Efficacy of Organic Insecticides and Repellents against Brown Marmorated Stink Bug in Vegetables

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ABSTRACT

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is a major pest of vegetable crops, fruit crops, and even ornamental plants in the Mid-Atlantic States. Organic growers have limited chemical options to manage this pest, and are in need of better management options. Several organically-approved insecticides including pyrethrins (Pyganic), azadirachtin (Aza-Direct), azadirachtin + pyrethrins (Azera), spinosad (Entrust), potassium salts of fatty acids (M-Pede), sabadilla alkaloids (Veratran D), extract from *Burkholderia* sp. (Venerate), and one experimental product, potassium salts + spinosad (Neudorff 1138), were evaluated for toxicity to BMSB nymphs and adults using lab bioassays and field trials on tomatoes and peppers. Another potential control option is to use natural chemicals to deter BMSB feeding in vegetables. Kaolinite [$Al_4Si_4O_{10}(OH)_8$] (Surround WP); a white, plate-shaped, aluminosilicate mineral that is sprayed on plants to alter the appearance, feel, and smell of a plant to an insect. Essential oils (Ecotec) are chemicals produced by plants which are repellent and even toxic to certain insects, and by mimicking octopomine these chemicals disrupt the insect's neurotransmitters. Treatments were evaluated in choice test bioassays and field experiments on peppers using weekly applications of the highest labeled rates of the products. The results showed that, although some organically-approved insecticides demonstrate a high level of activity on BMSB in lab bioassays, none of these products appear to be effective at reducing stink bug damage to fruiting vegetables in the field. However, kaolinite provided significant control of BMSB nymphs ($p=0.03$) and adults ($p=0.01$) in both choice test bioassays and in field trials. Essential oils did not provide any significant control of BMSB in choice test bioassays or in field trials. Further research is needed to determine if the efficacy of kaolinite holds up under heavy pest pressure.

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ORIGIN AND SPREAD

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is native to China, Japan, The Republic of Korea, and Taiwan (Lee et al. 2013). The earliest reports of BMSB in the United States start in 1996, but at the time, the bug was misidentified as brown stink bug, *Euschistus servus* (Say), a native species. In 2001, this invasive stink bug species was correctly identified at Cornell University, after specimens from Allentown, PA were sent in by Karen Bernhard, Pennsylvania State Cooperative Extension agent, who was following up on home invasion reports (Hoebeke and Carter 2003). BMSB were already being collected in black light traps in New Jersey as early as 1999 (Hamilton 2009). This invasive BMSB population is thought to have come from a single introduction from Bejing, China (Xu et al. 2014). In the 2000s, BMSB quickly proliferated and spread across the mid-Atlantic U.S. It spread to Delaware, Maryland, Virginia, and West Virginia by 2005, and by 2016 BMSB had been reported in 42 states and the District of Columbia (Leskey et al. 2016). Currently, it is reported as a serious agricultural pest in 17 states, and an urban nuisance pest in 24 states (www.stopbmsb.org/where-is-bmsb/state-by-state/). The stink bug has also recently become established in Ontario, Canada and several European countries (Gariepy et al. 2013, Haye et al. 2015).

DESCRIPTION OF LIFE STAGES

Adult

Adult BMSB are easily recognizable by the white and black banding on their antennae and the edges of their abdomen, which sets them apart from other brown-colored native stink bugs in the U.S. They are relatively large stink bugs ranging from 12-17 mm in length (Hoebeke and Carter 2003). Males can be distinguished from females by a pair of claspers on the last ventral abdominal segment (Fig. 1.1).

Egg

Generally laid in clusters of approximately 28 eggs on the underside of leaves, but numbers may vary (Kawada and Kitmura 1983). Eggs are bright green in color, barrel-shaped, approximately 1.6mm long, and 1.3 mm in diameter (Fig 1.2) (Hoebeke and Carter 2003). Egg stage typically requires 3-6 days (53.3 Degree Days (DD)) to complete development (Nielsen et al. 2008a). Full development from egg to adult takes approximately 32-35 days at 30°C. Egg development occurs between 15°C and 35°C (Nielsen et al. 2008a).

Nymphs

After egg hatch, first instars huddle around the egg mass. It is thought that they use this time to acquire endosymbionts (Taylor et al. 2014) that were placed on the eggs by their mother (Prado et al. 2006). First instars are approximately 2.4 mm long (Hoebeke and Carter 2003), with a black head, reddish eyes, and a rounded red-orange abdomen with black markings. Three to five days after the egg hatch first instars molt into second instars. At this point they begin to disperse and feed upon host plants using piercing sucking mouth parts. Second instars are approximately 3.7 mm in length (Hoebeke and Carter 2003), with black heads, dark eyes, and a flattened black body. The white banding on the legs and antennae becomes clearer at this stage. Twelve to thirteen days after egg hatch third instars appear. They are very similar to second instars in coloration and shape. They are approximately 5.5 mm in length (Hoebeke and Carter 2003). Fourth instars appear 19-20 days after hatch, and are approximately 8.5 mm in length (Fig 1.3) (Hoebeke and Carter 2003). They closely resemble the previous two instars. Fifth instars appear 26-27 days after hatch, and are approximately 12 mm in length (Hoebeke and Carter 2003). They closely resemble the previous instars, and have now developed wing pads (Fig 1.4). Nymphs also possess a serrated humeral area (Fig 1.3) which is smooth once they reach the adult stage, this is important to note as it may cause them to be confused for *Brochymena* spp. (Hoebeke and Carter 2003).

BIOLOGY

In most of China, BMSB is reported to generally have 1-2 generations per year (Zhang et al. 1993; Chu and Zhou 1997); however, Hoffman (1931) reported as many as 4-6 generations in southern China. In the Republic of Korea, BMSB is reported to have up to 2 generations per year in the southern regions (Bae et al. 2008, 2009; as reported by Lee et al. 2013). BMSB is reported to have 1-2 generations in Japan (Fujiie 1985; Katayama et al. 1993; Funayama 2008).

BMSB overwinters as an adult in both natural and man-made structures. These non-reproductive adults seek shelter from late September to early November, which is when reports of nuisance behavior occur (Kobayashi and Kitmura 1969). Yanagi and Hagihara (1980) showed that diapause is likely driven by photoperiod (14.8-15.5 light phase); however, as Niva and Takeda (2002) showed, nymphs in later instars (2nd to 5th) respond to cooler temperatures and shorter day length and enter diapause after they become adults. This means that temperature may be very important to BMSB development and could override photoperiod requirements (Rice et al. 2014). Overwintering adults emerge from overwintering sites from late march to mid-May (Wang and Wang 1988; as referenced in Lee et al. 2013). Adult females require 148 DD to become reproductively active in the spring (Nielsen et al. 2008a). Female BMSB mate multiple times, which increases the number of fertile eggs they can lay and helps to prolong their fertility as they age (Kawada and Kitamura 1983).

