under Driver's Seat	Engine Compartment
40	39.1 ± 0.2	42.2 ± 0.3	2.5 ± 2.5 b	17.5 ± 17.5 b
50	50.9 ± 0.1	54.2 ± 0.1	100.0 ± 0.0 a	100.0 ± 0.0 a
60	65.0 ± 0.7	67.1 ± 0.8	100.0 ± 0.0 a	100.0 ± 0.0 a

\*Observed for 24 h after 15 min of exposure to temperatures.

Means within columns followed by the same letter are not significantly different (ANOVA, LSD test,  $\alpha = 0.05$ ).

2015.

## Chapter Four

### Using Citizen Scientists to Evaluate Light Traps for Catching Brown Marmorated Stink Bugs in Homes in Virginia

(As Published: Aigner, J.D. and T.P. Kuhar. 2014. Journal of Extension. Vol. 52, No. 4, Art. # 4RIB5)

#### Abstract

More and more, citizen scientists are playing an integral role in research studies. This has been particularly evident as entomologists unravel the biology, spread, and management of the brown marmorated stink bug, which has plagued many homeowners in the mid-Atlantic U.S. in recent years. We used citizen scientists to evaluate different indoor light traps for catching the bugs in houses. Throughout the late winter and early spring months, these traps were tested inside homes and enabled us to determine that the most efficacious trap was an aluminum foil water pan trap, developed by—you guessed it—a citizen scientist.

#### Introduction

Citizen science, or public participation in research, can be a useful and efficient strategy for collecting certain scientific research data (Salmon et al. 2008). Collaborations between research scientists and citizen scientists have the potential to broaden the scope of research and enhance the ability to collect data (Cohn, 2008). Moreover, based on the type of data required, labor needs, budget limitations, and the urgency of the situation, citizen involvement may be the only feasible means for conducting specific studies (Jimmerson 1981, Silvertown 2009).

We discovered this recently with our research on the invasive pest insect, brown marmorated stink bug (BMSB), *Halyomorpha halys*. After its accidental introduction into eastern Pennsylvania in the mid-1990s, BMSB spread rapidly through the mid-Atlantic U.S., becoming an

ever-increasing nuisance pest during the fall and winter months in households and commercial buildings as well as a serious agricultural pest of tree fruit, vegetables, and other crops (Hamilton 2009, Kuhar et al. 2012, Leskey et al. 2012). Citizen scientists have contributed valuable data for tracking the spread of this insect into new regions. Websites for reporting the insect have been established at Rutgers University (<http://njaes.rutgers.edu/stinkbug/>) and Pennsylvania State University (<http://www.Stinkbuginfo.org>), and these data have enabled scientists to follow the bug's invasion across North America and to estimate the rate of spread. Also, with the help of Extension-run Master Gardener programs and communication networks, researchers have been able to obtain useful information on which plants this invasive stink bug utilizes for food and development (Bergmann et al. 2013).

Citizen scientists have contributed in other ways as well. Because BMSB uses man-made structures such as attics, garages, offices, and other buildings to overwinter (Hamilton 2009, Inkley 2012), citizens have monitored when, where, and how the bugs enter houses as well as how many. In 2011, one homeowner from Maryland collected over 26,000 BMSB in his house from January to June (Inkley 2012). Throughout the winter and spring, especially on warmer days, BMSB become active indoors, often finding their way into living areas. This nuisance factor has inspired citizens to develop creative ways of ridding their homes of these creatures. While the best defense for BMSB is sealing windows, doors, and other small access points (Day et al. 2011), this may not always be effective or possible because of the importance of airflow and the functionality of building products designed for expansion and contraction.

Like many other insects, BMSB are attracted to lights at night (Nielsen et al. 2013). Citizen scientists in the U.S. recognized this behavioral phenomenon within their homes and explored ways to trap BMSB using lights in a dark room. Various trap designs and homemade devices

began appearing on the Internet, including a reflective pan of water with a light source directed down on it and a plastic two-liter bottle trap with an inverted top and LED light inside to attract the bugs (<http://www.youtube.com/watch?v=zKc5acECuQk>). One homeowner from Pennsylvania designed and patented his stink bug light trap and started a business, Strube's Stink Bug Traps LLC, Columbia, PA (<http://www.stinkbugtrapsonline.com/>). Other commercial light traps began appearing in hardware stores in 2010, including the Rescue Trap (<http://www.rescue.com/product/reusable-indoor-stink-bug-trap>).

Our study was designed to evaluate the effectiveness of various light traps for the trapping of BMSB inside of homes. Two traps were homemade, while two others were commercially available. In order to achieve the necessary replications for our experiment, we involved Virginia Cooperative Extension agents to help us recruit 14 citizen scientists who were interested in conducting the study in their homes in 2012 or 2013.

### **Materials and Methods**

Experiments were conducted during the winter and spring in homes in Giles, Loudoun, or Montgomery Counties, Virginia in 2012 and 2013. Study sites were selected by identifying 16 volunteers who had sufficient stink bug infestations based upon phone or email complaints to Virginia Cooperative Extension agents and were interested and willing to conduct the experiment. Because BMSB is the only known stink bug in North America that overwinters in man-made structures, we were certain that participants were able to positively identify the insect.

In 2012, four traps were tested, including the Sterling Rescue® Stink Bug Trap (Figure 4.1), the Strube Stink Bug Trap (Figure 4.2), aluminum pan trap with soapy water and a desk lamp shining on it (Figure 4.3), and a 2-liter bottle trap (Figure 4.4). The costs to either purchase or make

these traps were approximately \$30, \$50, \$10, and \$7 for the Sterling Rescue, Strube, aluminum pan, and 2-liter bottle traps, respectively. The Rescue® Stink Bug Traps and light attachments were purchased from Lowes. The Strube Stink Bug Traps were donated by the company. The 2-liter bottle trap was constructed according to Smith (2011) by cutting the top off of a 2-liter soda bottle and inverting it to make a funnel. A Sylvania Dot-It battery powered LED light was placed in the bottom of the bottle, and masking tape on the sides of the bottle allowed for a rough surface for the BMSB to grasp. The bottom of the bottle was wrapped in black 3M electrical tape to ensure that the light was coming from the top of the trap (Smith 2011). First brought to our attention by the Catoctin Creek Scenic River Advisory Committee of Loudoun County, Virginia, the water pan trap was simply an aluminum foil roasting pan (29.8cm x 23.8cm x 5.9cm) with approximately 1-liter of water: 1 mL Dawn dish detergent mixture in the bottom of the pan. A desk lamp with a 13W CFL light bulb was placed next to the pan and pointed into the pan. In 2013, the Strube Stink Bug Trap was excluded from the experiment because the trap was discontinued and not available in 2013.

All locations were provided with one of each trap and all materials necessary to conduct these tests, including batteries and extension cords. Within each home, individual traps were each placed in a separate room for approximately 1 week. The light source for traps were either manually turned on for LED light in the 2-liter bottle trap, or automatically turned on using a Brinks Timer set for 12 hours daily from 7PM – 7AM. Each week throughout the duration of the experiment, the total number of BMSB adults was counted and reported by the homeowners. After each count, traps at location 1 were moved to location 2; location 2 to location 3; location 3 to location 4; and location 4 to location 1. This was repeated each week for the duration of the project.



All data were analyzed using JMP Pro 10 (SAS Inst. 2007, Cary, NC). ANOVA was conducted and followed by a Student's t-test to evaluate significant difference of the number of BMSB caught per trap ( $\alpha=0.05$ ).

### **Results**

From 2012 to 2013, there were a total of 14 participants (72%) who reported stink bug trap catch data for a minimum of 4 weeks (Figure 4.5). These data were used for statistical analysis. In 2012, significantly more BMSB were caught in both the Strube and aluminum foil pan traps over the weekly observation period (Figure 4.6;  $F = 11.12$ ;  $dF = 3$ ;  $p < 0.0001$ ). In 2013, the aluminum foil water pan trap caught 19 times more BMSB adults than the other two traps (Figure 4.7, a difference that was highly significant;  $F = 25.91$ ;  $dF = 2$ ;  $p < 0.0001^*$ ). The Strube trap was omitted from our analysis because of its exclusion in 2013.

### **Discussion**

Excluding the Strube trap, which discontinued the model that we tested in 2012, the aluminum foil pan trap was the most effective device for trapping BMSB in homes during the winter and spring. Some individuals reported bug catch as high as 144 per week using this homemade trap. Although this trap may not remove every stink bug from a building, it will provide homeowners with some relief from their stink bug infestations, as well as a sense of satisfaction from removing large numbers of the bugs from their homes. This information should be of immediate value to many Extension personnel in the mid-Atlantic U.S., who have been asked the popular question, "How do I get rid of stink bugs?"

Our study is also valuable for Extension in other ways. As Extension agents seldom have the resources or time to adequately conduct research studies that could provide answers to problems that are plaguing their clientele, the study reported here shows them that using citizen scientists may enable them to conduct practical applied research. Based on the nature of our study, which involved trapping stink bugs from infested houses, there was no more efficient way to collect these data than to use citizen scientists. Because our participants cohabitate with these insects, they had a vested interest in the experiment. Some of our participants used the experience as a teaching tool for their families, such as an opportunity for children to learn how to count or to learn about the scientific process.

