Using Pheromone Lures, Insecticide Netting, and a Novel Food-Grade Repellent to Develop IPM Strategies for the Brown Marmorated Stink Bug

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Scientific Abstract

The invasive brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), has resulted in increased use of insecticides on horticultural crops in the Mid-Atlantic US, which has diminished integrated pest management (IPM) programs that worked well otherwise. The research herein explored the use of three new tools in the development of BMSB management strategies. In one study, a BMSB pheromone lure was placed on insecticide-incorporated mesh netting to be used in an attract-and-kill strategy to protect bell peppers. The pheromone and netting deployed within a pepper field resulted in more BMSB feeding on plants within 6.1 m to the attract-and-kill screen than in further peppers or peppers in the weekly insecticide treatment. In another study, the insecticidal netting used as a row cover reduced stink bug damage to peppers, but also caused significant yield losses, possibly due to lack of light and/or pollination. The BMSB pheromone lure was also utilized in a sticky trap-based action threshold for insecticide application decisions. The trap and lure predicted densities of bugs on pepper plants and the use of an action threshold of 5 bugs per trap per week to trigger an insecticide spray reduced insecticide applications by 50% at one location, however population densities were lower at the other two locations and significance was not found among treatments. Lastly, BMSB is a nuisance pest to homeowners so we tested the exclusion efficacy of repellents on overwintering shelters and found an 8-fold reduction in BMSB that entered shelters treated with geranyl cyclopentanone (apritone). The use of BMSB pheromones paired with insecticide netting for attract-and-kill of BMSB, the development of action thresholds using captures in pheromone-baited sticky cards, and the use of apritone as a repellent are all promising IPM strategies worth refining in future studies.

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General Audience Abstract

The brown marmorated stink bug (BMSB) has become a serious household nuisance and agricultural pest in the US. BMSB feeds on and damages a large variety of crops including, fruits, vegetables, and field crops. Most growers of high value crops have increased their insecticide usage to battle this bug; thus, more sustainable integrated pest management (IPM) approaches need to be established. One main vegetable crop that BMSB damages is bell peppers. One study tested the efficacy of an attract-and-kill approach involving a BMSB pheromone lure paired with insecticide-impregnated mesh netting. When deployed in pepper fields, it was found that BMSB stayed on plants rather than receiving a lethal dose from the netting. Another study tested the netting as a row cover or as a fence between peppers and found that it did little at preventing damage. Furthermore, pepper yields were significantly lower under the row cover treatment. In another study, a clear sticky panel placed on a stake and paired with a BMSB pheromone lure was used to assist in making management decisions based on relative densities of bugs in plots. This can help determine if insecticide applications are needed. Not only is BMSB a pest of agriculture, but it is also a nuisance pest to homeowners when adults seek winter shelter during their fall dispersal period, sometimes entering homes by the thousands. The tactile repellent, geranyl cyclopentanone (apritone), was found to elicit an 8-fold reduction in the number of overwintering BMSB in overwintering shelters.

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Chapter One

Literature review: Biology, ecology, distribution, and pest management of brown marmorated stink bug, *Halyomorpha halys*

Origin and distribution. The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is native to Japan, Korea, China, and Taiwan and was introduced to the United States in Pennsylvania the late 1990s and first properly identified in 2001 (Hoebeke and Carter 2003). This introduction to the Mid-Atlantic region was likely from Beijing, China (Xu et al. 2014). After the initial introduction, the pest continued to spread throughout the Mid-Atlantic states and into Canada (Fogain and Graff 2011, Stop BMSB 2017). Separate introductions into Eastern and Western USA have led to multiple haplotypes in North America (Valentin et al. 2017). The estimated number of females when introduced to the Eastern US was only 2-18, making it likely that genetic bottlenecking occurred and explains the separate haplotype in the eastern US. This haplotype has been documented to potentially become an outbreak pest more easily than other haplotypes around the world (Leskey and Nielsen 2018).

Not only has BMSB established as an invasive pest in the US, but has also been detected throughout Europe and western Asia in Switzerland, Italy, France, Greece, and Hungary, Spain, Serbia, Romania, Austria, Bulgaria, Russia, Abkhazia, and Georgia (Milonas and Partsinevelos 2014, Vetek et al. 2014, Haye et al. 2015, Šeat 2015, Macavei et al. 2015, Rabitsch and Friebe 2015, Dioli et al. 2016, Gapon 2016, Maistrello et al. 2016, Maurel et al. 2016, Simov 2016, Leskey and Nielsen 2018). The first European introduction was to Switzerland in 2004, followed by its spread to surrounding countries. A separate introduction to Europe occurred in Italy in 2012, where high populations developed rapidly, as they did in the Mid-Atlantic US (Maistrello et al. 2016). It was later found that the haplotype that invaded Italy was the same as in the Mid-

Atlantic US, suggesting that the US was likely a point of origin for BMSB in Italy (Valentin et al. 2017). This pest has most recently been introduced into Chile, thus continuing its global spread (Faúndez and Rider 2017).

Biology and Ecology. Where it is currently established, BMSB has one to two generations per year, dependent on temperature and day length in a given region (Nielsen et al. 2008a). Eggs are deposited in masses or clutches containing 20-30 eggs (Hoebeke and Carter 2003) that are typically found on the undersides of leaves. First instars feed on the egg mass and acquire essential endosymbionts that aid in their survival and development as nymphs (Bansal et al. 2014). From the second instar onward, development and survivorship depend on diet (Acebes-Doria et al. 2016a). BMSB requires 538 DD at temperatures 15-30°C to complete development from egg to adult, and females require an additional 148 DD to become reproductively mature (Nielsen et al. 2008a). A dynamic phenological model was used to determine the critical photoperiod for BMSB establishment and potential, finding 13.5 h of daylight to be the most optimal time for development across locations (Nielsen et al. 2016).

BMSB overwinter as adults in natural settings or human-made structures. Common natural overwintering hosts include large diameter standing dead trees (Lee et al. 2014). When overwintering in man-made structures, it can be an extreme nuisance pest to homeowners, gathering in aggregations that can contain thousands of bugs, particularly in attics and crawl spaces (Inkley 2012, Bergh et al. 2017). The adults emerge from overwintering sites between mid-April through late June and seek suitable plant hosts on which to feed and reproduce (Watanabe 1994, Inkley 2012, Nielsen et al. 2016). The first sign of BMSB in orchards begins in mid-April and it is present for the rest of the growing season. In locations where two generations occur, trapping typically detects a large population spike of foraging adults in September before they seek overwintering sites (Leskey et al. 2015a).

Host plants. Leskey and Nielsen (2018) summarized the host range of BMSB, stating that it has more than 170 ornamental and commercial crop hosts in North America, causing damage to fruit and vegetables specifically. Feeding by piercing the host tissue causes wounds on a variety of fruit and vegetable crops that create a discoloration on the fruit surface, which later may turn into softening of the produce (Kuhar et al. 2012, Zobel et al. 2016, Acebes-Doria et al. 2016b). During feeding, digestive enzymes including amylases, proteases, and esterases are secreted into the fruit to break it down, then the contents are consumed. A salivary sheath can be left behind, which has been found to increase plant defensive compounds in tomatoes (Peiffer and Felton 2014).

BMSB Management. Chemical control with broad-spectrum insecticides such as pyrethroids, carbamates, or organophosphates has been the most common tactic for managing stink bugs in agricultural crops for decades. With the introduction of BMSB, the number of insecticide applications from 2010 to 2011 increased 23% in apple orchards and 36% in peach orchards (Leskey et al. 2012a). This increase in insecticides lowered the amount of stink bug injury in apples but is unsustainable for growers as it interferes with integrated pest management (IPM) programs, increases control costs (Leskey et al. 2012a), and has caused frequent secondary pest outbreaks (Kuhar et al. 2017).

Numerous insecticide efficacy tests on BMSB have been conducted in the US (Nielsen et al. 2008b, Leskey et al. 2012b, Kuhar et al. 2012a-d, 2013a-c, 2014). The main insecticide classes evaluated in these bioassay and field trials included organophosphates, pyrethroids, carbamates, and neonicotinoids. Kuhar and Kamminga (2017) provide a review of these insecticide evaluations. Nielsen et al. (2008b) found that pyrethroids were more efficacious than other insecticide classes. Leskey et al. (2012b) later found high initial knockdown for pyrethroids, and all but bifenthrin and fenpropathrin resulted in bug recovery following the initial intoxication. Kuhar et al. (2012a-d) showed that several broad-spectrum insecticides can reduce damage caused by BMSB to peppers; however conventional applications can cause outbreaks of secondary pests such as the green peach aphid, *Myzus persicae,* which cause a build-up of honeydew on the peppers and leaves, impacting crop quality. Similarly, in tree fruit, frequent use of broad-spectrum insecticides can also cause aphid, mite and scale outbreaks, causing higher needs for additional pest management (Croft 1990, Kuhar and Kamminga 2017). Today, depending on the type of crop, select organophosphates including dimethoate, malathion, and methidathion; pyrethroids including etofenprox and bifenthrin; the carbamate methomyl; and the neonicotinoid dinotefuran are the most effective insecticides for the control of BMSB (Kuhar and Kamminga 2017).

Researchers have also explored ways to use insecticides for BMSB other than by foliar application. Aigner et al. (2015) demonstrated that drip chemigation of neonicotinoids can effectively reduce stink bug damage to pepper and tomato. Insecticide applied as border sprays or attract-and-kill approaches have also demonstrated to be highly effective for BMSB management and will be discussed in more detail later (Blaauw et al. 2015, Herbert et al. 2015, Morrison et al. 2016). More recently, long-lasting insecticidal netting (LLIN), which has a pyrethroid incorporated into the netting fibers, has been found to be highly toxic to BMSB adults and nymphs, however its use in agricultural pest management strategies still needs to be researched further (Kuhar et al. 2017).

Sampling/monitoring. Pest monitoring can also help to limit the use of broad-spectrum insecticides in IPM programs. Prior to the BMSB invasion, native stink bugs were monitored in orchards using yellow pyramid traps with a plastic, ventilated jar top (Leskey and Hogmire 2005). Leskey et al. (2012c) found that BMSB was more attracted to black wooden pyramid traps than other colors and Morrison et al. (2015) found that corrugated plastic traps also were effective for monitoring it. Until recently, black pyramid traps have been standard monitoring tools for BMSB. Commonly, the pyramid trap top is baited with a pheromone lure and a killing agent such as a 10% dimethyl 2,2-dichlorovinyl phosphate (DDVP)-infused kill strip. More recently long-lasting insecticidal netting has been used as a killing agent in trap tops, with fewer bugs escaping and season-long effectiveness (Leskey et al. 2015b, Morrison et al. 2015, Kuhar et al. 2017). The large, bulky pyramid trap has not been widely accepted by growers, so recent studies have evaluated pheromone-baited, clear sticky panels deployed on a wooden stake as a new trapping method. A correlation showed similarity between captures from these and pyramid traps, so due to the smaller size and the easier ability to deploy the sticky panel, this trap will likely become the new standard surveillance and monitoring tool for BMSB (Acebes-Doria et al. in press).

The trap is baited with a lure that is composed of a two component stereoisomeric mixture of (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*) 10,11-epoxy-1-bisabolen-3-ol (BMSB pheromone) combined with a synergist, methyl (2*E*,4*E*,6*Z*)-2,4,6-decatrienoate (MDT) (Weber et al. 2014, Weber et al. 2017). Leskey et al. (2015a) found that Murgantiol is attractive to adults and nymphs through the duration of the season and MDT is attractive to nymphs throughout the season, but MDT is only attractive to adults in the late season. The combination of the two components creates a season-long synergism. One pheromone lure commonly used

Ifor research purposes is now commercially available and is deemed the BMSB Dual Lure (Trécé, Inc., Adair, OK, USA).

Due to the variety of host crops in vegetable planting regimes, visual counting has been a popular method to determine pest densities (Zobel et al. 2016; Kuhar et al. 2012a-d, 2013a-c, 2014; Philips et al. 2017). In addition to traps and visual counts, sweep netting can be used to sample for BMSB on some crops. In soybeans, there is a high correlation in BMSB counts between visual counts for two minutes and 15 sweeps with a sweep net (Herbert et al. 2015).

Action thresholds. Sample-based action thresholds can reduce the number of insecticide applications by using a predetermined relative population density to trigger applications, rather than using preventive measures (Zadoks 1985). The pyramid trap and lure established for BMSB has been found to be an effective monitoring tool for deploying action thresholds in apples. Short et al. (2017) deployed traps at the center and exterior row of apple orchards and found an effective action threshold of 10 adults cumulatively in either trap, based on counts taken weekly. If the action threshold was reached, two consecutive alternate row middle insecticide treatments were made. Insecticide applications triggered by this action threshold reduced applications by 40% and resulted in lower injury in apples ≥ 10 m away from the traps, i.e., the greater orchard plot (Short et al. 2017). Similar success was found in soybeans when sweep nets and visual counts were used to determine pest densities (Herbert et al. 2015). Action thresholds for BMSB are currently lacking for vegetable crops.