ECOLOGY

Dispersal

BMSB has a reported host plant list of over 100 plants, which include many tree fruit, small fruit, vegetables, ornamentals, and field crops (Bergmann et al. 2013; Leskey et al. 2016). This polyphagous behavior is likely aided by its strong capacity for dispersal on a landscape scale (Rice et al. 2014). It is possible that BMSB moves between host plants as the plants mature and their reproductive structures become available, as these structures are likely the preferred food sources (McPherson and McPherson 2000). Hardwood trees and shrubs could be important intermediate hosts for overwintering BMSB before they move into crops (Nielsen and Hamilton 2009). Zhang et al. (1993) showed that BMSB has the capacity to travel 2 km in a 24 hour period, and this was confirmed in flight-mill studies with wild caught adults here in the US (T. Leskey unpublished data). Adult BMSB are strongly attracted to white, blue, and black light based stimuli under field and laboratory conditions (Leskey et al. 2016). A black light trap grid in New Jersey showed BMSB also fly at night looking for host plants and mates. This knowledge supports evidence that black light traps can be an effective monitoring tool for BMSB to track movement and document populations on a landscape level for the entire season (Nielsen et al. 2013). Aggregations of BMSB can be observed near outdoor lighting in the summer months, though this behavior has not been seen in nymphs (Rice et al. 2014).This grid also showed a variety of flight patterns throughout the year, with the largest peak being at 685 DD (Nielsen et al. 2013). BMSB's dispersal potential peaks in late summer when they seek shelter for overwintering and when they leave overwintering sites in the spring (Wiman et al. 2014). Late instar nymphs (4th to 5th) have been shown to travel 20 meters over grass to reach a trap baited with pheromone lures (Lee et al. 2014a). Brown marmorated stink bug is most known for its nuisance behavior, as it invades homes in numbers that can reach the thousands (Inkley 2012; Kobayashi and Kimura 1969). BMSB's tendency to invade homes likely improves survivorship over the winter months.

Effect of Temperature

BMSB is sensitive to freezing temperatures (Cira et al. 2015). Kiritani (2007) showed that mortality due to cold temperatures decreased by 13.5% for every 1°C increase in mean temperature. More recently, Cira et al. (2015) showed that exposure to temperatures below -15°C resulted in immediate death. Like native stink bugs, adult BMSB also find shelter in the natural environment in cool, dry places often found in dead standing trees with thick bark (e.g. oak *Quercus* spp. and locust *Robinia* spp.) in densities of about 6 adults per tree (Lee et al. 2014b). These preferred conditions are also found in man-made structures (Lee et al. 2014b). It is reported that in their native range, BMSB also utilize dry, high elevation mountain terrain for overwintering (Wang and Wang 1988; as referenced in Lee et al. 2013; 2014b). BMSB is also sensitive to heat. BMSB requires a temperature of 14.17 °C to develop (Nielsen et al. 2008a), and a recent study by Venugopal et al. (2016) showed that BMSB populations were low in areas with an average temperature of < 23.5 °C in the month of June. It has also been shown that exposure to a temperature of 50 °C for 15 min results in 100% mortality (Aigner and Kuhar 2016).

Chemical Ecology

The BMSB aggregation pheromone was recently identified as (3S,6S,7R, 10S)-10,11 epoxy-1-bisabolen-3-ol and (3S,6S,7R,10R)-10,11-epoxy-1-bisabolen-3-ol. This two component compound is released by the males to attract other stink bugs to a location. (Zhang et al. 2013;

Khiriman et al. 2014). There are several key differences between an aggregation pheromone and a sex pheromone. Aggregation pheromones target all motile life stages of an organism and announce the presence of a food source, a mate, or a potential overwintering site, but sex pheromones elicit a response in only one sex of the adult lifestage, and identify a potential mate. Aggregation pheromones draw individuals into an area around the point source, but individuals may be arrested in response to other stimuli. An organism responding to a sex pheromone will seek out the source of the pheromone (Rice et al. 2014). BMSB in the Mid-Atlantic are most responsive to aggregation pheromone traps in late summer and spring when they are moving into and out of over wintering sites (Funayama 2008). BMSB have also been shown to respond to 2,4,6, E,E,Z methyl decatrienoate (Funayama 2008; Khrimian 2005; Khrimian et al. 2008), which is the aggregation pheromone released by another pentatomid species *Plautia stali* Scott (Sugie et al. 1996). This aggregation pheromone was used in early studies, but it is only attractive to BMSB in early August (Aldrich et al. 2009, Nielsen et al. 2011, Leskey et al. 2012). 2,4,6, E,E,Z methyl decatrienoate has also been shown to function as a synergist for the BMSB aggregation pheromone, which increased the effectiveness of baited traps for the whole season (Weber et al. 2014).

Host plants

Of the >100 reported host plants of BMSB (Bergmann et al. 2013), some are important for development and some may be utilized briefly as a food source. Nielsen and Hamilton (2009) reported high numbers of eggs deposited on the trees, *Ailanthus altissima* (Mill.) Swingle, *Paulownia tomentosa* (Thunberg) Steudel, *Acer* spp., and *Fraxinus* spp. However, BMSB appear to seldom utilize a single host plant during their lifetime. Having access to multiple host plants appears to be vital for BMSB development and survival, which can only complete its development on tree of heaven, *Ailanthus altissima* (Mill.) Swingle), empress tree, *Paulownia tomentosa* (Thunb.), Steudel), and peach, *Prunus persica* L. (Acebes-Doria et al. 2016). Apple, *Malus domestica* Borkh, despite being fed upon heavily towards the end of the season, is not a suitable host for BMSB development (Funyama 2002; 2004). BMSB has a number of important crops on its host list, including: peach, apple, filbert nut (*Corylus avellana* L.), pear (*Pyrus* spp.), wheat (*Triticum aestivum* L.), grapes (*Vitis* spp.), small fruit, field corn (*Zea mays* L.), soybean (*Glycine max* L. Merrill), sorghum (*Sorghum bicolor* L.), and many vegetable crops such as sweet corn, tomato (*Solanum lycopersicum* L.), pepper, okra (*Abelmoschus esculentus* (L.) Moench), and eggplant (*Solanum melongena* L.) (Bergman et al. 2013). In addition to the commercially important hosts, several surveys have been conducted to identify a number of wild hosts that are preferred by BMSB, which include: tree of heaven, *Catalpa* spp., yellowwood (*Cladrastis kentukea* (Dum. Cours.) Rudd), paulownia, cherry (*Prunus* spp.), walnut (*Juglans* spp. L.), redbud (*Cercis canadensis* L.), and grape (*Vitis* spp. L) (Bakken et al 2015). These surveys identified regional differences in BMSB populations and identified a number of partial and non-host plants (Bergmann et al. 2016). The reproductive structures seem to be the focus of BMSB feeding on host plants, and they often move from one host to another in synchrony with one host reaching peak reproduction when another host's reproduction ends. (McPherson and McPherson 2000). For example, BMSB do not move into soybeans in large numbers until the beans have reached the R3 stage and those populations will not peak until the beans enter the R5 stage, which is a crucial point since the beans are filling at that stage (Nielsen et al. 2011).