Another benefit to using citizen scientists was that the experiment had an immediate impact because participants knew the outcome of the experiment and disseminated the results themselves via word of mouth. Although the outcome of the experiment was published in some local newspapers, many citizens within the counties where the testing took place had already heard about the experiment.

### **Acknowledgements**

We would like to thank the Catoctin Creek Scenic River Advisory Committee, Tami Carlow, and all of the volunteers who allowed us to use their homes for their dedication and assistance with this project. This research was supported in part by USDA-NIFA SCRI award #2011-51181-30937.

### **References**

**Bergmann, E., K. M. Bernhard, G. Bernon, M. Bickerton, S. Gill, C. Gonzales, G. C. Hamilton, C.**

**Hedstrom, K. Kamminga, C. Koplinka-Loehr, G. Krawczyk, T. P. Kuhar, B. Kunkel, J. Lee,**

**T. C. Leskey, H. Martinson, A. L. Nielsen, M. Raupp, P. Shearer, P. Shrewsbury,**

- J. Walgenbach, J. Whalen, and N. Wiman. 2013.** Host plants of the brown marmorated stink bug in the U.S. Northeastern IPM Center Technical Bulletin.  
(<http://www.stopbmsb.org/where-isbmsb/host-plants>) (Accessed:
- Cohn, J. P. 2008.** Citizen science: Can volunteers do real research? *Bioscience*, 58(3). 192. doi: 10.1641/b580303.
- Day, E. R., T. McCoy, D. Miller, T. P. Kuhar, and D. G. Pfeiffer. 2011.** Brown marmorated stink bug. Virginia Cooperative Extension. Pub. No. 2902-1100. Retrieved from:  
<http://www.pubs.ext.vt.edu/2902/2902-1100/2902-1100.html>
- Hamilton, G. C. 2009.** Brown marmorated stink Bug. *Am. Entomol.* Spring 2009. 55: 19-20.
- Inkley, D. B. 2012.** Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). *J. Entomol. Sci.* 47: 125 - 130.
- Jimmerson, R. M. 1981.** What determines citizen involvement? *J. Extension* [On-line], 19(5).  
Available at: <http://www.joe.org/joe/1981september/81-5-a3.pdf>
- Kuhar, T. P., K. L. Kamminga, J. Whalen, G. P. Dively, G. Brust, C. R. R. Hooks, G. Hamilton, and D. A. Herbert. 2012.** The pest potential of brown marmorated stink bug on vegetable crops. *Plant Health Progress*, doi: 10.1094/PHP-2012-0523-01-BR
- Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. Kuhar, D. Pfeiffer, G. Dively, C. Hooks, M. Raupp, P. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. Hoelmer, D-H Lee, and S. E. Wright. 2012.** Pest status of the brown marmorated stink bug,

Halyomorpha halys in the USA. *Outlooks on Pest Management*, 23: 218-226. doi:  
10.1564/23oct07

**Nielsen, A. L., K. Holmstrom, G. C. Hamilton, J. Cambridge, and J. Ingerson-Mahar. 2013.** Use of  
black light traps to monitor the abundance, spread, and flight behavior of *Halyomorpha*  
*halys* (Hemiptera: Pentatomidae). *J. Econ. Entomol.* 106: 1495-1502. doi:  
10.1603/EC12472

**Salmon, T. P., B. Faber, G. Bender, D. Shaw, V. J. Kowalski, and A. Berentsen. 2008.** Volunteer  
researchers: Moving beyond cooperators. *J. Extension.* 46(2) Article 2FEA7.  
Available at: <http://www.joe.org/joe/2008april/a7.php>

**SAS Institute, Inc. 2007.** JMP user's guide. Cary, NC: SAS Institute Inc.

**Silvertown, J. 2009.** A new dawn for citizen science. *Trends in Ecology and Evolution*, 24(9):  
467 – 470.



**Figure 4.1.** Aluminum foil pan trap with soapy water in action in a home in Loudoun County, Virginia.



**Figure 4.2.** Commercially available 2011 Strube's Stink Bug Trap.

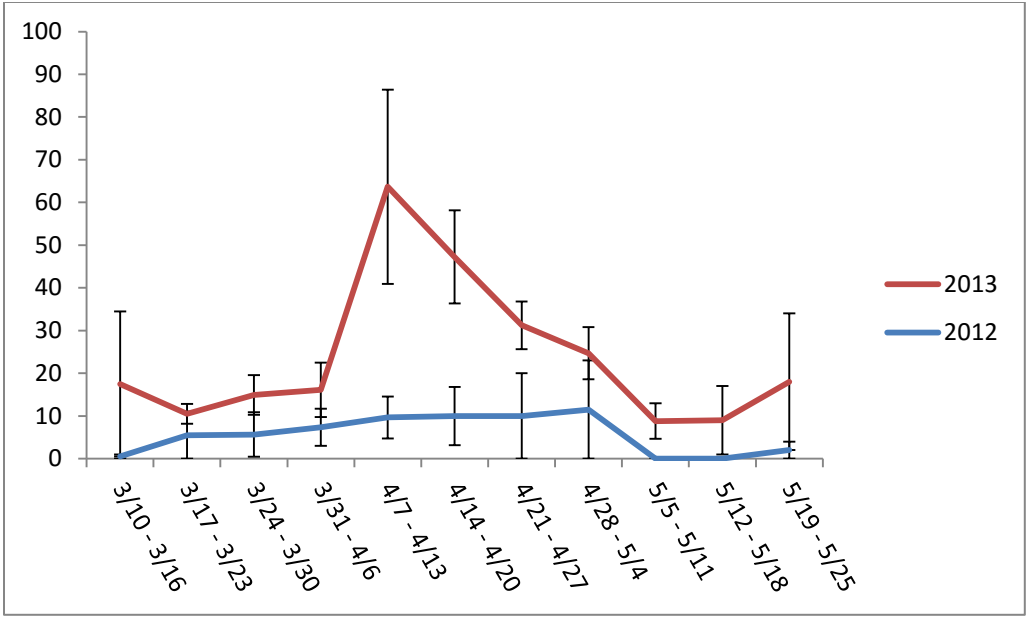


**Figure 4.3.** Homemade 2-liter bottle trap with LED press-on light in the bottom.

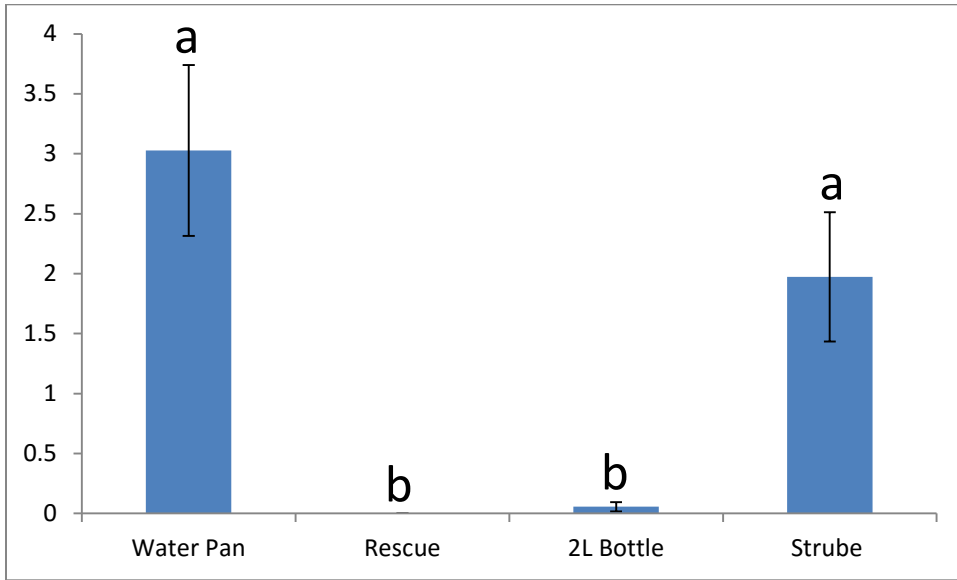


Figure 4.4. Rescue® stink bug trap in a home in Loudoun County, Virginia in 2013.