Attract-and-kill strategies. Many different pheromone types have been used in agriculture to disturb the behaviors of lepidoptera, coleoptera, diptera, and other orders, and can be successful in attract-and-kill approaches (El-Sayed et al. 2009). Lures formulated with the BMSB pheromone and pheromone synergist are highly attractive to BMSB and have the potential to be used in an attract-and-kill method. This method for BMSB was conducted in apple orchards by Morrison et al. (2016). Orchard perimeter trees baited with the BMSB aggregation pheromone and synergist and neighboring trees within a 10.73 m distance were sprayed weekly with insecticides effective against BMSB. It was found that baited traps and trees contained significantly more BMSB than unbaited trees. This reduced the number of trees in the orchard that were treated with insecticides by 93%, while also significantly reducing damage to trees without lures (Morrison et al. 2016). This retention of BMSB on host plants has also been documented in tomato plants containing a trap baited with MDT nearby (Sargent et al. 2014).

Border sprays. It is well known that BMSB commonly exhibits an edge-effect in a variety of cropping regimes, including orchards (Blaauw et al. 2014), row crops (Venugopal et al. 2014, Aigner et al. 2017), vineyards (Basnet et al. 2015), and ornamentals (Venugopal et al. 2015, Weber et al. 2017, Leskey and Nielsen 2018). Border insecticide applications have been incorporated into IPM strategies to reduce the number of insecticide applications to the entire crop field. One timely application to soybean field edges was effective for managing BMSB populations for the entire season (Herbert et al. 2015).

Trap cropping. Trap cropping with highly attractive plants, such as sunflower and sorghum millet, has been investigated for BMSB control in vegetable crops. These have been shown to retain bugs on trap crop field edges for a period of time, although spillover into the cash crop was also noted and the tactic did not significantly reduce stink bug feeding injury to the cash crop, typically bell peppers (Soergel et al. 2015, Mathews et al. 2017). Refinements to that approach are being investigated.

Stink bug IPM for peppers. Bell peppers (*Capsicum annuum*) are an ideal crop to evaluate pest management strategies because bugs can easily be counted on the crop, it produces fruit throughout the summer and fall season, and, in the Mid-Atlantic region, the crop routinely suffers infestations from BMSB (Philips et al. 2017). When left untreated, pepper plots at Kentland Research Farm in Whitethorne, VA sustained between 15 and 65% stink bug feeding damage in studies conducted from 2011 to 2015 (Kuhar et al. 2012a-d, 2013a-c, 2014; Aigner et al. 2015; Morehead et al. 2015). Philips et al. (2017) found no significant difference in infestations of nymphs or feeding injury among sweet bell, sweet banana, and hot chili pepper varieties. Thus, pest management results for one type of pepper would be relevant for other varieties. IPM strategies for BMSB could follow similar management approaches to those found effective in orchards, however must be researched to know how to efficiently employ these approaches.

Objectives. In this thesis, I investigate IPM strategies for BMSB in peppers as well as evaluate a potential repellent for use in urban situations. Specifically, my objectives were:

1. To evaluate the efficacy of the BMSB pheromone lure paired with long-lasting insecticidal netting (LLIN) as an attract-and-kill management strategy in bell peppers.
2. To evaluate the efficacy of deltamethrin-incorporated netting as a hoop row cover and as a vertical fence between plants to protect bell peppers from BMSB.
3. To determine if BMSB captures in pheromone-baited clear sticky panels deployed on a wooden stake can be used to predict infestations on bell peppers, and aid in effectively guiding insecticide spray applications.
4. To evaluate the repellency of three natural compounds, geranylcyclopentanone (Apritone), delta-dodecalactone FCC, and methyl-dihydrojasmonate (Jasmine) on BMSB in laboratory bioassays and field experiments.

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Chapter 2

Efficacy of an attract-and-kill management strategy for *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in peppers using aggregation pheromone lures and long-lasting insecticidal netting

Abstract

Attract-and-kill pest management approaches are commonly used by deploying a combined attractant, such as a pheromone, with a killing agent. Tools such as the BMSB aggregation pheromone lure and deltamethin-incorporated long-lasting insecticidal netting (LLIN) are practical options to test in an attract-and-kill approach against BMSB. This study determined the efficacy of the BMSB aggregation pheromone paired with LLIN in pepper plots to reduce damage in surrounding plants. A strip of pheromone-baited netting was stapled onto wooden stakes and deployed in pepper plots. Plants at different distances away were compared to a weekly insecticide treatment and it was found that peppers located the intermediate away from this attract-and-kill station sustained more stink bug feeding damage than peppers the furthest distance from the station and peppers treated with a weekly insecticide. This follows trends from previous studies where more damage was found in plants closer to the pheromone lure.

Introduction

In the Mid-Atlantic states, the invasive brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), has become a serious pest of various important high-value horticultural crops (Rice et al. 2014, Leskey and Nielsen 2018). Because peppers (*Capsicum annum*) continuously fruit throughout the summer and fall, the crop is regularly attacked by this bug, which appears to find the crop as it moves in the agricultural landscape during the summer (Zobel et al. 2016, Philips et al. 2017). If control measures are not taken, over 50% of harvested pepper fruit may be injured by BMSB in the Mid-Atlantic US annually (Kuhar et al. 2012 a-d; 2013 a-c; 2014; Aigner et al. 2015, Morehead et al. 2015; Zobel et al. 2016; Philips et al. 2017). Weekly applications of foliar insecticides are used to prevent BMSB damage in peppers and other vegetable crops (Kuhar and Kamminga 2017). Researchers are actively seeking alternative management strategies for this invasive bug that are less disruptive to IPM programs, rather than relying on preventive insecticide sprays that can cause secondary pest outbreaks and increase costs to growers.

Tremendous progress has been made in recent years in the identification, synthesis, and production of semiochemical attractants for BMSB. Synthetic derivatives of the BMSB aggregation pheromone, (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*) 10,11-epoxy-1-bisabolen-3-ol, have been shown to be highly attractive to BMSB nymphs and adults (Khrimian et al. 2014). Moreover, when the aggregation pheromone is combined with another stink bug attractant, methyl (2*E*,4*E*,6*Z*)-2,4,6-decatrienoate (MDT), there is a synergistic effect on attraction (Weber et al. 2014), resulting in enhanced season-long attraction of BMSB to traps (Leskey et al. 2015). One commercial lure containing these compounds, the BMSB Dual Lure (Trécé, Inc., Adair, OK, USA), has been shown to attract large numbers of BMSB adults and nymphs to a specific location (or tree) around the farm or orchard. This capability has led to the development of attract-and-kill strategies which have shown success in apples, where only pheromone-baited trees and or areas around those trees are treated with insecticides rather than the entire orchard (Morrison et al. 2016).

Here, I evaluate the efficacy of a BMSB lure paired with an attract-and-kill device made from deltamethrin-incorporated netting (Kuhar et al. 2017) as a management strategy for BMSB in bell peppers.

Materials and Methods

The experiment was conducted at Virginia Tech's Kentland Farm near Blacksburg, VA in 2016. This location has regularly suffered infestations of BMSB on peppers since 2011. Aristotle bell peppers (*Capsicum annum*) were transplanted on 1 June from greenhouse-grown seedlings into raised beds covered with black plastic mulch and drip irrigation. Individual plots were 4 rows x 6.1m long beds with 1.5m unplanted alleys to separate treatments. The experiment had three treatments (untreated control, attract-and-kill, and weekly insecticide applications) arranged in a randomized complete block design with four replicates.

For the attract-and-kill plots, deltamethrin-incorporated netting was obtained from Vestergaard-Frandsen (Lausanne, Switzerland). The 1.8m wide netting contains ~3.85 mg deltamethrin/g fiber and 32-33 holes per cm^2. Kuhar et al. (2017) tested the efficacy of this specific netting on BMSB adults and nymphs in the lab and found that after 10 seconds of exposure to the netting, 40% of adults and 90% of nymphs were found to be moribund or dead.

Netting was cut into 0.3m × 1.8m strips and stretched and fastened between to two wooden tomato stakes, allowing the netting to touch the ground (Fig. 2.1). A white sheet was fastened beneath the insecticide netting using landscape staples and used to count dead BMSB. This experiment utilized a pheromone lure containing 5 mg of the BMSB aggregation pheromone and 50 mg of the MDT pheromone synergist (Trécé Pherocon®), described as the low dose lure in Acebes-Doria et al. (unpublished data). The lure was attached to the top of the netting and herein will be referred to as the A&K treatment (Fig. 2.1). Each A&K screen was placed between two 6.1 m long plots on the outside rows.

For the conventional insecticide treatment, bifenthrin (Brigade 2EC, FMC Corp.) was used as the insecticide at a rate of 11.49 g a.i./ha per application. A solo-backpack sprayer equipped with a 2-nozzle boom that delivered 280 liters/ha was used to apply bifenthrin to the pepper plots. Insecticide applications were initiated at the first sign of fruit (14 July 2016) and continued weekly until one week prior to the final harvest (22 Aug 2016).

Data collection

All data were collected based on proximity of the peppers to the A&K stations. Data from peppers within a 4.6 m radius around each A&K screen were referred to as “Nearest”, data from peppers outside of the 4.6 m radius, but within the 6.1 m plot were referred to as “Intermediate”, and plots >6.1 m away from the station were referred to as “Furthest”. In addition, counts from the insecticide treated peppers were referred to as “Weekly Insecticide”. For all designated plots, counts of BMSB adults and nymphs in pepper plants began on 7 August 2016 and continued weekly until 29 August. Ten random plants per treatment were visually inspected and any stink bugs were counted. Pepper fruit were harvested on 8 Aug, 15 Aug, and 29 Aug and assessed for stink bug feeding. Peppers that were clean of stink bug feeding injury was analyzed for differences among treatments. Data were pooled across harvests for statistical analysis, which consisted of Kruskal-Wallis non-parametric test for significance and Steel Dwass all pairs was used for separation of means.

Results

Weekly counts of BMSB adults ($\chi_2 = 26.61$, df = 3, $P < 0.0001$) and nymphs ($\chi_2 = 26.61$, df = 3, $P < 0.0001$) per 10 plants in each plot were found to be significant between treatments (Fig. 2.2). More BMSB were observed on plants nearest to the A&K station, and fewer bugs were seen as the distance from the station increased. The lowest counts were observed in the weekly insecticide treatment (Fig. 2.2).

The percentage of peppers clean from stink bug feeding was found to be significantly different between treatments ($f = 14.04$, df = 3, $P < 0.0001$) (Fig. 2.3). The peppers in the plot at the intermediate distance (4.6 to 6.1m) from the A&K station sustained more feeding injury than the furthest distance (>6.1 m) and the weekly insecticide treatment (Fig. 2.3).

Discussion

The purpose of this A&K approach was to attract and retain BMSB to the insecticide-treated netting (Kuhar et al. 2017) and away from pepper plants. It seemed, however, that bugs were more apt to be drawn to the plants surrounding them and caused the most severe damage to plants 4.6 to 6.1m from the A&K screen. These distance categories were classified as such because they fit in well with the layout of the pepper rows, however it has been found that the area of arrestment for BMSB in orchards is 2.5 m (Morrison et al. 2016, Leskey and Nielsen 2018). Categorizing treatments into more specific distances will help determine the exact range of attraction and retention in peppers, but the attraction of ≤6.1 m found in this study could lay the foundation for future work deploying the pheromone lure and netting in vegetable and field crops. The (2017) Annual Report for Management of Brown Marmorated Stink Bug in US Specialty Crops discusses the use of a similar design to the one in this experiment, where deltamethrin-incorporated netting was draped over a shepherd’s hook and baited with a BMSB lure. Hundreds of dead bugs were counted on or beneath this trap between early August and early October 2017, with counts increasing throughout August and September. This design may be employed in future attract-and-kill studies in vegetables.

Determining the most optimal location for this pheromone lure and insecticide incorporated netting in different landscapes can be deciphered from past and current research on BMSB landscape ecology. BMSB is an edge effect species, meaning they enter agricultural fields from forested edges and have been recorded to remain on plants within 15 m to the field edge in soybean (Aigner et al. 2017). Border sprays in peach orchards have also proven to successfully reduce injury to fruit within the plot (Blaauw et al. 2015). This being said, field edges are the likely location for deployment of the paired insecticidal netting and BMSB pheromone lure.

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Fig. 2.1. Attract-and-kill netting (0.3 × 1.8 m) placed on the outer row between two 6.1m long pepper plots at Kentland Farm.