FEEDING

When BMSB move into fields to feed, they can cause serious injury to a variety of vegetable crops. BMSB feed by inserting their stylets into plant tissue and sucking out fluids. In crops like beans (*Phaseolus* spp.) and okra, feeding injury may cause scarred, faded sunken areas, and deformed pods (Kuhar et al. 2012f). In crops like tomatoes and peppers injury looks like faded or sunken areas; these are fleshy fruits and much of the injury is internal. The injury may result in discoloration that is visible (Rice et al. 2014, Kuhar et al. 2015).

Feeding injury reduces the quality and marketability of the fruit. Feeding injury may also cause early fruit set or abortion of the fruit if the injury is severe enough. BMSB have also been known to transmit bacteria or yeast infections such as *Eremothecium coryli* to plants when feeding as a way of indirectly damaging the plant (Brust and Rane 2013). BMSB was also shown to transmit the pytoplasma disease witches' broom to *paulownia tomentosa* (Thunb.) (Hiruki 1999). Crop losses of >50% have commonly been caused by heavy infestation of BMSB in fields. It appears that vegetables like sweet corn, okra, and pepper (Fig 1.4A) are highly preferred as host plants in vegetable cropping systems and suitable for reproduction (Kuhar et al. 2012f; 2015; Rice et al. 2014). Tomatoes and raspberries (*Rubus* spp.) (Basnet et al. 2014) do not appear to be suitable for BMSB reproduction, but can suffer heavy damage (Fig 1.4B). BMSB attacks most vegetable crops that are present from late July to October in the Mid-Atlantic region (Kuhar et al. 2012f).

Mixed plantings of vegetables or farms with diverse crops often see a substantial amount of movement of BMSB adults and nymphs between crops. The attractiveness of the crop or its proximity to an attractive host may determine how much BMSB feeding activity occurs. Crops near overwintering shelters or wooded areas are at high risk of being utilized by BMSB, and vegetables planted near more attractive hosts may be at lower risk of injury than those who are not planted near more attractive hosts (Rice et al. 2014; Kuhar et al. 2015).

CHEMICAL CONTROL

Repeated insecticide applications are often the only management tactic for the control of BMSB in vegetable cropping systems, because BMSB is a new pest and almost no alternative tactics exist for the control of BMSB (Lee et al. 2014b). In 2010 and 2011, it was reported that in the Mid-Atlantic increased the number of insecticide applications up to four times the amount sprayed in previous years (Leskey et al. 2012).

There are several methods that have been used to show the effectiveness of insecticides against BMSB adults and nymphs. These methods include treated glass surface assays (Nielsen et al. 2008b; Lee et al. 2012; Leskey et al. 2013), topical bioassays (Krawczyk unpublished data), bean dip feeding assays (Kuhar et al. 2012e), and field efficacy trials (Kuhar et al. 2012 abcd; Krawczyk et al. 2012; Leskey et al. 2016). These methods have been used to show that compounds like pyrethroids, neonicotinoids, carbamates, and organophosphates all provide effective control of BMSB; however, these insecticides are broad spectrum toxicants that can also be harmful to beneficial organisms such as natural enemies and pollinators. Secondary pest outbreaks of aphids and mites frequently occur in tree fruit and vegetables from repeated use of some of these insecticides (Kuhar et al. 2011; Leskey et al. 2012). The same techniques used to confirm that these synthetic insecticides worked against can be used to confirm if materials certified by the Organic Materials Review Institute (OMRI-certified) show any efficacy against BMSB.

ORGANIC AGRICULTURE

Organic agriculture is a rapidly growing area of agriculture. According to the Organic Trade Association, organic sales has risen from 8.1 billion in 2002 and to 29 billion in 2012, and as of 2011 there are 1.25 billion hectares of certified organic cropland (Willer and Lernoud 2014). The goal of pest management in organic cropping systems is to implement ecologically sound practices as the primary methods of controlling pests (IFOAM Standards 2005). These practices, and the order they should be implemented, were proposed in a model by Wyss et al. (2005). They propose four phases in which arthropod pest management in organic systems should be implemented. The first phase is to implement cultural practices such as crop rotation, field location, and host plant resistance. These are a form of indirect control, but unfortunately there are no known cultural control practices that work on BMSB. The second phase is to manage the vegetation around the field. This phase also involves indirect control tactics, but BMSB is a polyphagous species that has been shown to fly up to 2 km in a 24 hour period. Clearing all potential host plants in a 2 km radius around a field is an impractical control tactic. Work has been started on trying to identify plants that may serve as a trap crop for BMSB, and this work shows some promise (eOrganic.info/node/10003). The third phase is to release biological control agents. After the indirect methods fail the direct methods can be implemented. Unfortunately there are no native parasitoids that thrive on BMSB (Biddinger et al. 2012), and no parasitoids from BMSB's native range are approved for release in the United States. However, *Trissolcus japonicus* Ashmead, considered to be the most effective of BMSB's native parasitoids (Yang et al. 2009), is being examined, and has recently been discovered in Maryland and Virginia (Talamas et al. 2015). Finally, the fourth phase is to resort to the use of mating disruption and approved insecticides. BMSB does not use a sex pheromone which makes mating disruption difficult, and so we are left with the last resort of control measures in organic agriculture systems approved insecticide applications.

ORGANIC INSECTICIDES

One group of biologically derived insecticides is pyrethrins, which are derived from chrysanthemum flowers, *Chrysanthemum* spp. (Casida 1980). There are three species of *Chrysanthemum* recognized by the United States Department of Agriculture as suitable for use in the manufacturing of pyrethrins, but the most important is *C. cinerariaefolium* (Gnadinger 1933;

Head 1973). Although the entire plant contains pyrethrins, the greatest concentration is in the flowers. Pyrethrins work as a contact poison that delays the closure of sodium ion channels in the nervous system (National Pesticide Information Center 1998). Pyrethrins are popular because of the low toxicity of the compounds to humans and mammals (Barthel 1973; National Pesticide Information Center 1998).

Another group of biological insecticides is spinosyns, which are derived from the fermentation of *Saccharapolyspora spinosa*, a soil microbe (Horowitz and Ishaaya 2004). Spinosyns have shown activity against several pests including: lepidopterans, thysanopterans and dipterans. Like pyrethrins, they have low environmental impact and low toxicity against non-target species. Spinosyns work by exciting the neurons and causing muscle tremors and contractions, which results in in paralysis and loss of body fluids (Thompson et al. 2000).