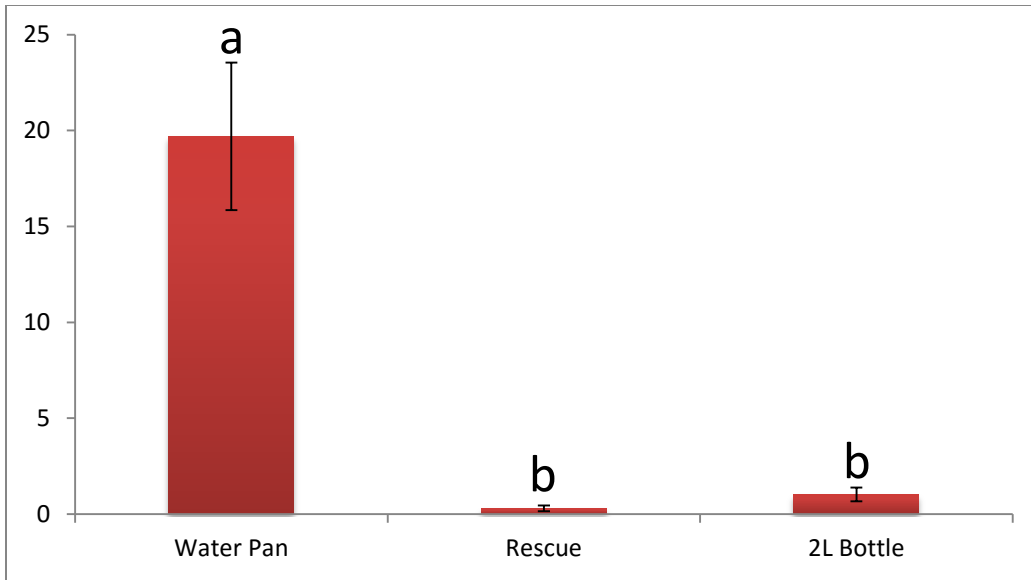




**Figure 4.5.** Mean ( $\pm$ SE) number of stink bugs caught by week in all light traps placed in houses in Virginia by citizen scientists in 2012 and 2013.



**Figure 4.6.** Cumulative Mean ( $\pm$ SE) Number of BMSB Adults Captured per Trap per Week in 2012.



**Figure 4.7.** Cumulative Mean ( $\pm$ SE) Number of BMSB Adults Captured per Trap per Week in 2013.

## Chapter Five

### Control of Brown Marmorated Stink Bug with Insecticide-Treated Window Screens

(Mooneyham, K., J.D. Aigner and T.P. Kuhar. 2016. Control of Brown Marmorated Stink Bug with Insecticide-Treated Window Screens, 2015. Arthropod Management Tests. In Press.)

The brown marmorated stink bug (BMSB) is a major nuisance pest, aggregating in and on buildings as they seek overwintering habitat during the winter months. Due to this activity, control efforts have focused on safeguarding entryways on structures to keep these insects from entering. While there has been a considerable amount of work conducted surrounding the efficacy of insecticides against BMSB in agricultural cropping systems, very little work has been documented focusing specifically on the evaluation of labeled insecticides in urban settings. Herein we intend to evaluate the efficacy of insecticides currently labeled for restricted use by pest management professionals against BMSB and the residual activity of each to determine if these insecticides do, in fact, provide adequate control of the pest.

This trial was conducted in Blacksburg, VA, starting on 24 Sep 2014 and focused on screen treatments to kill BMSB before they are able to enter a building by creating mesh bags (8 × 16 inches) from polyethylene window screen with three sides sewn together. Bags were treated with 9 currently labeled BMSB insecticide products (Table 5.1) by mixing each in 1 gal of water according to the highest labeled rate. Four bags were then dipped for 5 s into each of the insecticide solutions placed in a pan and all were allowed to dry approximately 1 h. There were also 4 bags dipped into a water control. The experiment consisted of a total of 40 treated screen bags that were arranged in a completely randomized design hung on a nylon clothesline, which allowed exposure to ambient weather conditions, including temperature, wind, rainfall, and sunlight for the duration of the experiment. This allowed us to simulate typical degradation

of each insecticide under normal conditions outside. Starting at 1 h after treatment, 10 BMSB adults were placed in each bag, which was then fastened closed with a clothespin. After exposure to each treatment for 48 h, mortality of the bugs was assessed by categorizing bugs as alive, dead, or moribund (intoxicated and unable to right themselves when on their dorsal side). This process was repeated approximately each week until none of the treated bags were killing bugs anymore. The bags remained in the field for the duration of the experiment.

Window screens dipped into an insecticide solution seemed to be an effective delivery method for this experiment. After 2 d in the field, all of the insecticide products except indoxacarb resulted in >80% mortality of bugs. After 10 d in the field however, only lambda-cyhalothrin, lambda-cyhalothrin + thiamethoxam, beta-cyfluthrin, beta-cyfluthrin + imidacloprid, and Alpine provided effective control (mortality >80%). The aforementioned insecticide products contain a pyrethroid (i.e., lambda-cyhalothrin and beta-cyfluthrin), a neonicotinoid (i.e., imidacloprid and dinotefuran), or both (i.e., lambda-cyhalothrin + thiamethoxam and beta-cyfluthrin + imidacloprid). After 22 d in the field, only beta-cyfluthrin, beta-cyfluthrin + imidacloprid, and lambda-cyhalothrin provided mortality above 80%. It should also be noted that lambda-cyhalothrin, beta-cyfluthrin, beta-cyfluthrin + imidacloprid, lambda-cyhalothrin + thiamethoxam and dinotefuran all showed some level of activity even after 40 d in the field. All of the other insecticides lost their entire efficacy by 29 d. We were able to show with this study that several commercial products containing pyrethroids alone and in combination with a neonicotinoid have the greatest residual activity for control of BMSB. We did not receive any gifts of insecticides or money from any company in order to conduct this experiment.

**Table 5.1.** Residual efficacy of window screens treated with commercial insecticides for control of BMSB.

Active Ingredient	Trade Name	Concentration (amt of product/gal)	% BMSB Mortality					
			2 DAT	10 DAT	22 DAT	29 DAT	37 DAT	44 DAT
Untreated	n/a	n/a	17.5 c	22.5 d	15.0 c	0.0 d	0.0 c	0.0 c
lambda-cyhalothrin	Demand	24 ml	100.0 a	97.5 a	50.0 a	75.0 bc	67.5 a	65.0 a
lambda-cyhalothrin + thiamethoxam	Tandem	32 ml	100.0 a	95.0 a	0.0 d	87.5 ab	10.0 c	12.5 c
beta-cyfluthrin	Tempo	16 ml	100.0 a	97.5 a	35.0 b	97.5 a	42.5 b	35.0 b
beta-cyfluthrin + imidacloprid	Temprid	16 ml	100.0 a	80.0 ab	42.5 ab	80.0 bc	55.0 ab	35.0 b
esfenvalerate	Fenvistar Plus	24.5 ml	93.3 a	40.0 cd	0.0 cd	0.0 d	0.0 c	0.0 c
fipronil	Termidor	47.3 ml	95.0 a	65.0 abc	0.0 d	0.0 d	2.5 c	0.0 c
imidacloprid	Premise 2	17.7 ml	94.0 a	74.0 abc	0.0 d	6.0 d	0.0 c	0.0 c
dinotefuran	Alpine	30 g	100.0 a	100.0 a	0.0 d	70.0 c	17.5 c	7.5 c
indoxacarb	Arilon	18.71 g	65.0 b	45.0 bcd	0.0 d	0.0 d	0.0 c	0.0 c

## Chapter Six

### Toxicities of Neonicotinoid Insecticides for Systemic Control of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Fruiting Vegetables

(As Published: J.D. Aigner, J.W. Walgenbach, and T.P. Kuhar. 2015. Journal of Agricultural and Urban Entomology. Jan. 2015. Vol. 31, Issue 1, pg(s) 70-80 doi: 10.3954/JAUE15-06.1)

#### Abstract

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest of various crops, including fruiting vegetables, throughout the mid-Atlantic U.S.A. Current control strategies for this pest rely almost exclusively on foliar applications of broad-spectrum insecticides, which disrupt IPM programs and cause secondary pest outbreaks. Systemic neonicotinoids applied to the root-zone via soil drench or chemigation may be a more IPM friendly tactic for insect control in vegetables. Laboratory bioassays that utilized a plant uptake method showed that the neonicotinoid insecticides clothianidin, dinotefuran, imidacloprid, and thiamethoxam were all toxic to *H. halys* nymphs, with estimated LC<sub>50</sub> values of 0.077, 0.013, 0.068, and 0.018 ppm, respectively. Field efficacy experiments in Virginia showed that two soil applications of each of the aforementioned neonicotinoid insecticides significantly reduced stink bug damage to pepper and tomato. Field experiments conducted on tomatoes in North Carolina in 2012 and 2014 revealed a similar reduction in stink bug damage with a single drip chemigation application of either dinotefuran or imidacloprid. In those trials, clothianidin was not efficacious and thiamethoxam was only effective in 2012. Our studies demonstrate the potential for soil applications of neonicotinoids to reduce stink bug damage to fruiting vegetables.

#### Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest native to eastern Asia (Lee et al. 2013). It was first discovered in the U.S.A. near Allentown, Pennsylvania, in the mid-1990s (Hoebeke & Carter 2003), and has since been detected in most of the

continental U.S.A., parts of Canada, and Europe (Leskey et al. 2014). *Halyomorpha halys* is highly polyphagous and feeds on a wide variety of host plants, preferring to attack reproductive structures, such as developing and mature fruit and pods (Hoebeke & Carter 2003, Bergmann et al. 2013, Lee et al. 2013). In the mid-Atlantic U.S.A., this species has become a serious pest of fruiting vegetables, including pepper, *Capsicum spp.* (Solanales: Solanaceae); tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae); okra, *Abelmoschus esculentus* (L.) Moench (Malvales: Malvaceae); and eggplant, *Solanum melongena* L. (Solanales: Solanaceae) (Kuhar et al. 2012a). Adults and nymphs invade fields during the summer months when fruiting bodies appear on plants. Feeding by *H. halys* causes white spongy areas on the skin and internal tissue damage to developed fruit, and it also may reduce fruit set and subsequent yield via abortion of flower buds and young fruiting bodies. Where this insect is established, such as southwest Virginia, damage to bell pepper has averaged between 20% to 40% in the absence of control measures (Kuhar et al. 2012b,c,d, 2013a,b,c).