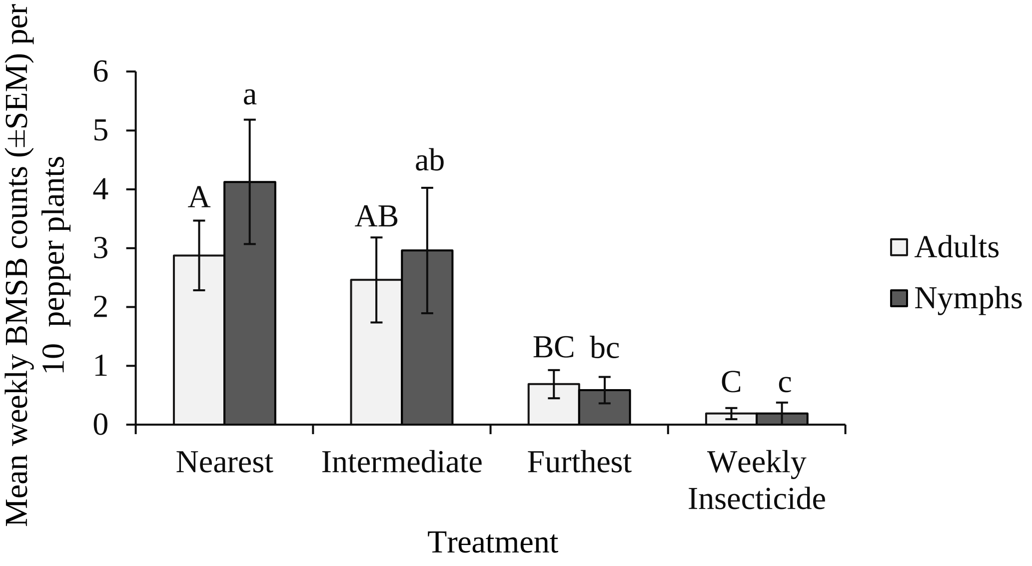

Fig. 2.2. Mean (± SEM) weekly BMSB counts per 10 random pepper plants from a 2016 field experiment in Whitethorne, VA. BMSB and native stink bug adults were pooled for the adult analysis. The Kruskal-wallis non-parametric test for significance was used and Steel-Dwass all pairs was used for separation of means.

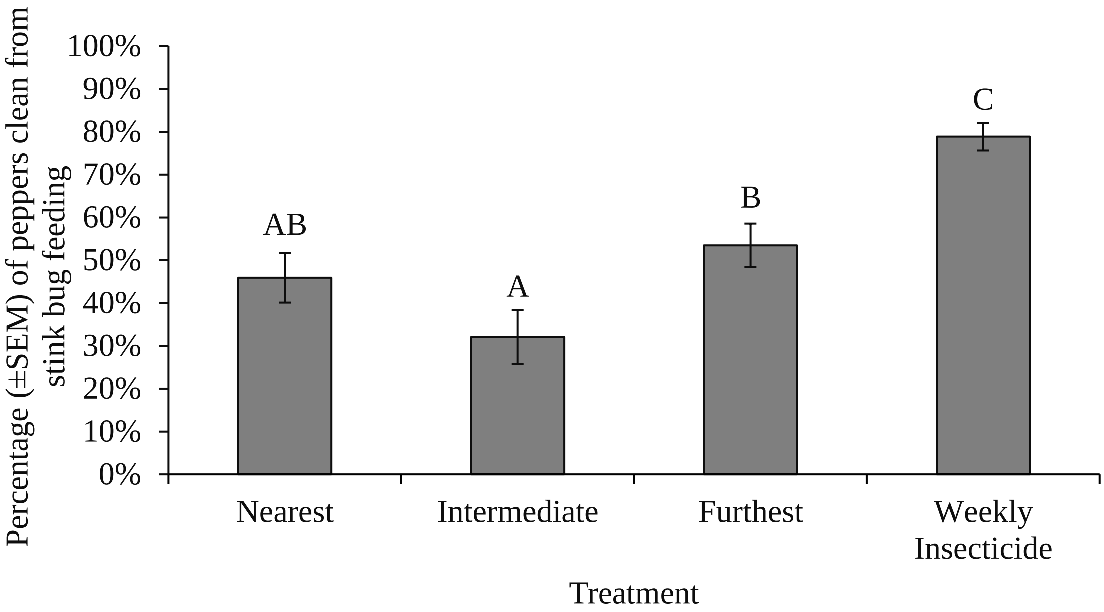

Fig. 2.3. Combined percentage of clean peppers from three pepper harvests in 2016. Significance was found using an ANOVA and means were separated using Tukey's HSD.

Chapter 3

Insecticide-incorporated netting for control of *Halyomorpha halys* in bell peppers

Abstract

Insecticidal netting has been used for decades as bed netting against mosquitoes and other biting insects in regions where arboviruses thrive. The use of this type of netting has been growing in agriculture to reduce the incidence of disease in cattle, as well as to reduce damage to crops from arthropod pests. BMSB lethality on deltamethrin long-lasting insecticidal netting (LLIN) has previously been determined, so this study tested the efficacy of LLIN against BMSB in peppers when deployed as row covers or as a fence within pepper rows. Results found significantly lower yields from plants covered with the row cover, possibly due to the lack of sunlight and pollination. Damage was minimally avoided by the LLIN deployed within the pepper row. Economic damage was not reduced with presence of the LLIN in pepper plots, but feeding injury was found to be lower in the row cover treatment at two locations.

Introduction

Ever since the invasive brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål became a serious pest of fruit and vegetable crops in the Mid-Atlantic United States, beginning around 2010 (Kuhar et al. 2012, Leskey et al. 2012, Rice et al. 2014), entomologists and growers have been exploring strategies and tools to manage it effectively and minimize crop damage. Insecticide-incorporated netting, which has been used primarily in the medical and veterinary field to control biting flies and the diseases that they transmit, is now being explored as a crop protection tool (Dáder et al. 2015, Wallingford et al. 2018). Kuhar et al. (2017) found that commercially-available polyethylene netting infused with deltamethrin at ~3.85mg a.i./g fiber caused 90% of BMSB nymphs and 40% of adults to become moribund or dead after a 10 second exposure. Herein, we evaluate the use of the LLIN listed above as a hoop row cover and as a vertical fence between plants to protect bell peppers (*Capsicum annuum*) from BMSB.

Materials and Methods

Field plots

The experiment was conducted in 2017 at three locations in southwest VA: 1) Virginia Tech's Kentland Farm in Whitethorne; 2) Virginia Tech's Dining Services Farm, also located in Whitethorne; and Garrett Farms near Glenvar, VA. At each site, Aristotle bell pepper were grown in a greenhouse and transplanted on 8, 12, and 15 June at Kentland, Dining Services, and Garrett Farms, respectively. Peppers were planted in raised beds covered in black plastic mulch with drip irrigation. Each experiment was arranged in a randomized complete block with four replications and individual plots were 1 row bed × 6.1m long and had a 1.5m unplanted alley between replications.

Treatments

The experiment had four treatments: 1) untreated control; 2) conventional weekly insecticide applications; 3) insecticide netting as a row cover; and 4) insecticide netting as a vertical fence between plants. The weekly insecticide treatments were foliar spray applications of either bifenthrin (Brigade 2EC, FMC Corp.) at 11.49g a.i./ha at Kentland and Garrett Farms, or the organic insecticide, Azera (MGK, Minneapolis, Minnesota), a mixture of 1.2% azadirachtins + 1.4% pyrethrins, applied at a rate of 28.0 + 30.0 g a.i./ha for azadirachtins and pyrethrins, respectively. A solo-backpack sprayer equipped with a 2-nozzle boom that delivered 280 liters/ha was used to apply insecticides. Insecticide sprays were initiated one week following placement of netting treatments and were repeated weekly until approximately one week prior to the final pepper harvest (Table 3.1). The insecticide netting deployed in this study was described by Kuhar et al. (2017) and provided by Vestergaard-Frandsen (Lausanne, Switzerland). The black polyethylene netting is known by different commercial names such as Zero-fly™ and D-Terrence™, comes packaged in rolls 1.8m wide with a mesh size of 32-33 holes per cm², and contains ~3.85mg a.i./g fiber (Kuhar et al. 2017). Mesh netting was cut into 6.1m long strips to deploy in the field. The row cover treatment consisted of two 6.1m strips of netting fastened together length-wise, creating a 6.1m × 3.6m sheet of screen. Galvanized steel wire (14-gauge) was cut into 2m long strips to be used as row cover hoops. Four hoops were used per 6.1m long plot, and the 6.1m × 3.6m long strip of screen was attached to the hoops using zip ties. The second treatment involving the insecticidal netting consisted of one 6.1m long strip of screen running vertically down the center of a staggered bed of bell peppers. These were stapled to wooden tomato stakes and continued down the entire plot length. The screens were placed in the plots at the first sign of flowering, which was on 3 June at Garrett Farms and 6 June at Kentland Farm and Dining Services.

Data Collection and Analysis

Each week following insecticide applications, 10 random pepper plants per plot were visually sampled and the number of live stink bug adults and nymphs recorded. Counts continued for a duration of six weeks during July and August and were pooled for data analysis. Beneficial arthropods were also visually counted in 10 plants per plot during this time to determine the effect of the insecticidal netting of the beneficial community.

Three pepper harvests occurred at each location throughout the season to assess for the presence of stink bug feeding (Table 3.1). At each harvest, a subsample of 25 fruit per plot were inspected for stink bug feeding wounds, which appeared as characteristic white dimpling on the fruit (Kuhar et al. 2015). The percentage of peppers clean of stink bug feeding were analyzed for significance among treatments. All ripe peppers remaining on plants were counted at the end of the season to assess total crop yield (cumulative number of peppers per plot).

Because stink bug counts and feeding assessment data were not normally distributed after transformation, they were analyzed using Kruskal-Wallis test for significance and means were separated with Steel Dwass all pairs at the 0.05 level of significance. Final pepper yields were analyzed using ANOVA and means were separated using Tukey's HSD at a 0.05 significance level.

Results

Stink bug counts on pepper plants were highest at Garrett Farms but relatively low in general at all locations. All stink bug counts were combined when analyzed due to the low counts. BMSB consisted of 83% of stink bugs counted, and the native brown stink bug (*Euschistus servus*) consisted of the remaining 17% of stink bugs. There was a significant treatment effect on stink bug densities at Garrett Farms ($\chi^2 = 9.64$; df = 3; $P = 0.0219$), but no significance was found at Kentland Farm ($\chi^2 = 2.03$, df = 3, $P = 0.565$) or Dining Services ($\chi^2 = 1.08$, df = 3, $P = 0.782$ (Fig. 3.1). Significantly more stink bugs were counted in the control treatment compared with the row cover treatment at Garrett Farms, and the vertical fence treatment and weekly insecticide treatments did not differ from any other treatment (Fig. 3.1).

Dead beneficial arthropods observed on pepper plants or on the ground directly below plants included coccinellids (60-80%), arachnids (10-30%), and other generalist predators (5-10%), such as species from the families Asilidae, Carabidae, Nabidae, Sarcophagidae, Pentatomidae, Anthocoridae, Reduviidae, and Syrphidae. For simplicity, counts of all dead beneficial arthropods were combined for analysis, and there was a highly significant treatment effect on this variable at three sites, with higher counts of dead beneficial arthropods in the vertical fence and row cover treatments than in the control plots across all locations (Fig. 3.2). Garrett Farms ($\chi_2 = 47.61$, df, 3, $P = <0.0001$) and Dining Services ($\chi_2 = 43$, df = 3, $P = <0.0001$) followed a significantly similar treatment trend with both LLIN treatments having higher counts of dead beneficals; however, at Kentland Farm, the LLIN treatments did not differ from the control treatment ($\chi_2 = 13.89$, df = 3, $P = 0.0031$) (Fig. 3.2).

The only location with significant differences in the percentage of peppers clean of stink bug feeding among treatments was Dining Services ($\chi_2 = 11.28$, df = 3, $P = 0.0103$) (Fig. 3.3). At this location, the row cover treatment had a significantly higher percentage of clean peppers than in the control. Significant differences were not found in the percentage of clean peppers among treatments at Garrett Farms ($\chi_2 = 4.38$, df = 3, $P = 0.223$) or Kentland Farm ($\chi_2 = 7.11$, df = 3, $P = 0.068$) (Fig. 3.3).

Final yield counts of peppers remaining on plants at the end of the season found that there was a significant difference among treatments at Kentland Farm ($f = 12.67$, df = 3, $P = 0.0005$) and Dining Services ($f = 7.28$, df = 3, $P = 0.0049$) (Fig. 3.4). The row cover treatment at these two locations had significantly fewer total peppers produced than all other treatments. Garrett Farms had a low count across all treatments and did not show a significant response ($f = 2.64$, df = 3, $P = 0.0972$), however still showed a trend of fewer peppers counted in the row cover treatment (Fig. 3.4).