Potassium salts of fatty acids or soap salts were first registered for use as a pesticide in 1947 (RED, 1992). By adding potassium hydroxide to fatty acids from palm (Arecales: Arecaceae), coconut (*Cocos nucifera* L.), olive (*Olea europaea* L.), castor (*Ricinus communis* L.), and cottonseed plants (*Gossypium* spp.), soap salts can be produced (NPIC, 2001). Soap salts cause the cells of insects to dehydrate ultimately causing death. This happens when the lipophilic carbon chains of soap salts disrupt the cellular membrane by breaking up the lipoprotein matrix (Purtrich 1981). Soft bodied insects and immature stages of insects are most vulnerable to soap salt (Purtrich 1981).

Sabadilla is an alkaloid insecticide extracted from the seeds of a South American lily from the *Schoenocaulon* genus. Sabadilla has a mode of action similar to pyrethrin, it disrupts the action potential of the nerves by targeting the sodium channels, which leads to repetitive nerve firing and depolarization of the nerve membrane (Bloomquist 2013). It has been shown to be effective against house flies, milkweed bugs, (Allen et al. 1945) and squash bug (Walton 1946).

One example is azadirachtins and neem oil derived from the neem tree *Azadirachta indica* (Meliaceae). This insecticide has been shown to have a wide range of effects on insects including insect growth disruption and repellency (Ionescu-Malancus et al. 2013). Three primary compounds found in neem oil are known as nimbin, nimbinin, and nimbidin. Neem oil is a popular biological insecticide because of its low toxicity to mammals and beneficial insects (Kashif and Ullah 2013). The effectiveness of neem-based products on pest insects however is mixed. Neem oil shows promising results by controlling an established population of mealybugs (Gowda et al. 2013) and against some Pentatomidae (Kamminga et al. 2009). In repellency tests conducted with filter paper half dipped into an insecticide solution, Kamminga et al. (2009) showed that neither the green stink bug, *Chinavia hilaris* (Say) nor the brown stink bug, *Euschistus servus* (Say) were repelled by a commercial formulation of azadirachtins (Aza-Direct, Gowan Co.).

A more recently developed natural insecticide is composed of an isolated strain of killed cells and fermentation broth containing a newly discovered *Burkholderia* spp. strain A396 isolated from the soil. It is reported to have broad pesticidal activity against insects, algae, arachnids, mites, and nematodes (Asolkar et al. 2013). It is reported to have multiple modes of action that include exoskeleton degradation and molting interference (http://marronebioinnovations.com/ag-products/brand/venerate/)

ORGANIC REPELLENTS

Organic repellents and/or antifeedants for stink bugs are an alternative approach that has not been well investigated. The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests continues to expand (Isman 2006), yet there is very little use of botanicals as repellents in agriculture. Zhang et al. (2014) examined the possibility of using essential oils as repellents for BMSB. Camphor from rosemary (*Rosemarinus officinalis* L.) and l-carvone from the mint family were identified as strongly repellent against BMSB in the laboratory setting. The chemical 1,8-cineole, which is shared by peppermint (*Mentha piperita* L.) (Mahboubi and Kazempour, 2013) and rosemary, was listed as weakly repellent.

Rosemary is a shrub-like aromatic herb that is native to the Mediterranean (Lograda et al. 2013). It is commonly used in cooking, and has been reported to have some medicinal uses including as an anti-inflammatory (Viuda-Martos et al. 2010), anti-carcinogenic (Cheung and Tai 2007), antifungal (Yang et al. 2011; Mugnaini et al. 2012), and inhibiting bacterial growth (Abutbul et al. 2004; Bozin et al. 2007). It is comprised of five major components: camphor, 1,8-cineole, α-pinene, camphene, and borneol (Lograda et al. 2013).

Peppermint is a hybrid mint from cross-breeding spearmint (*Mentha spicata* L.) and water mint (*Mentha aquatica* L.) (Dambrauskienė et al. 2008). Peppermint oil is comprised of seven major components: menthol, methyl acetate, menthofuran, 1,8 cineole, limonene, menthone and isomenthol (Kumar and Patra 2012). Based on research by Mkolo et al. (2011), peppermint oil can be used as a repellent against ticks.

Surround

Particle film technology is a combined synthesis of knowledge on mineral technology, insect behavior, and light physics as they apply to pest control and plant physiology. Particle film technology is largely based on kaolin [$Al_4Si_4O_{10}(OH)_8$]; a white, non-porous, non-swelling, low-abrasive, fine-grained, plate-shaped, aluminosilicate mineral. It disperses easily in water and is inert over a wide pH range. Aluminosilicate minerals or clays are secondary minerals derived from weathered feldspar and quartz. Raw kaolin must be processed to remove traces of Fe_2O_3, TiO_2, and SiO_2. Both Fe_2O_3 and TiO_2 are removed to enhance the brightness of the kaolin, and crystalline silica (SiO_2), which is a breathable human carcinogen, is removed to preserve human health (Harben 1995).

An effective particle film must be chemically inert, have particles less than 2 μm in diameter, spread to create a uniform film, not interfere with gas exchange from the leaf, allow photosynthetically active radiation to reach the leaf and block ultra violet light, change insect/ pathogen behavior on the plant, and can be easily removed from commodities. These characteristics are similar to natural plant defenses such as an increased cuticle thickness and pubescence that reduces water lass and heat stress. (Levitt 1980)

The purpose of particle film technology in insect control is to change the behavior of an insect pest either before or after the insect makes contact with the plant. Particle films alter the appearance and tactile nature of a plant to insects, and mask a plant's host cues (Puterka and Glenn 2005). Particle film may camouflage the plant by turning it white (Puterka et al. 2003; Puterka and Glenn 2005), and limit an insect's ability to move (Unruh et al. 2000) and grasp the plant surface (Puterka and Glenn 2005). Kaolin also aids in protecting the plant from heat stress, sun scalding, increases plant vigor, provides disease control, smoother fruit surface, reduced cracking of fruit or bark, and reduced russeting (Puterka et al. 2000).

JUSTIFICATION FOR RESEARCH

Control of BMSB is a tremendous challenge for organic farmers, who rely heavily upon alternative methods such as biocontrol, promoting natural enemies, and cultural control to prevent injury to crops (Zehnder 2007). Many of these control methods have not provided effective control of BMSB or are simply not available at this time. Consequently, it is important that we identify effective chemical control methods that organic growers can use. Relatively little research has been done to determine the efficacy of common OMRI-certified materials against BMSB in the laboratory setting and under field conditions. BMSB has been reported to cause heavy injury to a variety of vegetable crops, but we need to understand how their feeding effects the growth of plants and their reproductive structures.