Chemical control of stink bugs in agricultural crops has relied almost exclusively on the use of broad-spectrum insecticides, including pyrethroids, organophosphates, and carbamates (McPherson & McPherson 2000, Snodgrass et al. 2005, Temple et al. 2013). More recently, neonicotinoids have demonstrated a high level of activity against pentatomid pests, including various *Euschistus* species (Willrich et al. 2003, Cullen & Zalom 2007, Kamminga et al. 2009), *Chinavia* (=Acrosternum) *hilaris* (Say) (Willrich et al. 2003, Kamminga et al. 2009, 2012), *Murgantia histrionica* (Hahn) (Wallingford et al. 2012), and *H. halys* (Nielsen et al. 2008b, Kuhar et al. 2012c, Leskey et al. 2012a). In fruiting vegetable crop systems, several neonicotinoids, including imidacloprid, thiamethoxam, clothianidin, and dinotefuran, can be applied to root systems as a drench or injected directly into the drip irrigation system where they are translocated by the plant to green tissue to provide systemic control of above ground feeding pests (Elbert et al. 2008, Ghidui et al. 2012). Wallingford et al. (2012) achieved effective control of the harlequin bug, *M. histrionica*, using the aforementioned neonicotinoids applied to the



root zone of collards, *Brassica oleracea* L. (Brassicales: Brassicaceae) plants. However, the harlequin bug is primarily a leaf-feeding stink bug. The efficacy of this strategy against *H. halys* is not known. Herein we investigated the relative toxicities of four neonicotinoids applied via a plant uptake bioassay, and we evaluated their field efficacy when applied to the soil for systemic control of *H. halys* on pepper and tomato.

## Materials and Methods

**Insecticide plant uptake bioassay for LC<sub>50</sub> determination.** To start a colony of *Halyomorpha halys*, adults were collected from various locations throughout Virginia and Maryland in the fall of 2012 and 2013. These insects were held in artificial overwintering habitats consisting of 19-L buckets that were tightly packed with 12.7 mm polyethylene pipe insulation (Frost King®, Mahwah, NJ). Approximately 500 *H. halys* adults were placed into each overwintering container. Adults were maintained outdoors at Blacksburg, VA until early January for both years, at which time they were placed in a temperature chamber (Percival Scientific Inc., Perry, IA) and exposed to temperatures of 26°C, a 16:8 h L:D photoperiod, and a 50% relative humidity, which induced feeding and reproduction of the bugs after a few weeks (Nielsen et al. 2008a, Medal et al. 2012). Adults and nymphs *H. halys* were provided a water wick and maintained on a diet of snap beans, *Phaseolus vulgaris* L. (Fabales: Fabaceae); carrots, *Daucus carota* L. (Apiales: Apiaceae); and peanuts, *Arachis hypogaea* L. (Fabales: Fabaceae). Nymphs were starved for 24 h prior to use in bioassays.

'Ambrose' snap beans were planted in 1-L pots in a greenhouse located at Virginia Tech, Blacksburg, VA. *Phaseolus vulgaris* was chosen because of its ease of production in the greenhouse and because it is known that *H. halys* readily feed on the plant tissue when pods are not present. Commercial formulations of clothianidin (Belay®, Valent U.S.A. Corp., Walnut Creek, CA), dinotefuran (Venom® 70SG, Valent U.S.A. Corp., Walnut Creek, CA), imidacloprid (Admire Pro™, Bayer CropScience U.S., Research

Triangle Park, NC), and thiamethoxam (Platinum<sup>®</sup> 75SG, Syngenta Crop Protection Inc., Greensboro, NC) were obtained from manufacturers and were mixed with water to make concentrations of 0.0, 0.001, 0.01, 0.1 and 1.0 ppm of each active ingredient. Solutions were placed in 50 ml Falcon<sup>®</sup> (Becton Dickinson & Co., Franklin Lakes, NJ) sterile polystyrene conical tubes. At the three-leaf stage (17–21 d after germination), snap bean plants were excised at the base of the plant and the cut end submerged in each insecticide concentration for 24 h to allow the insecticide to be taken up by the plant and translocated throughout the tissue (Prabhaker et al. 2011). The plants were held upright in the centrifuge tubes using a disc of floral foam (FloraCraft<sup>®</sup>, Ludington, MI) cut to the inside diameter of the tube. For each experiment, 10 laboratory-reared 2nd–4th instars of *H. halys* were placed in 500 ml fine mesh bags that were fastened over the bean plants. Four bagged plants were established for each insecticide concentration (total 5 40 bugs) and were evaluated after 72 h for mortality (dead + moribund bugs). The experiment was replicated on five separate dates for each insecticide. Abbott's formula was used to correct for control mortality (Abbott 1925). Mortality and morbidity data were combined for analysis. Dose-mortality data were entered into PoloPlus<sup>®</sup> Version 1.0 (LeOra Software Co., Petaluma, CA) to determine LC<sub>50</sub> values (LeOra Software 2002).

**Field efficacy experiments.** Field efficacy experiments were conducted on bell pepper in 2012 and 2013 in Virginia, and on tomato in 2013 in Virginia and in 2012 and 2014 in North Carolina. In early June 2012 and 2013, transplants of 'Aristotle' bell peppers were planted on raised beds covered with black polyethylene mulch at Virginia Tech's Kentland Farm near Blacksburg, VA. 'Baby Cake' tomatoes also were tested in 2013 at the Virginia site. Pepper and tomato plants were spaced 0.3 and 0.5 m, respectively, within rows. Plots were one row by six meters long. Each experiment was set up in a randomized complete block design replicated four times, and each of the four neonicotinoids was applied at the highest labeled soil application rate. Treatments included an untreated control, imidacloprid (0.426 kg ai/ha), clothianidin (0.224 kg ai/ha), dinotefuran (0.291 kg ai/ha), and

thiamethoxam (0.190 kg ai/ha). In addition, we included a soil drench application of clothianidin (0.224 kg ai/ha) with a weekly grower-standard foliar application of pyrethroid fenprothrin (0.347 kg ai/ha) (Danitol®, Valent U.S.A. Corp., Walnut Creek, CA). The first soil applications were made approximately 21 d after planting by placing 40 ml of the insecticide solution directly at the base of each plant within its respective plot using a single nozzle pump action Solo® sprayer (Newport News, VA). A second application of all treatments was made 30 d after the first application. All plots were irrigated approximately once per week using 10-mil Aqua-Traxx® drip tape (Toro®, Bloomington, MN) with 30.5 cm spacing and were given approximately the same volume of water. Foliar applications of fenprothrin were made on 6, 14, 21, and 29 August in 2012, and on 30 July, 6, 14, and 26 August in 2013 with a 3-nozzle boom equipped with D3 spray tips and 45 cores and powered by a CO<sub>2</sub> backpack sprayer at 275.8 kPa delivering 355 L/ha. On three harvest dates in August in both years of the study, twenty randomly-selected mature fruit were hand-picked from each plot and inspected for stink bug feeding damage. This damage is indicated by a whitish or yellow spongy area on the fruit (Kuhar et al. 2012a).

Tomato trials were conducted at North Carolina State University's Mountain Horticultural Crops Research Station near Mills River, NC. Five-week-old 'Biltmore' and 'Florida 47' tomato transplants were set on 31 and 15 May in 2012 and 2014, respectively. Plants were set in raised beds covered with black plastic mulch and Chapin Drip Tape™ (Jain Irrigation Inc., Watertown, NY) buried about 6 cm below the soil surface. Drip tape emitters were spaced 30 cm apart and they delivered 372 L/h/100 m at a flow of 68.9 kPa. Plots consisted of two 7.6-m long rows on 1.5 m centers, with a non-treated row separating treatment rows. Plants were spaced 0.4 m within rows, and treatments were replicated four times and arranged in a randomized complete block. Treatments consisted of an untreated control and the following insecticides applied to the soil via drip tape: imidacloprid at 0.423 kg/ha, clothianidin at 0.224 kg/ha, dinotefuran at 0.294 kg/ha, and thiamethoxam at 0.193 kg/ha. Insecticides were applied on 2 and

8 July in 2012 and 2014, respectively, with a CO<sub>2</sub> injector into one-inch poly tube connected to individual treatment drip lines. In 2012, chlorantraniliprole (Coragen® Insect Control, DuPont® Crop Protection, Wilmington, DE) was applied at 0.058 kg/ha via drip tape to all treatments except the control on 20 June and to the imidacloprid and dinotefuran treatments on 25 July. In 2014, the same rate of chlorantraniliprole was applied via drip tape to all treatments, including the control, on 2 June and 4 August. Chlorantraniliprole was applied to control lepidopteran pests; however, this insecticide does not exhibit activity against *H. halys* (JFW unpublished). The entire plot was on the same drip irrigation schedule, which consisted of irrigating two times per week during which 1 to 3 cm of water was applied per week. Mature fruit were harvested from all plots on 8 and 22 August and 6 and 20 September in 2012, and on 31 July, 14, 21 and 28 August in 2014. The time intervals between treatment applications and harvests in 2012 and 2014 were 37 to 80 d and 23 to 51 d, respectively.