Discussion

Alternative pest management strategies are needed for stink bug control in agricultural crops such as peppers, which in the Mid-Atlantic US, are attacked regularly by BMSB and can suffer significant feeding damage to fruit when not controlled (Kuhar et al. 2012a-d, 2013a-c, 2014; Philips et al. 2017). The native brown stink bug (*Euschistus* servus) also appeared in pepper plots in this study. Damage seen on peppers between BMSB and the brown stink bug is similar which is why those counts were included in these data (Day and Kuhar 2009). Although weekly applications of broad-spectrum insecticides such as pyrethroids can effectively control BMSB, they are also disruptive to IPM programs and can lead to secondary pest outbreaks (Kuhar and Kamminga 2017). Our study showed that deltamethrin-incorporated screening could potentially be used as a pest management tool, but results were highly variable. At two of three locations, there was a significant reduction in stink bug feeding injury to fruit with row covers made from the screening. Bell peppers are self-pollinating but can be supplemented by pollinators, so peppers covered with the insecticidal netting may not have received as much pollinator supplementation than other treatments, explaining the lower yields (Franceschetti 1971, Pereira 2015). Prior attempts to exclude BMSB and other pests from bell peppers using black screens as row covers have found similar results to this study, where shading caused a decrease in marketable yield (Dobson et al. 2016, Jolliffe and Gaye 1995). The insecticidal netting also had a negative effect on beneficial arthropods at all locations, compared with the control treatment. Visual counting cannot fully describe the intricacies of beneficial arthropods in these pepper plots, but these data do show that the vertical fence within the bell pepper rows resulted in a high number of deaths in the family Coccinellidae. This may be because coccinellids are easy to see, but also helps determine that other species are affected by this netting. Applying plots with pyrethroids also kill many beneficial insects, but sampling methods in this study did not show the effects of the broad-spectrum insecticide on other insects.

Insecticidal netting has been used against biting mosquitoes in bed netting for numerous years where arboviruses are prevalent (Hill et al. 2006). Not only have there been successes in reducing mosquito-transmitted diseases in humans, but insecticidal netting has also been proven successful in reducing diseases in cattle (Bauer et al. 2006). Martin et al. (2006) found that using insecticidal netting on cabbage also deterred a variety of pests in Africa, however they removed the netting during the day when pest movement was low and to reduce overheating. The negative effect the row cover had on yield, as well as the netting touching the pepper plants during this study are a few reasons to not deploy this technique on peppers to combat BMSB injury. Current research is leaning towards using the netting in an attract-and-kill approach.

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Table 3.1. Location, insecticide applications by treatment, and harvest dates for each site.

Location	GPS Coordinates	Insecticide Applications	Harvest Dates
Garrett Farms	37°16'06.9"N 80°08'01.4"W	7/11, 7/18, 7/25, 8/2, 8/9, 8/16, 8/23, 8/31	8/2, 8/23, 9/7
Kentland Farm	37°11'59.8"N 80°33'53.9"W	7/14, 7/20, 7/26, 8/4, 8/10, 8/16, 8/22, 8/30	8/1, 8/14, 9/5
Dining Services	37°12'11.5"N 80°33'47.7"W	7/14, 7/20, 7/26, 8/4, 8/10, 8/16, 8/22, 8/30	8/14, 8/29, 9/12

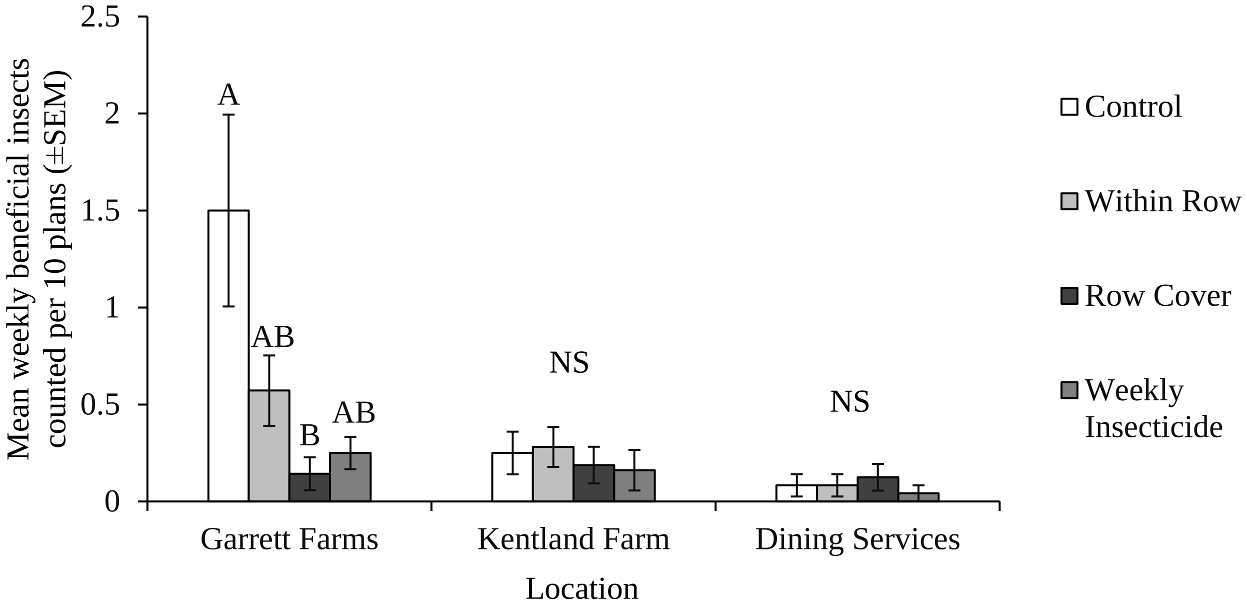

Fig. 3.1. Mean live stink bug count per 10 plants analyzed by treatment at each location. Bars with different letters are significantly different from each other (Steel Dwass, α = 0.05). NS represents data that are not significant.

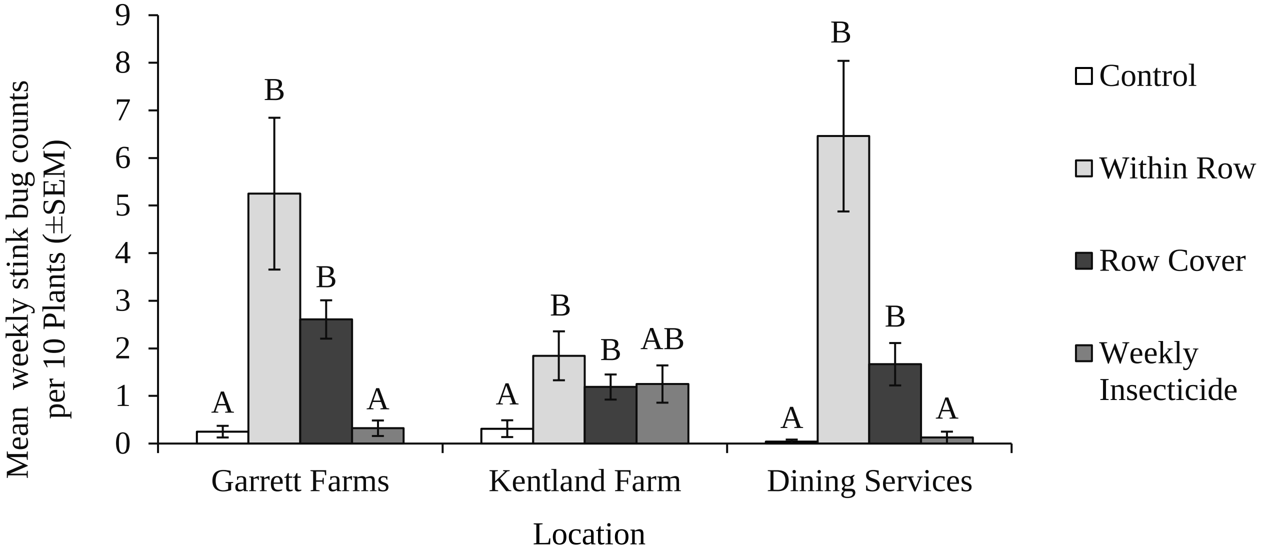

Fig. 3.2. Combined mean dead beneficial arthropod count per 10 plants analyzed by treatment at each location. Different letters represent significant difference between treatments (Steel-Dwass, α = 0.05).

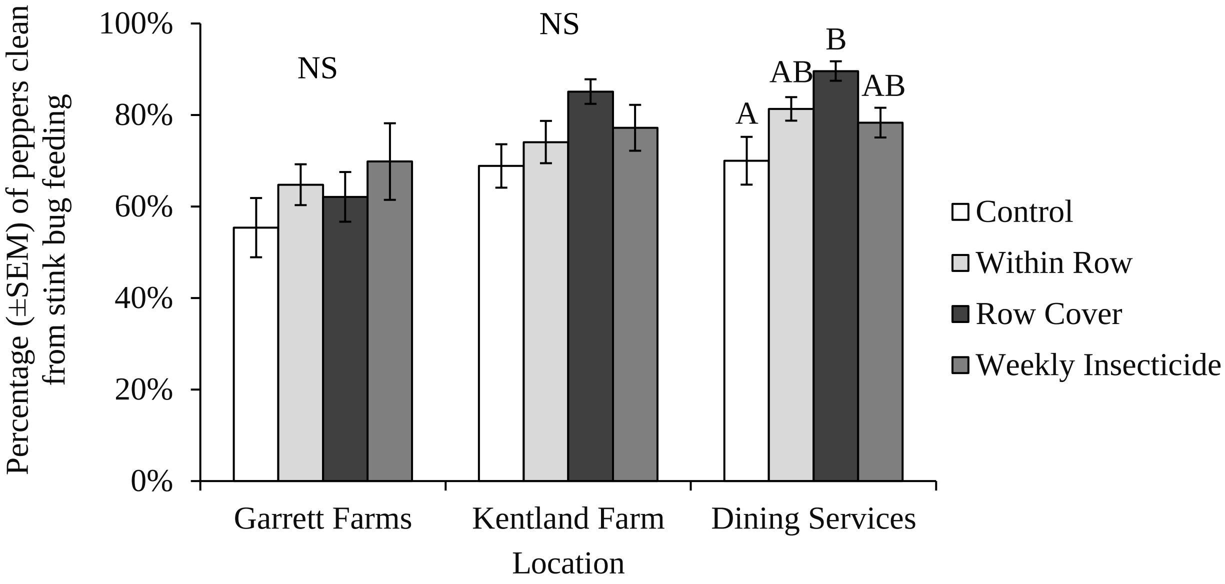

Fig. 3.3. Mean percentage of peppers clean of stink bug feeding (±SEM) analyzed by treatment at each location. Different letters represent significant difference between treatments (Steel-Dwass, α = 0.05).

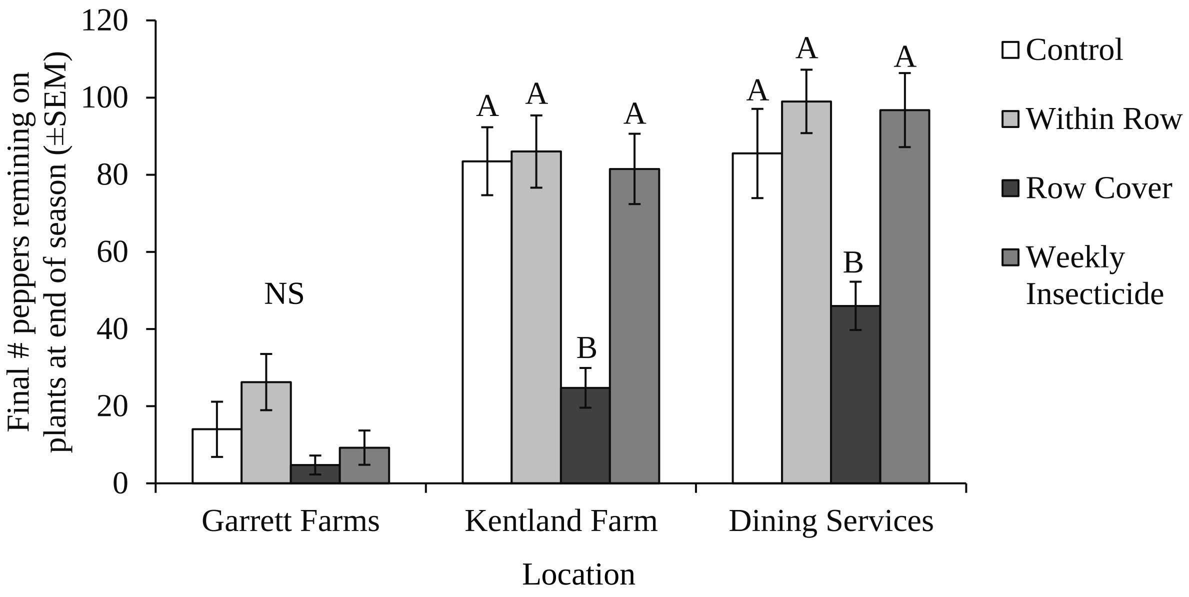

Fig. 3.4. Mean number of pepper fruit remaining on plants at the end of the season. Bars with shared letters are not significantly different from each other (Tukeys's HSD, α = 0.05). NS represents data that are not significant.