Insecticides are generally the last resort for organic growers, but it is important that we identify viable options for growers to rely on until alternative control methods can be developed. BMSB's highly polyphagous nature means it threatens a great number of crops both organically and conventionally grown. Conventional farmers have a large number of broad spectrum insecticides that can be sprayed that are effective at controlling BMSB populations in fields. Organic growers do not have many chemical control options, and because these insecticides are a huge investment they need to know if these treatments are going to effectively control BMSB. Chapter 2 examines the efficacy of 6 different active ingredients with two combinations of active ingredients against BMSB. Efficacy against adults and nymphs under laboratory conditions with topical and feeding bioassays, and then the chemicals were applied in baited plots to determine how effective they were at preventing damage to crops.

Alternatives to insecticides like kaolinite and essential oils may be another tool for organic growers. Evaluating the potential of these alternative solutions could allow growers to reduce the number of sprays required in a year to control pests at an acceptable level. Chapter 3 examines the efficacy of two commercially-available products against BMSB using choice test bioassays and field trials to determine if a particle film of kaolin or essential oils applied to crops may be useful in preventing BMSB feeding injury in vegetable crops.

Objectives

Obj. 1. To assess the toxicity and field efficacy of several biologically-derived insecticides on BMSB life stages.

Obj. 2. To investigate the repellent activity of two natural compounds to BMSB and evaluate their potential as crop protectants on vegetables.

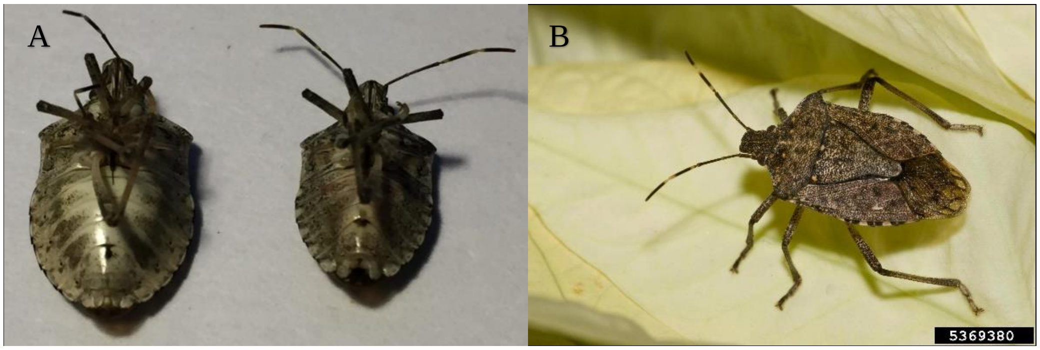

Fig. 1.1: Ventral side of a BMSB adult female on the left and male on the right (A), an adult BMSB (B) (photos by J. Adam Morehead)

Fig. 1.2: BMSB egg mass (photo by David R. Lance, Bugwood.org)

Fig. 1.3: BMSB first instars (Left), second instars (Middle), third or fourth instar (Right)(photos by Gary Bernon, USDA APHIS, Bugwood.org)

Fig. 1.4: Fifth instar BMSB (Photo by David R. Lance, USDA APHIS PPQ, Bugwood.org)

Figure 1.5: Stink bug feeding injury on bell pepper (left) and tomato (right) (photos by J. Adam Morehead)

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Chapter 2: Efficacy of organically-approved insecticides against brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)

ABSTRACT

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), has become a major pest of agricultural crops in the Mid-Atlantic U.S. Organic growers have limited options to effectively manage this invasive pest. Several organically-approved insecticides including pyrethrins (Pyganic), azadirachtin (Aza-Direct), azadirachtin + pyrethrins (Azera), spinosad (Entrust), potassium salts of fatty acids (M-Pede), sabadilla alkaloids (Veratran D), extract from *Burkholderia* sp. (Venerate), and one experimental product, potassium salts + spinosad (Neudorff 1138), were evaluated for toxicity to BMSB nymphs and adults using lab bioassays. These same products also were evaluated in field experiments on tomatoes and peppers using weekly applications of the highest labeled rates of the products. In topical bioassays that utilized a dipped mesh bag technique, high mortality (>70%) of BMSB nymphs was achieved with pyrethrins, azadirachtin, azadirachtin + pyrethrins, potassium salts + spinosad, and sabadilla alkaloids. Using the same topical bioassay for adult BMSB, only pyrethrins, azadirachtin + pyrethrins, and potassium salts resulted in high mortality (>70%). In bean-dip bioassays, none of the insecticides caused high mortality of nymphs; although pyrethrins caused significantly higher mortality than most of the other products. Pyrethrins also were the only product to cause high mortality of adults in bean-dip assays. In the field experiments, stink bug pest pressure was high with feeding damage to fruit averaging between 37 and 65% in the untreated control plots in the tomatoes and peppers in 2014 and 2015; there was no significant effect of insecticide treatment in any of the experiments. These results showed that, although some organically-approved insecticides such as pyrethrins demonstrate a high level of activity on BMSB in lab bioassays, none of these products appear to be effective at reducing stink bug damage to fruiting vegetables in the field.

INTRODUCTION

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is native to East Asia (Hoebeke and Carter 2003). This invasive highly polyphagous insect attacks a number of crops in the U.S. including tree fruit, fruiting vegetables, sweet corn, beans, small fruit, grapes, soybeans as well as some woody ornamental plants (Kuhar et al. 2012a, Leskey et al. 2012, Rice et al. 2014). BMSB feeds by inserting its stylets into the fruit, stem, or leaves of a plant and draining fluids (Hoffman, 1931). Feeding on the fruit and pods results in the greatest damage to vegetables and fruit (Kuhar et al. 2015a). Among vegetable crops, sweet corn, peppers, tomatoes, eggplant, okra, and beans appear to suffer the greatest damage from BMSB (Kuhar et al. 2012a, Rice et al. 2014, Cissel et al. 2015). BMSB has, in some cases, caused farmers to lose as much as 100% of their crops.

Control of this pest in tree fruit and vegetables has been challenging since its arrival (Leskey et al. 2012). Currently, chemical control remains the most effective and efficient strategy (Kuhar et al. 2015b). However, although a number of insecticides including most pyrethroids, several organophosphates and carbamates, and most neonicotinoids have been shown to be efficacious against BMSB (Nielsen 2008, Kuhar et al. 2012 b,c; 2013a,b,c; Leskey et al. 2012, 2013), these insecticides are all broad spectrum toxicants that can also be harmful to beneficial organisms such as natural enemies and pollinators. Many growers have greatly increased the number of sprays they apply per year just to deal with BMSB, as much as 4 times as many sprays (Leskey et al. 2012 b). The increased frequency of insecticide applications suppresses natural enemy populations, and causes secondary pest outbreaks of aphids and mites in vegetables and tree fruit (Kuhar et al. 2011, Leskey et al. 2012a).