When necessary, the proportion of damaged peppers and tomatoes from each treatment was transformed using an arcsine-square root transformation to normalize the variances (Sokal and Rohlf 1995), and then analyzed using ANOVA, JMP version 10.0 (SAS Institute, Cary, NC). Means were separated using Fisher's Protected LSD at the  $P < 0.05$  level of significance. Data are presented as original means.

## Results

**Insecticide plant uptake bioassay for LC<sub>50</sub> determination.** All four of the neonicotinoid insecticides were highly toxic to *H. halys*, killing nearly 100% of the nymphs at a concentration of 1.0 ppm. Systemic LC<sub>50</sub> values were lowest for dinotefuran (0.013 ppm) and thiamethoxam (0.018), but, based on non-overlapping 95% fiducial limits, only the LC<sub>50</sub> value for dinotefuran was statistically lower than that of clothianidin (0.068 ppm) or imidacloprid (0.068 ppm) (Table 1).

**Field efficacy experiments.** For all insecticides applied at the Virginia location to pepper in 2012 ( $F_{56.72}$ ,  $df_{55, 18}$ ,  $P < 0.05$ ) and 2013 ( $F_{53.09}$ ,  $df_{55, 18}$ ,  $P < 0.05$ ) and to tomato in 2013 ( $F_{55.02}$ ,  $df_{55, 18}$ ,  $P < 0.05$ ), there were significant treatment effects on *H. halys* feeding damage to fruit at harvest over the course of the season, resulting in less damage than the untreated control (Figures 1 and 2). On average, stink bug damage to fruit was reduced approximately 50% by the soil applications of neonicotinoids. In all experiments, the addition of four weekly foliar applications of the pyrethroid fenpropathrin to a systemic application of clothianidin did not result in significant additional reduction of stink bug damage except in peppers in 2012, where the combination of clothianidin and fenpropathrin had significantly less stink bug damage compared with imidacloprid.

In the tomato trials in North Carolina in 2012 ( $F_{55.28}$ ,  $df_{54, 12}$ ,  $P < 0.011$ ) and 2014 ( $F_{53.29}$ ,  $df_{54, 12}$ ,  $P < 0.048$ ), the lowest levels of fruit damage were in the dinotefuran and imidacloprid treatments (Figure 3). In 2012, under relatively low stink bug pressure, clothianidin was the only treatment that did not significantly reduce damage below the control. In 2014, when stink bug populations were higher, only dinotefuran significantly reduced damage below the control, although damage levels in the imidacloprid treatment did not differ from that of dinotefuran. The most effective drip line treatments in the North Carolina studies reduced damage by approximately 50% below the control, similar to that observed with drench applications in the Virginia peppers and tomatoes.

## Discussion

Chemical control of *H. halys* has been a topic of increasing interest ever since this invasive stink bug was recognized as a serious agricultural threat in the U.S.A. (Nielsen & Hamilton 2009, Leskey et al. 2012b). Several recent studies have assessed the efficacy of insecticides on *H. halys* using glass vial bioassays (Nielsen et al. 2008b), treated glass surface assays (Leskey 2012a), direct-contact topical applications (G. Krawczyk, unpublished data), bean dip bioassays (Kuhar et al. 2012f), and field efficacy trials (Kuhar et al.

2012b,c,d,e, Bergh 2013a,b,c, Hull et al. 2013a,b, Kuhar et al. 2013a,b,c). To our knowledge, this paper is the first published research on the systemic activity of neonicotinoid insecticides on *H. halys*. The plant uptake bioassay method, adapted from Prabhaker et al. (2011), was an effective and efficient method for discriminating insecticide concentrations to determine LC<sub>50</sub> levels for four neonicotinoids on *H. halys*. The effectiveness of this bioassay technique confirms *H. halys* will feed on leaves and stem tissue of snap bean and that this bioassay method is a sufficient way to measure mortality in future studies to achieve similar objectives.

While all neonicotinoids were toxic to *H. halys* nymphs in our bioassays, imidacloprid and clothianidin had slightly higher (4–6 times) LC<sub>50</sub> levels than those of dinotefuran and thiamethoxam. It is noted, however, that we do not know if the different neonicotinoids were taken up at the same rate by bean plants, so these values should not be used to compare relative toxicity of the products to *H. halys*.

Nielsen et al. (2008a) previously reported toxicity levels for dinotefuran and thiamethoxam on *H. halys*, but, because a treated glass vial assay was used, the LC<sub>50</sub> values are based on mg [AI]/cm<sup>2</sup>, rather than ppm concentration of ingested solution.

In the field, soil applications of the aforementioned neonicotinoids at their highest recommended labeled rates were efficacious, significantly reducing stink bug damage to peppers and tomatoes in Virginia by about 50%. However, it should be noted that we evaluated two soil applications of these products made approximately 30 d apart in order to compare the relative toxicities throughout multiple harvests. This application strategy would violate the label restrictions by the U.S.A. Environmental Protection Agency (EPA) for some of the products. For instance, clothianidin cannot be applied to the soil on fruiting vegetables more than once per season, and the postharvest interval for imidacloprid, dinotefuran, and clothianidin is 21 d, and thiamethoxam is 30 d to harvest. Those intervals would probably eliminate the possibility of a second application of these insecticides in order to not interfere

with proper harvest dates. However, in the tomato experiments conducted in North Carolina, a single application was made through the drip irrigation line more than 21 d before harvest. In those experiments, application of either dinotefuran or imidacloprid significantly reduced stink bug fruit damage; whereas, clothianidin was not efficacious, and thiamethoxam was only effective in 2012 and not 2013. Additional late season control of stink bugs could be provided by foliar insecticide applications. In our study, the percentage of stink bug damage to fruit in plots treated with a soil application of clothianidin followed by four weekly foliar applications of fenpropathrin was significantly less in one of the three experiments. However, foliar applications of pyrethroids also kill natural enemies and other beneficial insects (Croft & Whalon 1982), which can lead to outbreaks of secondary pests, such as spider mites and aphids (Leskey et al. 2012b). In peppers in the mid-Atlantic U.S.A., for instance, multiple applications of pyrethroids often result in serious pest outbreaks of green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Chapman et al. 2009, Ghidui & Kuhar 2012, Kuhar et al. 2012b). Thus, because systemic neonicotinoids are highly efficacious at controlling aphids and many other sucking insect pests (Elbert et al. 2008), their use as one of the insecticide applications for control of *H. halys* during the season should prevent the problems with aphid outbreaks in crops like pepper.

The use of neonicotinoid insecticides have been under scrutiny in recent years as being detrimental to honey bees, *Apis mellifera* L., and other pollinators (Laycock et al. 2012, Stoner and Eitzer 2012, Fairbrother et al. 2014). While the active ingredients of all of the soil-applied neonicotinoids that we tested are highly toxic to bees when applied topically or orally, the assumed risk to pollinators will likely be reduced when these products are applied to the root systems via chemigation or soil drench. Dively & Kamel (2012) found that the levels of neonicotinoid insecticides in pollen and nectar of cucurbit crops varied with the timing of soil applications, but, under worst case scenarios, levels were not acutely lethal to honeybees and were often below the no-observable-effect level. We believe that this strategy should pose less threat to pollinators than any of the alternative chemical control approaches, which invariably

include multiple foliar sprays of insecticides, such as pyrethroids, carbamates, organophosphates. It is our conclusion that soil application of the neonicotinoid insecticides imidacloprid, dinotefuran, clothianidin, or thiamethoxam is an effective method to manage *H. halys* in fruiting vegetables efficiently, while attempting to minimize impacts on non-target species.

### Acknowledgments

We would like to thank Doug Inkley, Doo-Hyung Lee, Katherine Kamminga, Christopher Philips, Adam Wimer, Anna Wallingford, Louis Nottingham, James Wilson, Benjamin Aigner, Elizabeth Fread, Emily Lawrence, and Ashley Lohr for their dedication and assistance with this project. This project was funded by the USDA-NIFA-SCRI Grant # 2011-51181-30937 and USDA-NIFA RIPM Grant #2011-34103-30726.

### References

- Abbott, W. S. 1925.** A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Bergh, J. C. 2013a.** Alternate-row-middle applications of single insecticides targeting brown marmorated stink bug in apple, 2012. *Arthropod Manag. Tests* 38: A3. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2013.A3].
- Bergh, J. C. 2013b.** Effect of an adjuvant on control of brown marmorated stink bug and other pests in apple, 2012. *Arthropod Manag. Tests* 38: A4. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2013.A4].
- Bergh, J. C. 2013c.** Single insecticides targeting brown marmorated stink bug in apple, 2012. *Arthropod Manag. Tests* 38: A2. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2013.A2].