Chapter 4

Using pheromone-baited sticky panels for *Halyomorpha halys* (Hemiptera: Pentatomidae) pest management decision-making in peppers

Abstract

The BMSB aggregation pheromone and pheromone synergist is highly attractive to adults and nymphs throughout the season and can be used in numerous IPM approaches. BMSB aggregation pheromone lures have been deployed in monitoring programs to determine the presence, relative abundance, and spread of the pest. More recently, the lure paired with a standard black pyramid trap was found to be an efficient monitoring tool to determine when trap-based insecticide applications should occur. This study evaluated whether captures in pheromone-baited clear sticky panel traps deployed in peppers can help predict damage-causing BMSB densities, which could then be used to determine proper timing of insecticide applications, rather than relying on preventive sprays. There was a high correlation between weekly BMSB counts in peppers and weekly captures in traps. The percentage of clean peppers was found to be equivalent between an action threshold and the weekly insecticide treatment at one out of three locations. The two other locations had overall low feeding injury and stink bug counts.

Introduction

Brown marmorated stink bug (BMSB), *Halyomorpha halys*, is an invasive species from Asia, specifically Japan, Korea, China, and Taiwan, and has become a serious pest of a number of important agricultural crops in the United States (Rice et al. 2014, Leskey and Nielsen 2018). In the Mid-Atlantic US, bell pepper (*Capsicum annuum*) is a valuable vegetable crop that can suffer severe damage from feeding by BMSB nymphs and adults (Kuhar et al. 2012e, Philips et al. 2017). Although weekly insecticide applications of either pyrethroids, neonicotinoids, an organophosphate (acephate), or a carbamate (methomyl) can significantly reduce stink bug feeding injury to peppers (Kuhar et al. 2012a-d, 2013a-c, 2014; Kuhar and Kamminga 2017), this control strategy is not compatible with integrated pest management (IPM) and can result in destruction of natural enemies and secondary pest outbreaks such as green peach aphid, *Myzus persicae* (Hemiptera: Aphidae) (Kuhar et al. 2012a-d).

BMSB pest pressures can change throughout the season which can be measured and incorporated into pest management decision making, leading to more efficient integrated pest management regimes and significantly reduced amounts of management inputs (Leskey et al. 2012a, Morrison et al. 2015). There has been tremendous progress in the US in recent years to develop pheromone-based monitoring tools for BMSB (Weber et al. 2017). Methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT), the aggregation pheromone of the brown-winged green bug (*Plautia stali*), was shown to have cross-attraction to BMSB, particularly later in the season (Funayama 2008, Leskey et al. 2012b). More recently, stereoisomeric libraries were discovered for BMSB, identifying the aggregation pheromone as the two stereoisomers (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*) 10,11-epoxy-1-bisabolen-3-ol (Khrimian et al. 2014). Weber et al. (2014) found that when the aggregation pheromone was combined with MDT, there was a synergistic effect on attraction, resulting in enhanced season-long captures of BMSB in traps (Leskey et al. 2015). One commercially available lure has been deemed the BMSB Dual Lure (Trécé Inc., Adair, OK, USA).

There has been success in establishing action thresholds for BMSB in apple orchards using the standard black pyramid trap paired with the pheromone lure (Short et al. 2017). When cumulative captures in traps reached a predetermined action threshold, insecticide applications were triggered. This ultimately reduced insecticide usage by 40% while maintaining similar damage to an aggressive insecticide program involving weekly insecticide applications (Short et al. 2017). Recently, it was found that captures on pheromone-baited 15.2 × 30.5 cm clear STKY™ Dual Panel Adhesive Trap (Trécé Inc., Adair, OK, USA) deployed atop a wooden stake were correlated with captures in pyramid traps (Acebes-Doria et al. in press). Herein, we evaluated whether the clear sticky panel baited with the BMSB Dual Lure could be used to assess BMSB infestations on bell peppers, and aid in effectively guiding insecticide spray applications.

Materials and Methods

Field plots

In 2017, plots of ‘Aristotle’ bell peppers (*Capsicum annuum*) were established at three sites in southwest, VA: two were spatially isolated plots at Kentland Farm in Whitethorne, VA and the third was at Garrett Farms in Glenvar, VA. At the two Kentland Farm sites, transplants were set in the field on 9 June, with one research plot grown on raised beds on 1.9 m row centers covered with black polyethylene mulch and the other plot grown on bare ground cultivated and harrowed prior to planting with rows spaced 1m apart. At Garrett Farms, transplants were set on raised beds covered with black polyethylene mulch on 15 June. At all three locations, pre-plant fertilizer (10:10:10 P:N:K) was applied, drip irrigation was run as needed and plants were spaced 0.3m apart within rows. At each location, treatment plots (about 1m wide and 6.1m long) were arranged in a Latin Square design with four replications per location. Each individual treatment plot contained 20 pepper plants and a 1.5 m unplanted alley was left between each replicate. The entire research plot was four rows wide, containing each of the four treatments described below.

To sample the relative density of BMSB, the clear sticky panel was stapled to the top of a wooden stake inserted into the ground and was about 1.5 m above the ground. The BMSB Dual lure was tied to a piece of string that was attached to the top wooden stake with the sticky panel and the trap and lure was placed in the center of each research plot. These were placed in each plot at the beginning of July, prior to the first formation of fruit. Traps were checked for BMSB adult and nymph captures and changed weekly before BMSB counts were taken on pepper plants. The pheromone lure has a field life of 12 weeks, so did not have to be changed for the duration of this study. Captures of adults and nymphs were combined to provide the total BMSB captures per week, to determine if a threshold-based spray was needed.

Treatments

The experiment had four treatments: 1) untreated control, 2) weekly insecticide applications, 3) insecticide application only if the number of BMSB captured per week exceeded 5 bugs, and 4) insecticide application only if the number of BMSB captured per week exceeded 10 bugs. The sticky panel was replaced every week after counts were taken. Bifenthrin (Brigade 2EC, FMC Corp.) was used as the insecticide treatment, at a rate of 11.49 g a.i./ha (4 fl. Oz./ acre). A solo-backpack sprayer equipped with a 2-nozzle boom that delivered 280 liters/ha, was used to apply bifenthrin to the pepper plots. Conventional “Weekly Insecticide” treatments were initiated at the first sign of fruit and concluded one week prior to final harvest (Table 4.1).

Data Collection and Analysis

Once per week, 10 pepper plants per treatment replication were randomly sampled for the number of BMSB adults and nymphs present. Peppers were harvested four times during the season and assessed for stink bug injury (Table 4.1). Approximately 25 harvestable-sized peppers were collected from each treatment plot and assessed for feeding wounds. The percentage of peppers clean of stink bug feeding were compared among treatments. A regression analysis was used to compare BMSB trap capture with number of BMSB observed on pepper plants in the untreated control plots. Data from BMSB count on peppers and feeding wound percentages were both not normal and were analyzed using the non-parametric Kruskal-Wallis, with mean separation by Steel-Dwass all pairs.

Results

At Garrett Farms, sprays triggered by the 5-bug per trap threshold resulted in 50% reduction of insecticide use compared with the conventional treatment. Both Kentland Farm plots reached the 5-bug per trap threshold only once, and neither Kentland Farm plots had a trap catch over 10-bug per trap throughout the 2017 field season (Table 4.1). After averaging BMSB counts in the control treatment across all research plots, a correlation was found ($R^2 = 0.5197$, df = 3, $P < 0.0001$) between weekly BMSB counts on plants and weekly trap captures (Fig. 4.1).

Significance was found at Garrett Farms on the number of bugs counted in different treatments ($\chi_2 = 11.4$, df = 3, $P = 0.0098$), with mean separation showing the lower counts in the weekly insecticide treatment than the control. Both threshold treatments showed no differences from the control or weekly insecticide (Fig. 4.2). Treatments at Garrett Farms showed a lower bug count in the treatments that were sprayed fewer times. There was not a significant effect of treatment at Kentland Farm 1 ($\chi_2 = 3.31$, df = 3, $P = 0.346$) or and Kentland Farm 2 ($\chi_2 = 2.86$, df = 3, $P = 0.414$). The spike in the number of BMSB in plants in the 10 BMSB per trap treatment at Kentland Farm 1 is the result of a high count one week, thus not differing from the other treatments. There was a four-fold difference in the average number of BMSB between control plots at Garrett Farms and Kentland Farm 1, and even fewer BMSB were recorded from Kentland Farm 2.

Significant differences were found among treatments in the percentage of clean peppers at Kentland Farm 1 ($\chi_2 = 9.92$, df = 3, $P = 0.0193$) and Garrett Farms ($\chi_2 = 14.93$, df = 3, $P = 0.019$) (Fig. 4.3). At Kentland Farm 1, the only differences seen were between the weekly insecticide treatment and the control. At Garrett Farms, there was a significantly higher percentage of clean peppers found in the 5 BMSB per trap and the weekly insecticide treatments than the control. There were no significant differences between treatments in the percentage of clean peppers at Kentland Farm 2 ($\chi_2 = 3.82$, df = 3, $P = 0.282$) (Fig. 4.3).

Discussion

Developing IPM strategies to reduce the amount of pesticide applications for invasive pests like BMSB is crucial for the sustainability of agriculture. The correlation between weekly visual counts and trap captures suggests that the sticky panel and lure combination is an accurate tool for measuring the density of BMSB in peppers throughout the season. More needs to be learned about the movement of this pest throughout vegetable farms. Zobel et al. (2016) found the peak times of BMSB in a variety of vegetables throughout the growing season and found in bell peppers, the highest abundance of BMSB adults were seen in late July, then again in mid- to late August. Our experiment found similar peak abundance times, with all threshold treatments triggered in August. BMSB counts on pepper plants were typically taken in the early afternoon, which has been found to be the most active time for the bugs in peach orchards in a 24 h period (Cambridge 2016). Conducting a similar study in vegetable farms would be optimal for determining the most optimal time for visual sampling.

The insecticide spray triggered by the 5 BMSB per trap threshold showed comparable damage to the weekly insecticide treatment at Garrett Farms, reducing sprays by 50%. The threshold treatment only showed to be different than the control at one out three locations. The two locations where differences were not seen in the threshold treatments may be due to the lower population density. This approach needs to be researched more in areas of high BMSB densities to determine if it can be considered an appropriate IPM approach in peppers. Philips et al. (2017) found that there was no host preference for BMSB between multiple varieties of pepper, so this IPM approach could potentially be applied to multiple pepper varieties. The overall goal is to lower the cost to the growers, reduce impacts on natural enemy populations, and decrease the likelihood of secondary pest outbreaks (Leskey et al. 2012b, Kuhar et al. 2017).

Monitoring pest densities can be accomplished by efficient trapping, visual sampling, sweep net sampling, or beat sheet sampling and the best method depends on the crop type being sampled. Monitoring is also useful to evaluate the spread of invasive species like gypsy moth (*Lymantria dispar*) and emerald ash borer (*Agrilus planipennis)* (Gage et al. 1990, Francese et al. 2008). Knowing the peak densities and how they relate to economic damage a key factor to improving IPM decision-making (Binns and Nyrop 1992). Success using action thresholds has been found for multiple pests in different crops like cucumber beetles (*Acalymma vittatum*) in cantaloupe using visual sampling or stink bugs in cotton monitored with ground cloths (Brust and Foster 1999 Greene et al. 2001). Successful action thresholds have been developed in soybean using visual and beat sheet sampling, finding that one timely spray significantly reduced crop damage and insecticide usage (Herbert et al. 2015).

The standard BMSB standard black pyramid trap was used in Short et al. (2017), where traps were placed in the center and exterior of the plot. It was found that when plots were treated when either trap reached 10 BMSB, damage was equivalent to apples treated weekly. Damage was not equivalent between the threshold treatment and plot always sprayed in apples surrounding the exterior trap due to the edge effect of BMSB in agricultural fields (Blaauw et al. 2014). The sticky panel and lure were placed in the center of the pepper plots in this study to evenly disperse BMSB among treatments in this Latin-square design plot. Data show that there were no differences in bug counts with distance from the trap and lure. The adoption of the clear sticky panel is an effective tool for growers and has the potential to be implemented into various IPM regimes in agriculture if studied thoroughly (Acebes-Doria et al. in press).

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Table 4.1. Location, insecticide applications by treatment, and pepper harvest dates.