Control of BMSB is an even greater challenge for organic farmers, who rely heavily upon alternative methods such as biocontrol, promoting natural enemies, and cultural control to prevent damage to crops (Zehnder 2007). Integrating organically-approved insecticides into a management strategy may reduce crop damage by this invasive bug. Some naturally-derived insecticides include: azadirachtins, which are derived from the neem tree, *Azadirachta indica* (Meliaceae) and have a wide range of insect growth and behavioral effects on insects (Ionescu-Malancus et al. 2013); pyrethrins, which are derived from chrysanthemum flowers, *Chrysanthemum* spp. and have neurotoxic effects on most insects (Casida 1980); sabadilla alkaloids extracted from the seeds of a South American lily from the *Schoenocaulon* genus have a mode of action similar to pyrethrins in that they disrupt the action potential of the nerves by targeting the sodium channels, which leads to repetitive nerve firing and depolarization of the nerve membrane (Bloomquist 2013); spinosyns, which are derived from the fermentation of *Saccharapolyspora spinosa*, a soil microbe (Horowitz and Ishaaya 2004) and have excellent activity against lepidopterans (Zhao et al. 2002), thysanopterans (Eger et al. 1998), and dipterans (Burns et al. 2001), and also have demonstrated some activity on pentatomids including *Chinavia hilaris* (Say) and *Euschistus servus* (Say) (Kamminga et al. 2009); potassium salts of fatty acids, which have been recommended by Trdan et al. (2006) as a control measure for cabbage stink bug, *Eurydema* spp. and shown by Durmusoglu et al. (2003) to control southern green stink bug, *Nezara viridula* L. when combined with azadirachtins; and extracts from the microbe *Burkholderia* sp., which have demonstrated insecticidal effects against various sucking insects (Asolkar et al. 2013).

Lee et al. (2014) recently examined the efficacy of most of the aforementioned OMRI-certified insecticides in treated glass surface (contact) bioassays against BMSB and showed significant mortality of BMSB nymphs and adults with pyrethrins, potassium salts of fatty acids, spinosad, *Burkholderia* sp. (MBI-206), and *Chromobacterium subtsugae* (MBI-203).

Herein, I further investigate the potential of several organically-approved insecticide products at controlling BMSB nymphs and adults in different types of bioassays and in the field on tomatoes and peppers. This information will help determine viable BMSB control options for organic growers.

Table 2.1. List of insecticides used in bioassays and field trials.

Active ingredient	**Trade Name (Manufacturer)**	**Recommended field rate: g ai. per ha**	**Concentration tested: g ai. per liter**
Water Control	-	0	-
Azadirachtin (1.20%)	AzaDirect (Gowan)	48.4	11.8

Azadirachtin (1.20%), Pyrethrins (1.40%)	Azera (MGK)	49.0 53.9	12.0 13.2
Spinosad (22.5%)	Entrust Dow Agrosciences)	175.1	239.7
Potassium salts of fatty acids (49%)	M-Pede SL (Gowan)	2861.7	456.0
Potassium salts of fatty acids 47% + Spinosad 0.1%	Neudorff 1138 (Neudorff)	2721.1 6.8	431.4 1.1
Pyrethrins (5%)	Pyganic (MGK)	58.1	46.7
***Burkholderia* spp. (94.4%)**	Venerate (Marrone BioInnovations)	14,230.3	905.5
Sabadilla alkaloids (0.20%)	Veratran D (MGK)	33.6	0.1

MATERIALS AND METHODS

Treatments

All insecticides used in these experiments were commercially-formulated products that were supplied by their manufacturers (Table 2.1). All treatments were prepared according to the highest recommended field application rate listed on the label. Treatments were mixed in 500 mL or 1400 ml of water for lab bioassays and field applications, respectively. The sabadilla alkaloids required a special preparation of placing the ground seeds in a fine mesh bag that was allowed to seep for > 2hr into the proper volume of solution for either bioassays or field application.

Insects

Adult and nymph BMSB and egg masses were collected from trees in Virginia from May to September in 2014 and 2015 in order to start a lab colony at Virginia Tech. Insects were maintained in 0.028 m^3 screened cages in a temperature chamber (Percival Scientific Inc., Perry, IA) and exposed to temperatures of 28°C ± 2, a 16:8 h L:D photoperiod, and a 50% relative humidity. Adults and nymphs 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 and adults were held and supplemented with field-collected insects from trees when found. Fresh egg masses were isolated from the cages and held in small Petri dishes until 2nd instars appeared, at which time they were returned to the cages. Nymphs and adults were starved for 24 hours prior to use in bioassays.

Submersion (dipped mesh bag) bioassay

For adults and nymphs, twenty insects each were placed in a fine mesh polyethylene bag and submerged in 500 ml of treatment solution for 3-5 seconds then allowed to air dry at (room temperature). The bugs were provided a fresh green bean to prevent death from dehydration. Percent mortality was recorded at 24 and 48 hours. Mortality was counted as dead plus moribund, upside-down and unable to right themselves, or unable to walk. At least three replicates (n=3) were conducted for each treatment. All treatments were compared to a water control to ensure that submersion was not killing the insects

Green bean dip assay

Following Kuhar et al. (2007) and (2012c), green bean dip assays were conducted on nymphs (2nd-4th instars) and adults. For each bioassay, four green bean pods were dipped in each selected treatment and allowed to dry for thirty minutes under a fume hood, then one treated bean pod, a filter paper disc, and five insects of the selected stage were placed in a 9-cm diam. Petri dish. Four dishes (twenty insect total) were set up per treatment per assay. Percent mortality was recorded at 24 and 48 hours as described above. Treatments were compared to a water control. Beans and filter paper were dipped in water and allowed to dry for 30 minutes.

Field trials

Field efficacy experiments were conducted on bell pepper as well as tomato at Virginia Tech's Kentland Farm near Blacksburg, VA in 2014 and 2015. In early June of both years, transplants of 'Aristotle' bell peppers and 'Baby Cake' tomatoes were planted on raised beds covered with black polyethylene mulch. Pepper and tomato plants were spaced 0.3 and 0.5 m, respectively, within rows. Plots were one row by 6 m long. Each experiment was set up in a randomized complete block design with four replicates (N=4).