**Bergmann, E., K. M. Bernhard, G. Bernon, M. Bickerton, S. Gill, C. Gonzales, G. C. Hamilton, C.**

**Hedstrom, K. Kamminga, C. Koplinka-Loehr, G. Krawczyk, T. P. Kuhar, B. Kunkel, J. Lee, T. C.**

**Leskey, H. Martinson, A. L. Nielsen, M. Raupp, P. Shearer, P. Shrewsbury, J. Walgenbach, J.**

**Whalen, and N. Wiman. 2013.** Host plants of the brown marmorated stink bug in the U.S.

Publication of the Brown Marmorated Stink Bug IPM Working Group in conjunction with the

Northeastern IPM Center. Available at: <http://www.stopbmsb.org/where-is-bmsb/host-plants/>;

accessed online 10 November 2015.

**Chapman, A., T. Kuhar, P. Schultz, T. Leslie, S. Fleischer, G. Dively, and J. Whalen. 2009.** Integrating

chemical and biological control of European corn borer in bell pepper. *J. Econ. Entomol.* 102:

287–295.

**Croft, B. A. and M. E. Whalon. 1982.** Selective toxicity of pyrethroid insecticides to arthropod natural

enemies and pests of agricultural crops. *Entomophaga* 27: 3–21.

**Cullen, E. M. and F. G. Zalom. 2007.** On-farm trial assessing efficacy of three insecticide classes for

management of stink bug and fruit damage on processing tomatoes. *Plant Health Progress,*

*Plant Manag. Network.* Available at:

<http://www.plantmanagementnetwork.org/pub/php/research/2007/stink/>; accessed online 10

November 2015 [DOI: 10.1094/PHP-2007-0323-01-RS].

**Dively, G. P. and A. Kamel. 2012.** Insecticide residues in pollen and nectar of a cucurbit crop and their

potential exposure to pollinators. *J. Agric. Food Chem.* 60: 4449–4456.

**Elbert, A., M. Haas, B. Springer, W. Thielert, and R. Nauen. 2008.** Applied aspects of neonicotinoid uses

in crop protection. *Pest Manag. Sci.* 64: 1099–1105.

- Fairbrother, A., J. Purdy, T. Anderson, and R. Fell. 2014.** Risks of neonicotinoid insecticides to honeybees. *Environ. Toxicol. Chem.* 33: 719–731.
- Ghidu, G. M. and T. P. Kuhar. 2012.** Pepper insects and their control, pp. 216–226. In V. M. Russo [Ed.], *Peppers: botany, production and uses*. CAB International, Wallingford, 280 pp.
- Ghidu, G., T. Kuhar, J. Palumbo, and D. Schuster. 2012.** Drip chemigation of insecticides as a pest management tool in vegetable production. *J. Integr. Pest Manag.* 3(3): 4. Available at: [https://www.researchgate.net/publication/233895804\\_Drip\\_Chemigation\\_of\\_Insecticides\\_as\\_a\\_Pest\\_Management\\_Tool\\_in\\_Vegetable\\_Production](https://www.researchgate.net/publication/233895804_Drip_Chemigation_of_Insecticides_as_a_Pest_Management_Tool_in_Vegetable_Production); accessed online 10 November 2015 [DOI: 10.1603/IPM10022].
- Hoebeke, E. R. and M. E. Carter. 2003.** *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): A polyphagous plant pest from Asia newly detected in North America. *Proc. Entomol. Soc. Wash.* 105: 225–237.
- Hull, L. A., D. Binddinger, and G. Krawczyk. 2013a.** Large plot evaluations of various lepidopteran and brown marmorated stink bug tactics, 2012. *Arthropod Manag. Tests* 38: A9. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2013.A9].
- Hull, L. A., G. Krawczyk, and D. Biddinger. 2013b.** Evaluations of products for internal Lepidoptera and brown marmorated stink bug control, 2012. *Arthropod Manag. Tests* 38: A7M. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2013.A7].

**Kamminga, K. L., D. A. Herbert, T. P. Kuhar, S. Malone, and A. Koppel. 2009.** Efficacy of insecticides against *Acrosternum hilare* and *Euschistus servus* (Hemiptera: Pentatomidae) in Virginia and North Carolina. *J. Entomol. Sci.* 44: 1–10.

**Kamminga, K. L., A. L. Koppel, D. A. Herbert, Jr., and T. P. Kuhar. 2012.** Biology and management of the green stink bug. *J. Integr. Pest Manag.* 3(3): 8. Available at: <http://jipm.oxfordjournals.org/content/jipm/3/3/C1.full.pdf>; accessed online 10 November 2015 [DOI: <http://dx.doi.org/10.1603/IPM12006>].

**Kuhar, T. P., K. L. Kamminga, J. Whalen, G. P. Dively, G. Brust, C. R. R. Hooks, G. Hamilton, and D. A. Herbert. 2012a.** The pest potential of brown marmorated stink bug on vegetable crops. *Plant Health Progress, Plant Manag. Network.* Available at: [http://offices.ext.vt.edu/rockingham/programs/anr/BMSB\\_veggie.pdf](http://offices.ext.vt.edu/rockingham/programs/anr/BMSB_veggie.pdf); accessed online 10 November 2015 [DOI: 10.1094/PHP-2012-0523-01-BR].

**Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012b.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 1. *Arthropod Manag. Tests* 38: E37. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2012.E37].

**Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012c.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 2. *Arthropod Manag. Tests* 38: E38. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2012.E38].

**Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012d.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 3. *Arthropod Manag. Tests* 38: E39. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2012.E39].

**Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012e.** Evaluation of insecticides for the control of brown marmorated stink bug in tomatoes in Virginia, 2011. *Arthropod Manag. Tests* 37: E72. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2012.E72].

**Kuhar, T., H. Doughty, K. Kamminga, and L. Lilliston. 2012f.** Evaluation of insecticides using a bean dip bioassay for control of brown marmorated stink bug, 2011. *Arthropod Manag. Tests* 37: L1. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2012.L1].

**Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2013a.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 1. *Arthropod Manag. Tests* 38: E39. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2013.E39].

**Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2013b.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 2. *Arthropod Manag. Tests* 38: E40. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI: 10.4182/amt.2013.E40].

**Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2013c.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 3. *Arthropod Manag. Tests* 38: E41. Available at: <http://www.entsoc.org/Pubs/Periodicals/AMT>; accessed online 10 November 2015 [DOI:10.4182/amt.2013.E41].

**Laycock, I., K. M. Lenthall, A. T. Barratt, and J. E. Cresswell. 2012.** Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology* 21: 1937–1945.

**Lee, D-H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. 2013.** Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environ. Entomol.* 42: 627–641.

**LeOra Software. 2002.** Polo plus: probit and logit analysis. Version 1.0. Berkeley, CA.

**Leskey, T. C., G. C. Hamilton, D. J. Biddinger, M. L. Buffington, C. Dieckhoff, G. P. Dively, H. Fraser, T. Gariepy, C. Hedstrom, D. A. Herbert, K. A. Hoelmer, C. R. R. Hooks, D. Inkley, G. Krawczyk, T. P. Kuhar, D.-H. Lee, A. L. Nielsen, D. G. Pfeiffer, C. Rodriguez-Saona, P. W. Shearer, E. Talamas, E. Tomasino, J. Tooker, P. D. Venugopal, J. Whalen, V. Walton, and N. Wiman. 2014.** Datasheet for *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae). *Invasive Species Compendium*, CAB International, Wallingford. Available at: [www.cabi.org/isc/datasheet/27377](http://www.cabi.org/isc/datasheet/27377); accessed online 10 November 2015.

**Leskey, T. C., D.-H. Lee, B. D. Short, and S. E. Wright. 2012a.** Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): Analysis of insecticide lethality. *J. Econ. Entomol.* 105: 1726–1735.

- Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. 2012b.** Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-Atlantic tree fruit orchards in the United States: Case studies of commercial management. *Psyche* 2012, Article 535062, 14 pp. Available at: <http://www.hindawi.com/journals/psyche/2012/535062/>; accessed online 10 November 2015 [DOI:10.1155/2012/535062].
- McPherson, J. E. and R. M. McPherson. 2000.** Stink bugs of economic importance in America North of Mexico. CRC Press, Boca Raton, FL, 253 pp.
- Medal, J., T. Smith, A. Fox, A. S. Cruz, A. Poplin, and A. Hodges. 2012.** Rearing the brown marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae). *Fla. Entomol.* 95: 800–802.
- Nielsen, A. L. and G. C. Hamilton. 2009.** Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. *J. Econ. Entomol.* 102: 1133–1140.
- Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008a.** Developmental rate and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* 37: 348–355.
- Nielsen, A. L., P. W. Shearer, and G. C. Hamilton. 2008b.** Toxicity of insecticides to *Halyomorpha halys* (Hemiptera: Pentatomidae) using glass-vial bioassays. *J. Econ. Entomol.* 101: 1439–1442.
- Prabhaker, N., S. J. Castle, S. E. Naranjo, N. C. Toscano, and J. G. Morse. 2011.** Compatibility of two systemic neonicotinoids, imidacloprid and thiamethoxam, with various natural enemies of agricultural pests. *J. Econ. Entomol.* 104: 773–781.
- SAS Institute, Inc. 2007.** JMP user's guide. SAS Institute Inc., Cary, NC.
- Sokal, R. R. and F. J. Rohlf. 1995.** Biometry, 3rd ed. W. H. Freeman & Company, New York, NY, 419 pp.