	Spray Dates			
Field (GPS Coordinates)	**Weekly Insecticide**	**5-bug per trap**	**10-bug per trap**	**Harvest Dates**
Garrett Farms 37°16'08.5"N 80°08'02.5"W	7/11, 7/18, 7/25, 8/2, 8/9, 8/16, 8/23, 8/31	8/2, 8/9, 8/16, 8/23	8/9, 8/23	8/2, 8/16, 8/31, 9/7
Kentland Farm 1 37°11'58.2"N 80°33'58.1"W	7/14, 7/20, 7/26, 8/4, 8/10, 8/16, 8/22, 8/30	8/16	-	8/4, 8/16, 8/30, 9/8
Kentland Farm 2 37°12'01.7"N 80°33'55.0"W	7/14, 7/20, 7/26, 8/4, 8/10, 8/16, 8/22, 8/30	8/16	-	8/4, 8/18, 8/31, 9/11

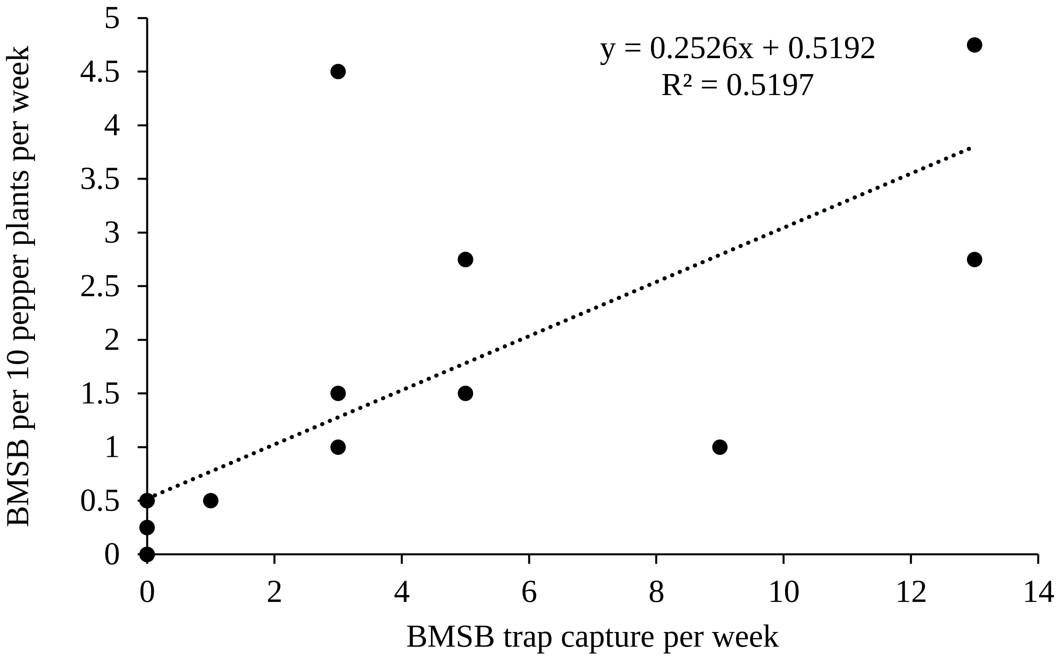

Fig. 4.1. Regression analysis of the relationship between weekly BMSB counts on pepper plants in control plots and weekly captures in pheromone-baited sticky panels across three field sites in Virginia, 2017.

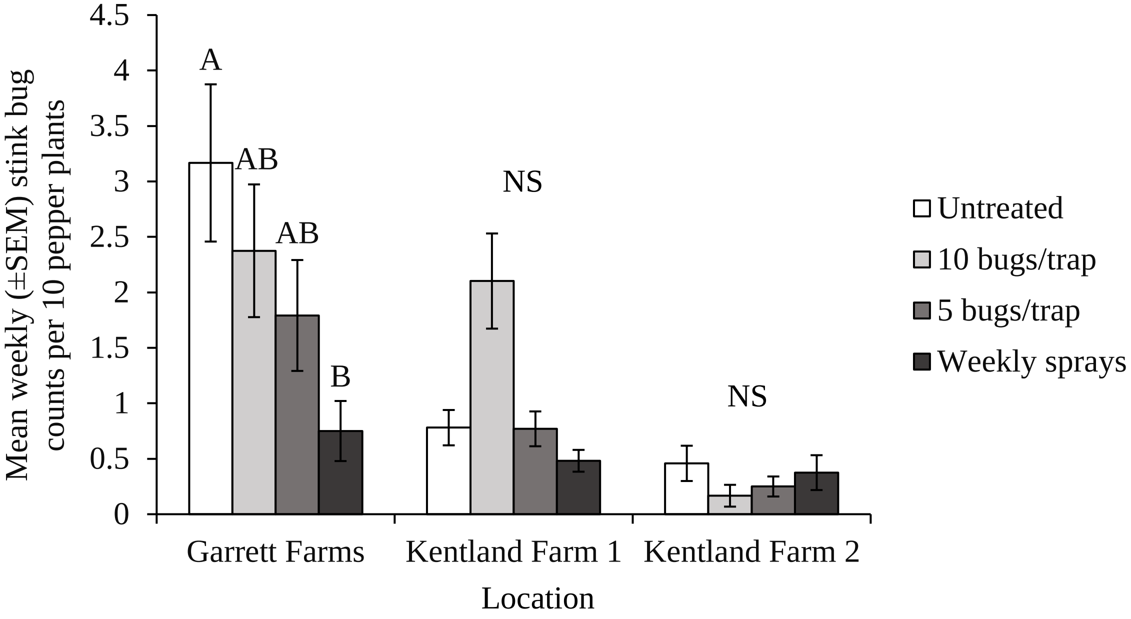

Fig. 4.2. Mean weekly visual count (±SEM) of BMSB per 10 pepper plants across 4 management regimens at three field sites in Virginia, 2017.

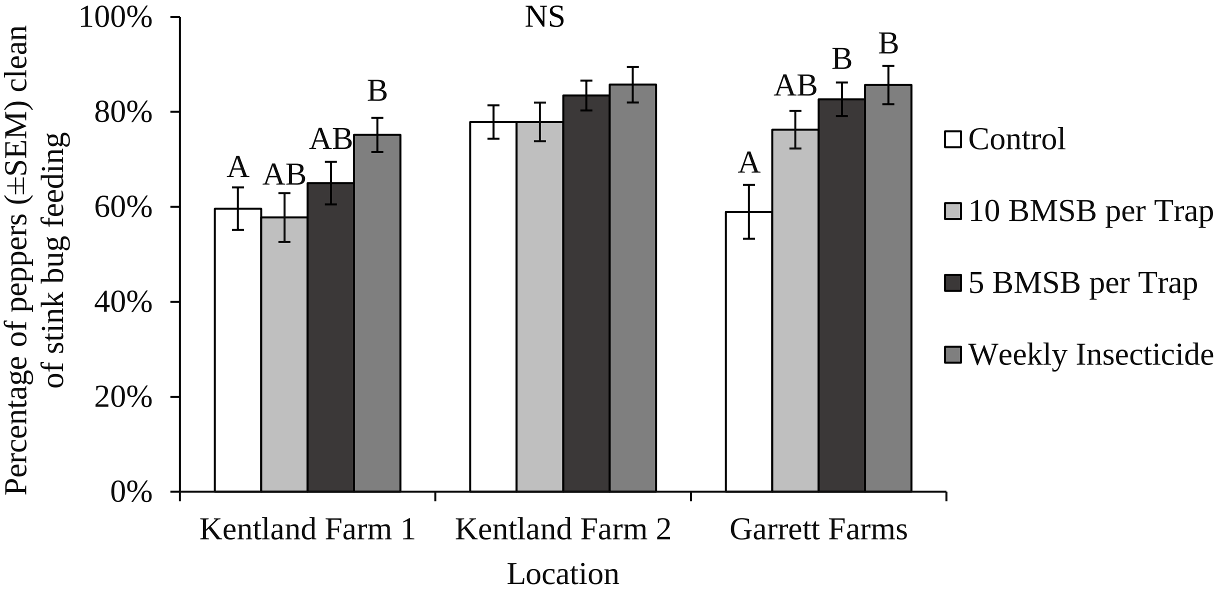

Fig. 4.3. Percentage of stink bug feeding (±SEM) on harvested peppers among treatments.

Chapter 5

Repellency of geranyl cyclopentanone (apritone) and other fragrant compounds on *Halyomorpha halys* (Stål) seeking overwintering shelter

Abstract

BMSB is an agricultural pest and a major nuisance pest to homeowners in the fall and winter when adults seek shelter in occupied dwellings. It can enter some homes by the thousands in its invasive regions throughout the US, and affected homeowners need solutions to manage this serious issue. Not much is known about excluding BMSB from houses other than sealing cracks and openings into the home, as well as timely insecticide sprays that are costly and may not always be effective. Bedoukian Research Inc. provided eight candidate repellents to be tested against BMSB. A Petri dish bioassay, a field cage experiment, and an outdoor experiment on overwintering bugs all found that the compound, geranyl cyclopentanone (apritone), is highly repellent to BMSB. BMSB seeking overwintering shelter in the fall were 8 times less likely to enter overwintering structures treated with apritone, making this one of the only known repellents for overwintering BMSB. In the future, this compound shows promise for use on homes during the fall dispersal period of BMSB, primarily at the unsealed entry points.

Introduction

For almost a decade in the Mid-Atlantic United States, and now many other regions of world, the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (BMSB), has been a high research priority for many entomologists. The pest was introduced in the late 1990s to Allentown, PA (Hoebeke and Carter 2003) and has since spread to 44 US states, 4 Canadian provinces (Fogain and Graff 2011), and has spread to parts of South America and Europe as well

(Wermelinger et al. 2008, Maistrello et al. 2016, Gapon 2016, Macavei et al. 2015, Faúndez and Rider 2017). The pest has caused significant damage to multiple crops; for instance, in 2010 it caused $37 million in crop losses to Mid-Atlantic US apples alone (Leskey et al. 2012). This has resulted in a primary research focus on its management in agriculture, however given its serious nuisance pest status (Inkley 2012), management solutions for affected home and business structures are comparatively limited.

BMSB seeks overwintering shelter when day length shortens and temperature drops, and homeowners typically see an influx of overwintering bugs for a few weeks during September and October in the Mid-Atlantic US (Leskey and Nielsen 2018). Usual places for insect harborage is the attic or crawl space of buildings and houses. The pest enters buildings through cracks in corner joints, behind siding on the home exterior, through shingle siding or roofing, as well as many other openings in vents and cracks in unsealed doors and windows (Inkley 2012).

To this extent, it has been anecdotally determined that sealing cracks in these doors and windows is an important first step to exclude BMSB from entering human-made dwellings (Frye 2016). Insecticide applications to building exteriors have also been widely used to reduce infestations with limited success unless properly timed in the early fall. Several pyrethroids or pyrethroid + neonicotinoid mixtures are widely used for this purpose (Kuhar and Kamminga 2017). However, applying toxic insecticides to sides of homes is not always a practical or desirable tactic. A compound that effectively repels BMSB from buildings or from entry points in buildings would be highly desirable. In Japan, Watanabe (1994) demonstrated that applications of the mosquito and tick repellent, diethyltoluamide (DEET), on window frames paired with cyphenothrin-treated polyethylene netting draped over windows dramatically reduced BMSB entrance to non-wooded houses (Watanabe 1994). Many terpenes and terpenoids that are produced by strongly-scented plants like *Rosmarinus officinalis* (rosemary) or *Mentha piperita* (mint) have repellent effects on insects (Nerioa et al. 2010). These essential oils, including rosemary and spearmint oil, as well as the individual compounds that comprise them, were shown to have spatio-repellent activity against BMSB in the lab (Zhang et al. 2014). Herein, I evaluate the repellency of three natural compounds, geranyl cyclopentanone (Apritone), delta-dodecalactone FCC, and methyl-dihydrojasmonate (Jasmine) on BMSB in laboratory bioassays and field experiments.

Materials and Methods

Laboratory bioassay

A laboratory bioassay was conducted using 3rd and 4th instar BMSB nymphs obtained from a colony maintained at Virginia Tech in a temperature chamber (Percival Scientific, Perry, IA, USA), exposed to temperatures of 26 ± 2°C and a 16:8 L:D photoperiod (Nielsen et al. 2008). Nymphs were used because they are less likely to be influenced by unwanted behaviors like mating. The colony was provided a water wick and a diet of snap beans or carrots, and raw peanuts or sunflower seeds (Medal et al. 2012).