All treatments were applied as foliar sprays with a CO_2 backpack sprayer at 276 kPa delivering 356 L/Ha through a three-nozzle drop down boom. Insecticides were applied four times in 2014: 19, 25 Aug and 3 and 9 Sept. In 2015 peppers were treated five times: 27 July; 3, 10, 17, 18, 24 Aug; and tomatoes were also treated five times: 28 July; 4, 11, 18, 25 Aug. Peppers were harvested 1 time in 2014: 29 Aug, and 2 times in 2015: 13 and 26 Aug. Tomatoes were harvested three times in 2014: 29 Aug, 8, and 12 Sep; and two times in 2015: 20 and 31 Aug. At each harvest, a sample of 20 or 25 fruit per plot rated for stink bug feeding damage (Kuhar et al. 2015a).

Data Analysis

For the bioassays, Abbott's formula (Abbott 1925) was used to correct for control mortality. When necessary, proportion data were 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 2007, 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.

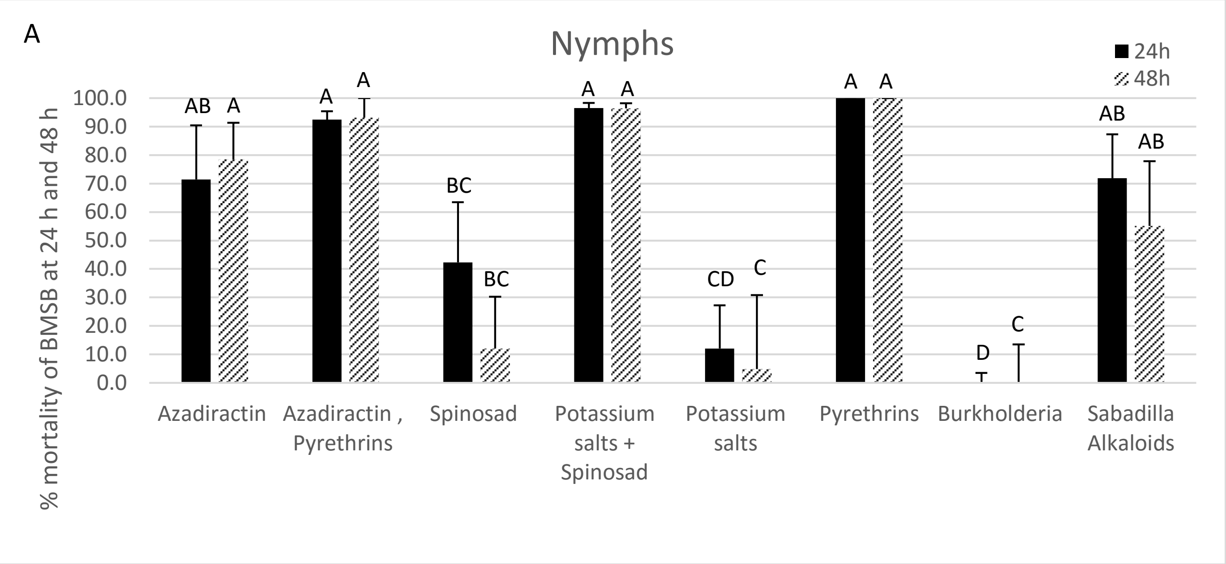
RESULTS

Submersion (dipped mesh bag) bioassays

In the bioassays conducted on BMSB nymphs, there was a significant treatment effect ($f = 9.23$; $df = 7$; $P < 0.0001$). Pyrethrins, azadirachtin + pyrethrins, and potassium salts + spinosad each resulted in significant mortality (>90%), which was significantly higher than that of *Burkholderia* sp., spinosad, and potassium salts alone ($P < 0.05$; Fig. 2.1A)). There also was a significant treatment effect for adults ($f = 5.57$; $df = 6$; $P < 0.0039$), with pyrethrins and azadirachtin + pyrethrins causing the highest mortality, which was greater than 90% (Fig. 2.1B).

Burkholderia sp., potassium salts + spinosad, and spinosad each caused relatively low mortality of BMSB adults after 48 hrs exposure.

Note that all data collected on the sabadilla alkaloids treatment in 2015 were excluded from analysis because we believe that the product lost its potency after a year of storage. This was evidenced by lack of activity in lab bioassays and field efficacy that was drastically different between the 2014 and the 2015 field season. To show a more accurate representation of the efficacy of the sabadilla alkaloids treatment we only display the 2014 data and an asterisk above the bars on the figures indicates where reps were insufficient to perform data analysis.



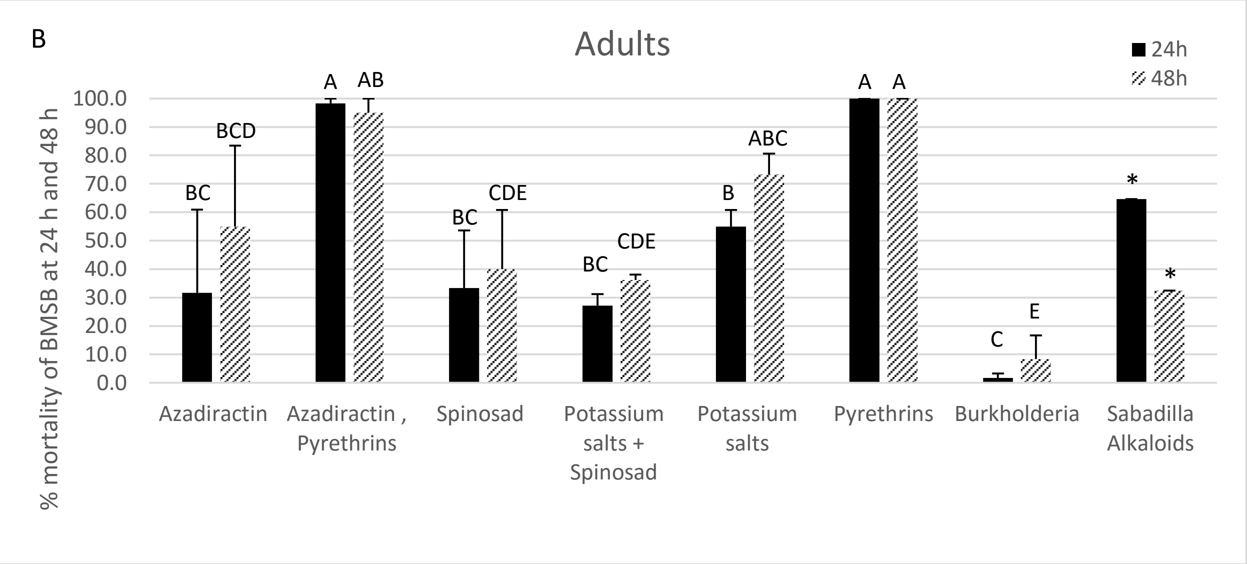
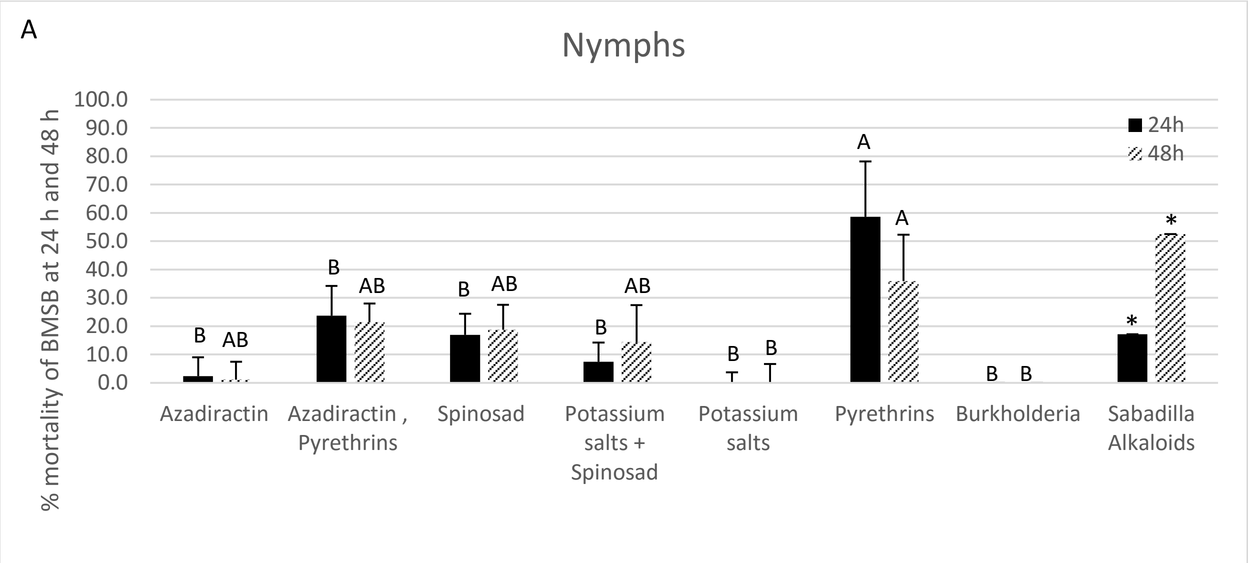