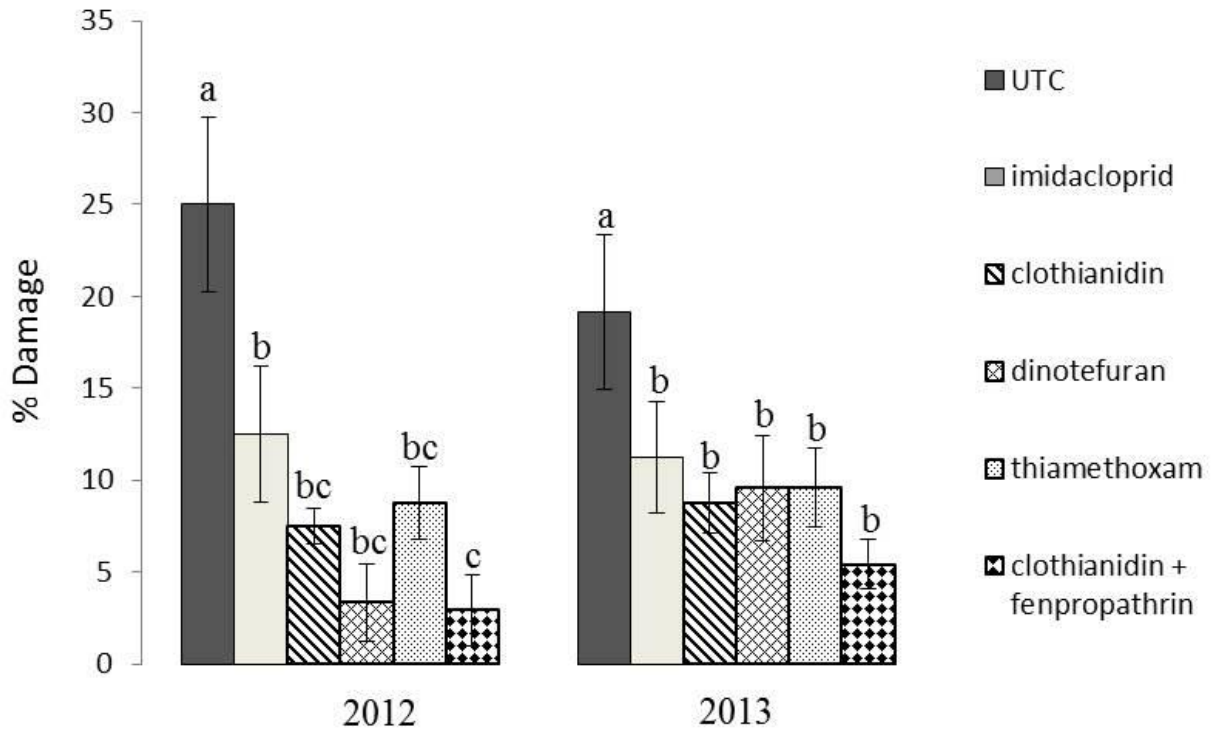
**Snodgrass, G. L., J. J. Adamczyk, Jr., and J. Gore. 2005.** Toxicity of insecticides in a glass vial bioassay to adult brown, green, and southern green stink bugs (Heteroptera: Pentatomidae). *J. Econ. Entomol.* 98: 177–181.

**Stoner, K. A. and B. D. Eitzer. 2012.** Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*). *PLoS One* 7(6): e39114. Available at: <http://www.plosone.org/article/fetchObject.action?uri5info:doi/10.1371/journal.pone.0039114> & representation5PDF; accessed online 10 November 2015 [DOI: 10.1371/journal.pone.0039114].

**Temple, J. H., J. A. Davis, J. Hardke, J. Moore, and B. R. Leonard. 2013.** Susceptibility of southern green stink bug and redbanded stink bug to insecticides in soybean field experiments and laboratory bioassays. *Southwest. Entomol.* 38: 393–406.

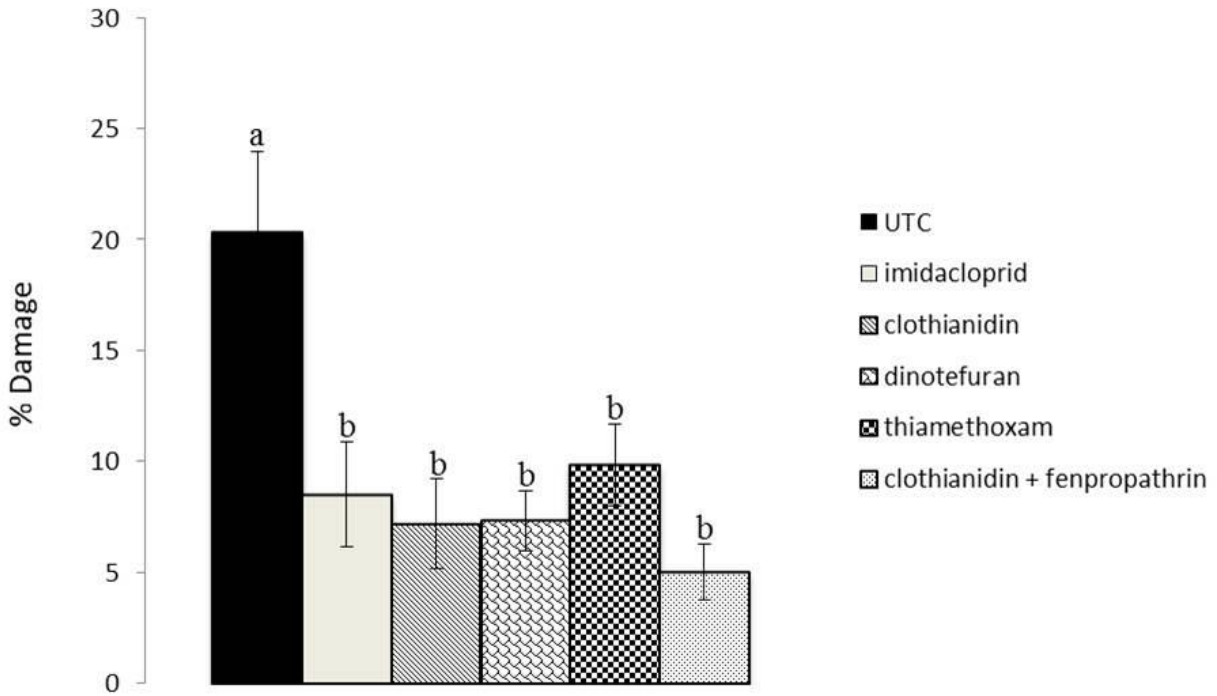
**Wallingford, A. W., T. P. Kuhar, and P. B. Schultz. 2012.** Toxicity and field efficacy of four neonicotinoids on harlequin bug (Hemiptera: Pentatomidae). *Fla. Entomol.* 95: 1123–1126.

**Willrich, M. M., B. R. Leonard, and D. R. Cook. 2003.** Laboratory and field evaluations of insecticide toxicity to stink bugs (Heteroptera: Pentatomidae). *J. Cotton Sci.* 7: 156–163.