Nine candidate repellents supplied by Bedoukian Research, Inc. (Danbury, CT, USA) were evaluated individually in a choice test experiment that assessed which side of a Petri dish (treated or control) that bugs were found. At the time of the experiment, all compounds were unknown. Jasmine, delta-dodecalactone, methyl apritone, and apritone were the compounds revealed at a later date. Apritone was not included in the initial experiment, but was formulated after methyl apritone showed high repellency then also tested in this Petri dish experiment. The bioassay followed the protocol of Kamminga et al. (2009) and utilized a 14-cm diameter Petri dish with two halves of Fisherbrand® filter paper (P8 No. 09-790-12c). Each half was drenched with one of the repellents or the other with a 90% ethanol control solution and allowed to dry for several minutes before exposing nymphs. Rain-X was applied to the sides and top of the Petri dish to prevent bugs from crawling away from the paper and a green bean was placed on both sides of the dish. Ten nymphs were placed in the center of the dish and the lid was placed to prevent bugs from escaping. The number of nymphs on each side of the dish was recorded at 5 min, 30 min, 90 min, 2h, 4h, 6h, and 24h after initiation. Dead nymphs and those that were not on either piece of filter paper were excluded from analyses. There were four replications of each treatment at a time and the test was repeated three times. Based on pooled data from all trials, the proportion of nymphs on the control versus the treated filter paper was and analyzed as a binomial proportion that was significantly different from the 50% probability, using the standard normal approximation (Ott and Longnecker 2001).

Field cage experiment

Based on the outcome of the previous experiment, this study evaluated the efficacy of apritone (repellent 6A), jasmine (repellent 4), and delta-dodecalactone FCC (repellent 3) for preventing bugs from entering an overwintering shelter box. BMSB adults were collected from the sides of buildings and from shelters in Virginia during their period of dispersal to overwintering sites in September and October 2016. The adult bugs were stored in buckets filled with Styrofoam pipe insulation, which served as overwintering harborage for the bugs. The buckets were stored in a dark a cold room at 4°C until the start of the experiment. The experiment was conducted in high tunnels located at Prices Fork Research Station in Blacksburg, VA in January and February 2017.

Wooden (19 × 22 × 24 cm) overwintering shelters were constructed following Bergh et al. (2017). These wooden shelters contain two openings on the front of the box, one slot at the top and one at the bottom. In Bergh et al. (2017), adults entering the shelters settled in 0.3 cm wide spaces between cardboard inserts within the box. A similar shelter design was used in this experiment with slight alterations. The shelter only contained one 0.5 cm opening along the top front side of the box and two sheets of balled up newspaper were used as a harborage source, rather than cardboard inserts (Fig. 5.1 A & B).

The apritone, jasmine, delta-dodecalactone FCC, and ethanol control treatments were applied with a spray bottle to two pieces of (23.8 × 15.2 cm) of kraft paper per shelter, using approximately 40mL of material. Repellent treatments were diluted to 5% with a 90:10 ethanol:water solution and were compared to a 95% ethanol control. Each piece of paper was folded over the entrance to the shelter to cover all surfaces the bug could walk on to enter, thus being exposed to the tactile treatment. The paper was allowed to dry before it was taped to the front and top of the shelter (Fig. 5.1). Each shelter was placed into individual 0.7 × 0.7 × 1.2 m mesh cages. BMSB adults used in this study were pulled from cold room storage 24 h prior to releasing them into cages and exposed to 26 ± 2°C in a temperature-controlled chamber. This allowed the bugs to regain activity before exposure to cold temperatures in the high tunnel, influencing their movement into the overwintering shelter. Twenty BMSB adults were released into each cage and the experiment evaluated the proportion of bugs that entered the overwintering shelter 24 h after release.

Three replications of each treatment were conducted per trial. Three trials were conducted, and a trial consisted of four releases over a four-day period (*N* = 36), with 20 bugs used per cage at each release. After the bugs were given 24 h to settle, they were counted and a second set of bugs was released and counted 24h later. The repellent was reapplied to the box at the start of each trial. The proportion of bugs that entered the box of each treatment was analyzed using a

Kruskal-Wallis non-parametric test with means separated using Steel-Dwass to determine which repellent was most effective at preventing BMSB from entering shelters. Dead bugs were excluded from analyses.

Efficacy of apritone on BMSB seeking overwintering shelter

Based on the results of the previous two experiments, apritone was used in this experiment to deter BMSB from entering human-made structures during the peak dispersal period in September and October. To determine this, five buildings or houses in Virginia that had been infested with dispersing BMSB annually were chosen as locations to deploy overwintering shelters (Table 1), using the same shelters used in the previous cage test (Fig. 5.1A & B). Two 38.1 × 30.5 × 45.7 cm apple crates, with the open side facing outward, were stacked and secured together using zip ties and weighted using cinderblocks. The overwintering shelter was placed in the top crate and fastened to the crate using bungee cords. The experimental design was a paired t-test where a treated box was next to an untreated control box placed against the same wall of a building (Fig. 5.1C). Three pairs of shelter were deployed at each location and each pair included one shelters treated with 5% geranyl cyclopentanone and one with the control solution. Weekly counts of BMSB in overwintering shelters were taken and the treatments were reapplied directly following counts each week. A paired t-test at $P < 0.05$ was used to compare the numbers of bugs entering the shelters.

Results

Laboratory bioassay

Six out of the nine compounds showed significant repellency (Fig.5.2). Methyl apritone showed the highest repellency ($f = 168.5$, df = 14, $P < 0.0001$), finding approximately 1 out of 10 of nymphs on the repellent side of the Petri dish. This was followed by jasmine (f = 72.6, df = 14, P < 0.0001), apritone (f = 20.6, df = 14, P = 0.0005), delta-dodecalactone (f = 19.3, df = 14, P = 0.0006), unknown 3 (f = 7.2, df = 14, P = 0.018), and unknown 4 (f = 5.6, df = 14, P = 0.033). The three compounds that did not show repellency were unknown 1 (f = 2.6, df = 14, P = 0.13), unknown 2 (f = 0.23, df = 14, P = 0.64), and unknown 5 (f = 0.14, df = 14, P = 0.71) (Fig. 5.2).

Field cage experiment

There was a significant treatment effect on the proportion of bugs entering overwintering boxes (χ_2 = 27.47; df = 3, 35; P <0.0001). The highest proportion of bugs entered the ethanol-treated control box (Fig. 5.3), with significantly fewer bugs entering boxes treated with apritone or jasmine. The delta-dodecalactone did not significantly repel BMSB from entering the overwintering boxes (Fig. 5.3). Because apritone demonstrated the highest repellency, it was selected for use in the next experiment.

Efficacy of apritone on natural shelter-seeking BMSB

BMSB entrance into boxes was low at most sites, with the greatest number of bugs counted in boxes at the Newport location (Table 5.1). In cases that the control box or apritone box in a pair did not have any bugs at the time of data collection, that replication was removed from the analysis. A total of 20 paired boxes with BMSB captures were included in this data. About 8-fold fewer BMSB entered boxes treated with apritone than boxes treated with the control solution, which was significant (f = 9.51; df = 1, 39; P = 0.0038) (Fig. 5.4).

Discussion

Tools for reducing BMSB infestations in human-made structures are badly needed. Although sealing cracks and entry holes is a useful first step (Frye 2016), many bugs still seem to find their way into buildings and homes, particularly older ones. An effective chemical repellent could aid in this battle. Among eight initial candidate repellents formulated from food-grade materials, three demonstrated high repellency including apritone, jasmine, and delta-dodecalactone. The latter (delta-dodecalactone) has demonstrated repellency against *Anopheles gambiae* (Pask et al. 2013). Jasmine (methyl-dihydrojasmonate) is a type of carboxylic ester that has been shown to have repellency against multiple species in the family Culicidae (Butler 2006, Kaufman et al. 2010). Apritone, which has also demonstrated repellency against bed bugs (*Cimex lectularius*), repelled 78% of BMSB nymphs in the treated filter paper bioassay (Bedoukian 2013).

Apritone showed the highest repellency against BMSB seeking shelter in a natural setting and resulted in an 8-fold reduction of entries to repellent-treated shelters. Thus, this compound has potential as a commercial repellent for BMSB. Much additional information is needed for the commercial development of this compound, such as formulation, field longevity, optimal and economical concentration of active ingredient, and release strategy. Bedoukian Research Inc. has a patent processing for commercial use of apritone on BMSB. Perhaps in the future, combining the application of this repellent to home openings with other exclusion strategies like sealing cracks in doors and window frames may help address the severe nuisance problem faced by homeowners.

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Table 5.1. Location of houses and buildings where BMSB overwintering shelters were placed to assess for repellency of overwintering bugs against apritone and the mean (±SEM) BMSB entry per box pair.

		Cardinal direction and mean capture (±SEM) of each box pair		
Town	GPS Coordinates	Box Pair 1	Box Pair 2	Box Pair 3
Blacksburg, VA	37°15'05.2"N 80°21'14.3"W	SE 0.3 ± 0.21	SW 0	NW 0
Newport, VA	37°17'28.5"N 80°30'17.6"W	E 5.4 ± 3.1	S 4.6 ± 3.1	N 3.6 ± 2.3
Salem, VA	37°16'37.8"N 80°06'06.2"W	N 0	E 0.5 ± 0.28	S 0
Whitethorne, VA	37°11'41.8"N 80°34'45.9"W	NW 0.2 ± 0.2	NE 0	SE 0
Linden, VA	38°54'07.9"N 78°05'01.7"W	E 0.7 ± 0.6	W 2.6 ± 1.8	S 0.3 ± 0.21

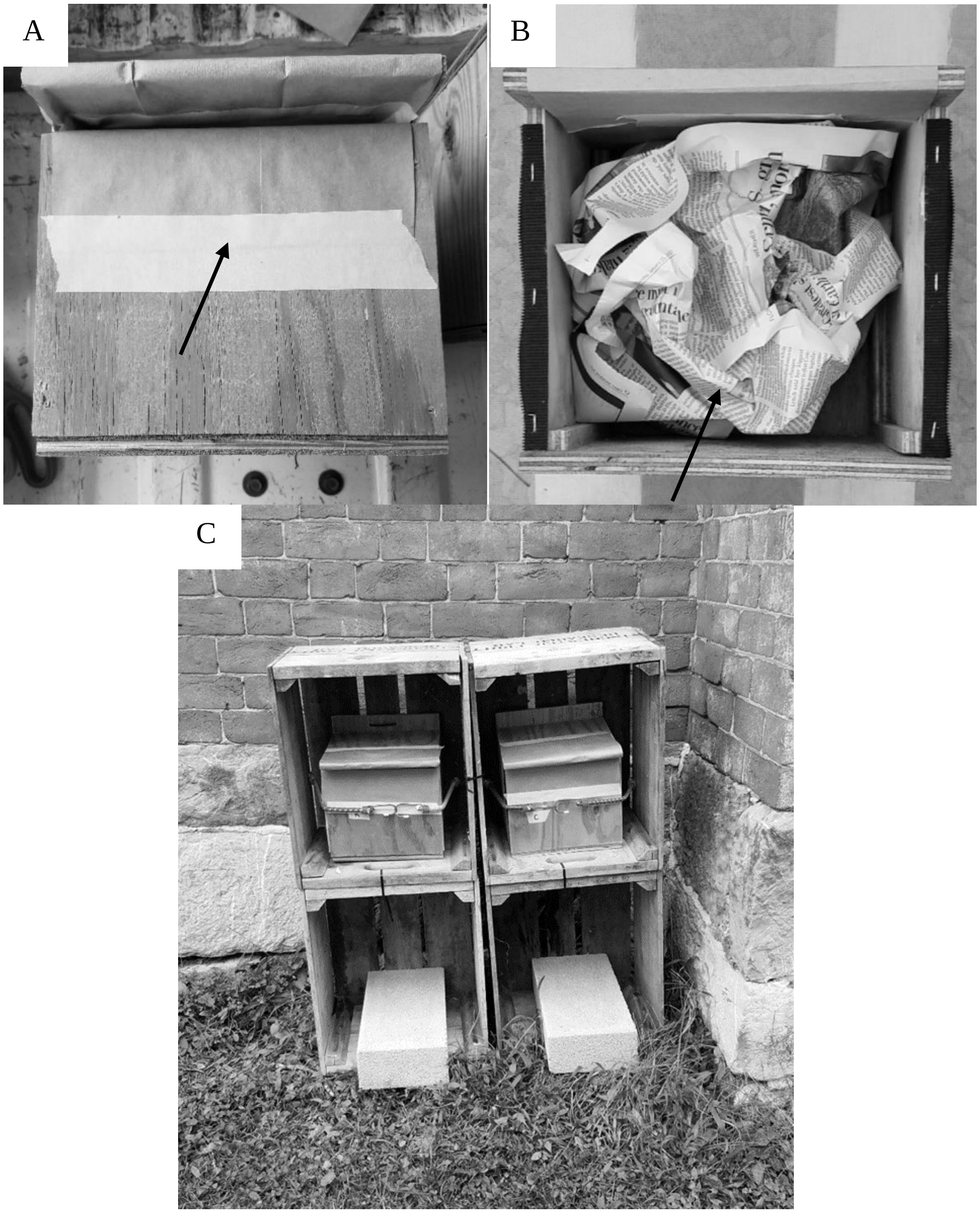

Fig. 5.1. Overwintering shelter setup showing A) the opening holding the repellent treated paper, B) the newspaper harborage source inside of the overwintering shelter, and C) the paired boxes placed against the wall of a building.