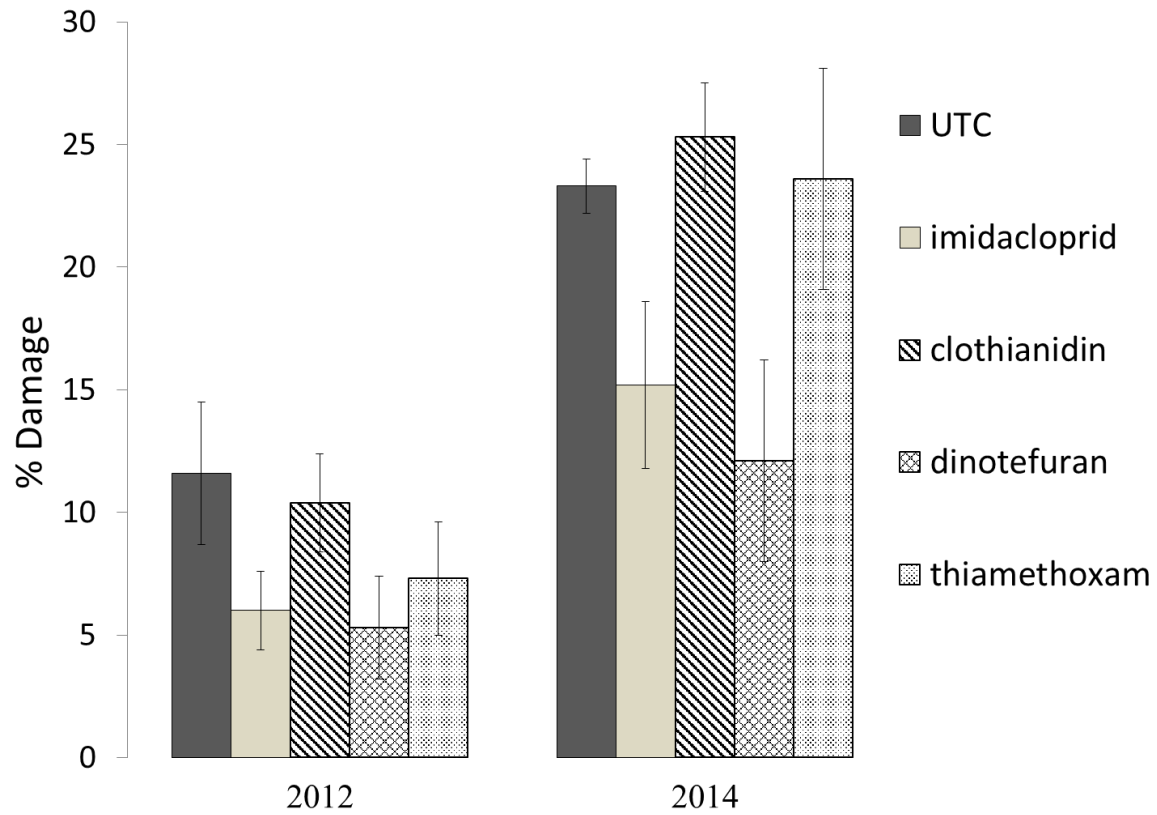


**Figure 6.1.** Mean ( $\pm$ SE) cumulative percentage stink bug damage to pepper fruit from soil-drench insecticide field-efficacy experiments conducted near Blacksburg, Virginia. All neonicotinoids were applied twice as a soil drench 30 days apart. Fenpropathrin was applied as four weekly foliar sprays. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ( $P > 0.05$ ).





**Figure 6.2.** Mean ( $\pm$ SE) cumulative percentage stink bug damage to tomato fruit from soil-drench insecticide field-efficacy experiments conducted near Blacksburg, Virginia, in 2013. All neonicotinoids were applied twice as a soil drench 30 days apart. Fenpropathrin was applied as four weekly foliar sprays. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ( $P > 0.05$ ).



**Figure 6.3.** Mean ( $\pm$ SE) cumulative percentage stink bug damage to tomato fruit resulting from drip-line application of soil insecticides conducted near Mills River, North Carolina. Bars within years with a letter in common are not significantly different according to Fisher's Protected LSD ( $P > 0.05$ ).

**Table 6.1.** Observed LC<sub>50</sub> levels of four neonicotinoid insecticides on *Halyomorpha halys* nymphs 72 h after exposure on treated snap bean plants in an insecticide plant uptake bioassay.

Active Ingredient	LC <sub>50</sub>	n	$\chi^2$	95% FL	Slope $\pm$ SE
Clothianidin	0.077	461	127.91	0.033 – 0.282	1.301 $\pm$ 0.111
Dinotefuran	0.013	400	74.61	0.004 – 0.031	2.160 $\pm$ 0.347
Imidacloprid	0.068	393	46.12	0.032 – 0.198	1.489 $\pm$ 0.141
Thiamethoxam	0.018	539	147.76	0.009 – 0.041	1.634 $\pm$ 0.166

## Chapter 7

### Summary

My overarching goal was to gain a better understanding of several questions surrounding the physiology and management of *H. halys* in urban and agricultural settings. My results show the diverse ways that we can manage the pest, as well as some of the physiological limits of the bug.

Collaborating with researchers in Minnesota, I was able to show that *H. halys* can acclimate to varying levels of climatic conditions, and that there are significant differences between season, sex, and location, and the bug's supercooling points. By identifying these critical points, we have gained an understanding on the *H. halys*' ability to survive the winter in various geographic locations. While Neilsen et al. (2008) noted the effects of high temperatures on the bug's development and fecundity, the sub-lethal effects of low temperatures on *H. halys* have not been well characterized. However, there have been studies showing that overwintering survivorship can increase by 13.5% for every 1°C increase above a mean of 4°C in January and February (Kiritani 2007, Lee et al. 2013). My data are consistent in confirming that overwintering *H. halys* adults can survive temperatures lower than 4°C (Kiritani 2007). Zhu et al. (2012) showed the potential distribution of *H. halys* in areas in the US and Europe based on comparable climates in the bugs native range. My data can be used to update this model by adding the physiological limits of *H. halys* to this model and can potentially add to the precision of this information. Additionally, global climate change can contribute to the further expansion of *H. halys* as we continue to see poleward temperature expansion.

Exposure to heat over time can have deleterious effects on *H. halys* adults. Internal combustion of proteins are one of the primary causes of mortality in insects (Neven 2000); however, there are other factors that can play roles in mortality such as dehydration (Benoit et al. 2009). Developing a better understanding of the exposure to high temperatures has given the pest management industry critical

knowledge to address infestations of *H. halys* in newly manufactured vehicles and other shipping cargo. Utilizing heat treatment as a tactic will help to offer a more eco-friendly alternative to fumigation treatments, while minimizing the chances of unintended exposure to humans. As society moves toward sustainable ideals, this treatment is one that will be more accepted because of its sustainability.

Identifying an indoor trap that was successful at catching overwintering *H. halys* adults was integral in answering homeowner complaints. By doing this, I provided some level of relief for homeowners who seek ways to mitigate the invasion. This discourages the use of insecticides inside of the home, particularly ready to use chemistries that are not known to be labeled for indoor use against stink bugs. Over two years of study, the water pan trap was 14 times more effective than any other trap tested. I must point out that this trap was only tested in the spring months. This trap would most likely not be as effective during the time of invasion because diapausing adults generally are not attracted to light sources at that time (Toyama 2011). Leskey et al. (2015) showed that *H. halys* adults are most attracted to light sources of a white wavelength, which was similar to the light used in my study. It has also been observed that *H. halys* adults tend to orient to areas in homes that are associated with water sources (i.e, kitchen sinks), therefore this trap could be attractive because of the light stimuli and the water source. This could be an interesting avenue to study in the future as questions evolve regarding this type of trap. It is interesting to note that after this study was completed, Strube Stink Bug Traps is no longer in business.

When recommending insecticides for use against a pest, it is important to continue to evaluate the efficacy of the insecticide against the target pest. I was able to determine that several of the insecticides recommended in Extension urban pest management guides did not have acceptable residual efficacy. While there is still more work to be done, we have identified several insecticides that may be

excellent candidates for continued recommendation. The best candidates have either a standalone pyrethroid or a pyrethroid in combination with a neonicotinoid.

I also identified the LC<sub>50</sub> values of various neonicotinoids against *H. halys* nymphs using a systemic application method and found that several neonicotinoid insecticides are effective at significantly reducing stink bug damage in peppers and tomatoes in Virginia and tomatoes in North Carolina.

Applications of neonicotinoid insecticides to the soil by way of drip irrigation are as effective as a foliar application of a pyrethroid insecticide and may cause less detriment to pollinators and natural enemies.

Also non-target effects to other animals, such as birds, could also be limited with this type of an application, as it has been shown that neonicotinoid-treated seed can be detrimental to several bird species consuming newly planted seeds (Goulson 2013). However, there is still a risk associated with birds that forage in areas where there can be high concentrations of neonicotinoids in surface water (Hallmann et al. 2014). This application method could be relevant in other cropping systems where neonicotinoids could be injected into drip irrigation systems where piercing-sucking insects might be problematic, such as peaches or grapes. While I was able to show adequate LC<sub>50</sub> values for the neonicotinoids tested, it would be valuable to continue this work, possibly evaluating pesticide residues within different parts of the plant, to adequately assess where on the plant the bugs were able to ingest a toxic dose of the pesticide.

### References

**Benoit, J. B., G. Lopez-Martinez, N. M. Teets, S. A. Phillips, and D. L. Denlinger. 2009.** Responses of the bed bug, *Cimex lectularius*, to temperature extremes and dehydration: levels of tolerance, rapid cold hardening and expression of heat shock proteins. *Med. Vet. Entomol.* 23: 418 – 425.

**Goulson, D. 2013.** Review: An overview of the environmental risks posed by neonicotinoid insecticides. *J. Appl. Ecol.* 50: 977–987.

- Hallmann, C. A., R. P. B. Foppen, C. A. M. van Turnhout, H. de Kroon, and E. Jongejans. 2014.** Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* 511: 341–343.
- Kiritani, K. 2007.** The impact of global warming and land-use change on the pest status of rice and fruit bugs (Heteroptera) in Japan. *Glob. Chang. Biol.* 13: 1586–1595.
- Lee, D.-H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. 2013.** Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environ. Entomol.* 42: 627-641.
- Leskey, T. C., D-H Lee, D. M. Glenn, and W. R. Morrison. 2015.** Behavioral Responses of the Invasive *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) to Light-Based Stimuli in the Laboratory and Field. *J. Insect Behav.* 28: 674 – 692.
- Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008.** Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* 37: 348–355.
- Neven, L. G. 2000.** Physiological responses of insects to heat. *Postharvest Biol. Tec.* 21: 103 – 111.
- Toyama, M., F. Ihara, and K. Yaginuma. 2011.** Photo-response of the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), and its role in the hiding behavior. *Appl. Entomol. Zool.* 46: 37–40.
- Zhu, G., W. Bu, Y. Gao, and G. Liu. 2012.** Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). *PLoS One.* 7: e31246.