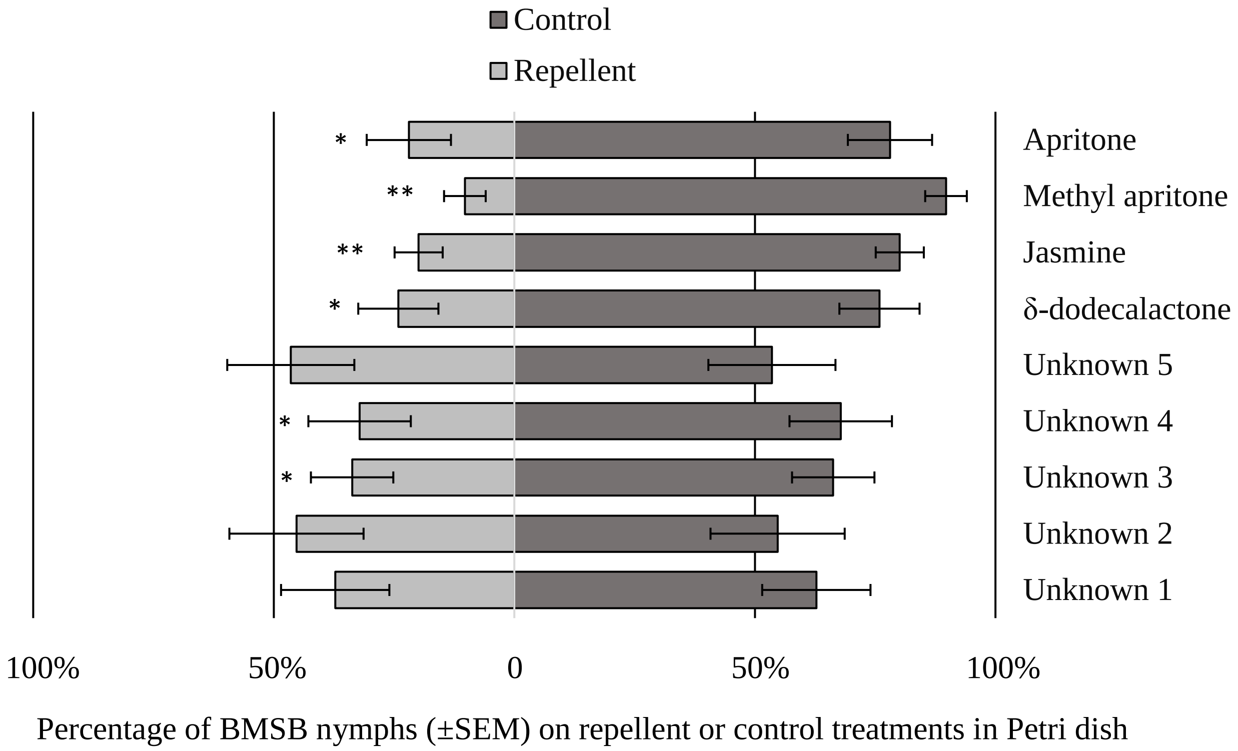

Fig. 5.2. Mean proportion of BMSB nymphs (±SEM) on the repellent and control side of a Petri dish choice test. * represents significance and ** represents a *P*-value <0.0001.

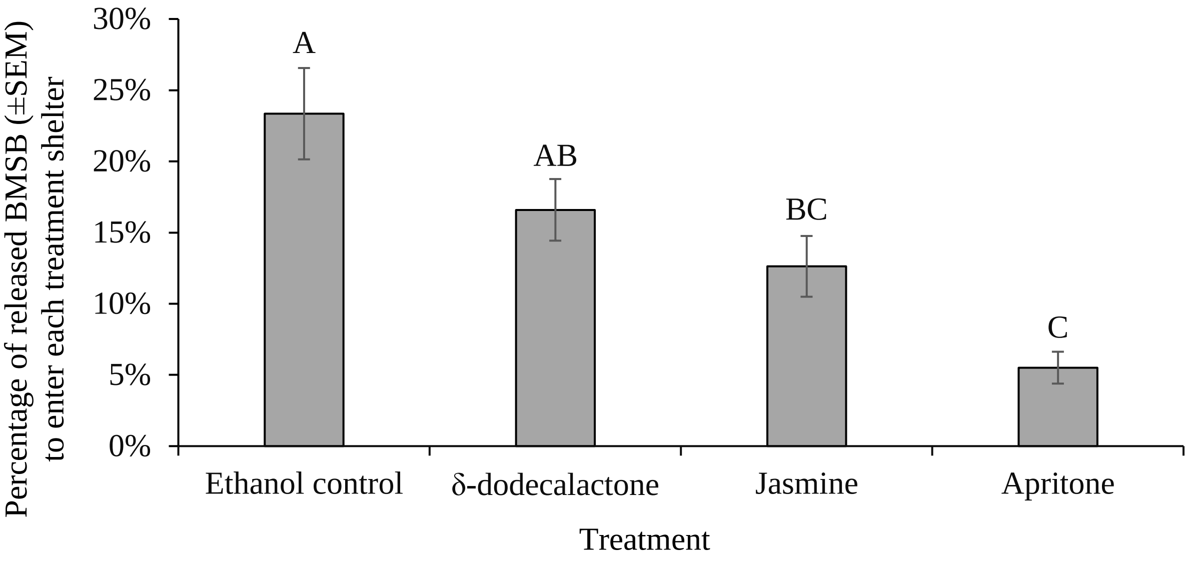

Fig. 5.3. Mean percentage of BMSB adults (±SEM) that entered treated overwintering boxes in a no-choice cage assay that compared the repellency of delta-dodecalactone, methyl dihydro jasmonate, and geranyl cyclopentanone/apritone.

Fig. 5.4

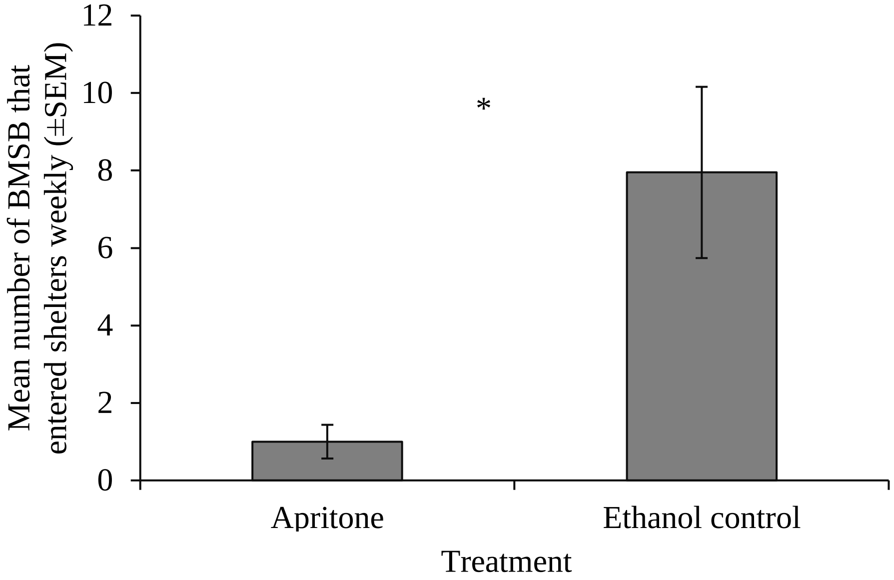

Fig. 5.4. Mean (±SEM) number adult BMSB found in overwintering boxes deployed against human-made structures during the fall dispersal period in September and October 2017.

Chapter 6

Conclusions and Final Remarks

During the BMSB outbreak in 2010, I was in high school living in Linden, VA, a town with a large BMSB infestation. Around February 2011, I opened the sliding glass door in our dining room and hundreds of BMSB in diapause fell on me from the curtain that they were overwintering in. After this, I gained an interest in entomology while pursuing my undergraduate degree and completed research studying behavioral preferences of the emerald ash borer (*Agrilus planipennis*) to visual and chemical stimuli. I wanted to bring this experience into the design of my thesis objectives.

Starting in May 2016, I was aware that there was a powerful aggregation lure available for BMSB, as well as the potential of deltamethrin-incorporated long lasting insecticidal netting for managing BMSB. Pairing the two seemed like an obvious method for an attract-and-kill approach. My advisor, Tom Kuhar, knew of my interest in IPM and asked me to work on strategies for managing BMSB in peppers, so I deployed the netting and lure in pepper plots to determine how BMSB react to the stimulus in the field. I hoped the bugs would be more attracted to the attract-and-kill station than the pepper plants, but it was soon realized that BMSB were attracted to the pepper plants directly surrounding the lure and retained on those plants more than the attract-and-kill station, so most BMSB did not crawl on the netting a receive a lethal dose of insecticide. These findings paved the way for the experiments conducted in peppers in 2017.

I separated the insecticidal netting and pheromone lure into two studies to compare the different management strategies more easily. BMSB have shown to be attracted to the color black, as seen in the standard pyramid trap design; therefore, I deployed the netting as a fence running within a pepper row. Though the black color of the netting is attractive to BMSB, the feeding injury on peppers surrounding netting deployed as the within row fence did not compare significantly to peppers treated weekly with insecticide. I also deployed the netting as a row cover over pepper plants to barricade BMSB from the plants. This approached seemed to have success in the significantly similar percentage of clean peppers to those in the weekly insecticide treatment, but overall yields were significantly lower due to over-shading and lack of supplemental pollination by pollinators. This significant reduction in yield makes this an inefficient management approach. The row cover was also time-consuming to deploy and may have an expensive cost for growers.

The second study I conducted in peppers in 2017 was to deploy the pheromone lure on a clear sticky panel as a tool for management decision-making. This trap design was deployed in the center of a pepper plot following a 4 × 4 Latin-square design. Weekly trap captures correlated to visual counts of bugs on plants, thus was an effective tool for monitoring the relative density of BMSB in the pepper plots. Treatments compared were trap-based action thresholds of 5 and 10 BMSB per trap per week, a weekly insecticide spray, and an untreated control. The 5 BMSB per trap action-threshold was found to have a significantly equal percentage of clean peppers to the treatment sprayed every week, reducing sprays by 50% at one location. The two other locations had lower BMSB population densities, only triggering the 5 BMSB per trap action-threshold spray twice, and the threshold treatments did not differ from the weekly insecticide or the untreated control. If conducting this study again, I would deploy this approach in areas of higher BMSB density so it would be easier to evaluate the efficiency of the trap-based action threshold.

My last objective was to evaluate repellents provided by Bedoukian Research, Inc. as a management approach for BMSB on man-made structures like homes. As mentioned in the first paragraph in these conclusions, my home in Linden is readily invaded by BMSB and is not the only one. Plenty of homeowners in the regions with heavy infestations have made claims that thousands of BMSB adults enter their homes every year to overwinter and have asked for any way to reduce the number of bugs that enter. Sealing entry-points has been one of the main techniques for reducing bug entry, but not all entries to homes can be sealed such as chimneys and ventilation pipes. Timely insecticide sprays are also an option, but are not always effective and can be expensive to homeowners. Repellents seem to be a practical approach to reduce BMSB entry into homes. Bedoukian Inc. provided candidate repellents and I evaluated their success on overwintering BMSB, finding that one compound, geranyl cyclopentanone (Apritone), was highly repellent towards overwintering BMSB, allowing 8-times less bugs to enter overwintering shelters treated with the repellent than the control. This compound is undergoing the patent process for future use to control BMSB from homes. The next steps of this research are to determine the most effective percentage of active-ingredient, as well as to determine the longevity of the repellent in field settings. Hopefully this compound can relieve homeowners of the yearly infestations of overwintering BMSB.

The results of this research have provided knowledge for future steps of the use of insecticidal netting and BMSB pheromone lures for management of the pest. The BMSB working group has adopted the pairing of insecticidal netting and pheromone lure as an attract-and-kill approach on agricultural field edges, and has deemed it the “Ghost trap”. Research to determine the distance of attraction for BMSB to this trap is underway, measuring more precise distances than in Chapter 2 of this thesis. Using the netting as an attract-and-kill approach is more practical than deploying the netting as a within row fence or row cover as seen in Chapter 3. I found successful results of the trap-based action threshold in 1/3 locations in Virginia, but has high potential as a management approach. Combing the trap-based action threshold with attract-and-kill insecticidal sprays has been discussed amongst BMSB experts, and may be a very effective IPM approach. These studies are to help provide growers with management approaches that are more economically and ecologically sound than conventional insecticide management to ultimately assist in more sustainable agricultural systems.