Fig. 2.1. Mean (±SE) percent mortality of BMSB nymphs (A) and adults (B) in submersion assay that utilized a dipped mesh bag technique. Data are corrected for control mortality with Abbott's formula. Bars within an assessment time with a letter in common are not significantly different according to Fisher's protected LSD (* above the sabadilla treatment bars indicate data with insufficient reps to generate a standard error or analyze).

Bean dip bioassays

In the bean-dip bioassays conducted on BMSB nymphs, there was a significant treatment effect (f = 4.47; df = 7; P <0.0083). Only pyrethrins resulted in significant mortality, albeit less than 60%, which was significantly higher than that of *Burkholderia* sp. and potassium salts alone, which caused practically zero mortality (P < 0.05; Fig. 2.2A). There appeared to be a little higher activity on adults with a significant treatment effect (f = 5.40; df = 7; P <0.0037). Pyrethrins, once again, caused the highest mortality just under 90%, which was significantly higher than azadirachtins, potassium salts with and without spinosad, and *Burkholderia* sp. (Fig. 2.2B).



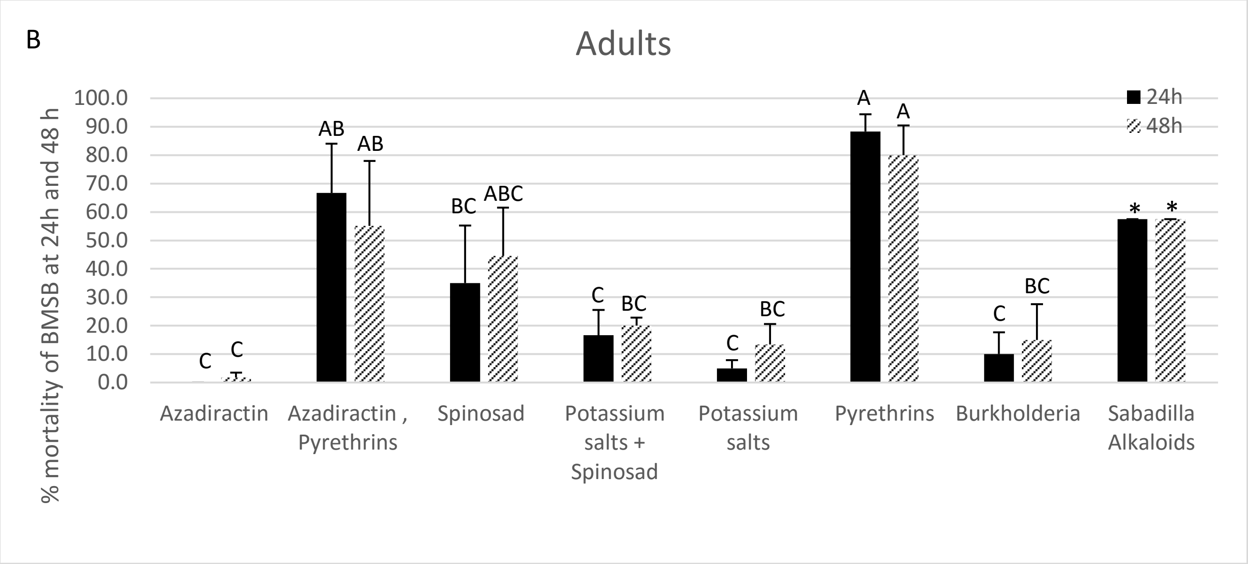


Figure 2.2. Mean (±SE) percent mortality of BMSB nymphs (A) and adults (B) in bean dip assay. Data are corrected for control mortality with Abbott's formula. Bars within an assessment time with a letter in common are not significantly different according to Fisher's protected LSD (* above the sabadilla treatment bars indicate data with insufficient reps to generate a standard error or analyze).

Field Experiments

Stink bug pest pressure in the 2014 field season was low in pepper untreated control plots with an average of 10% damage. Stink bug pressure was moderate in tomato untreated control plots averaging 34% damage to sampled subsets of fruit. There was no significant treatment effect of 2014 field experiments (Table2.2).

Pest Pressure was higher in the 2015 field season, with an average of 47% damage in pepper untreated control plots; and an average of 65% damage in tomato untreated control plots. There was no significant treatment effect of 2014 field experiments (Table2.3).

Table 2.2. Field evaluation of organic insecticides for the control of BMSB in bell peppers and tomatoes, Kentland Research Farm, Blacksburg, VA 2014.

Treatment	Rate (g a.i./ha)	Average % of harvested fruit with stink bug damage ± SE			
		Pepper	Tomato		
		29-Aug	29-Aug	8-Sep	12-Sep
Water Control	0	10.0 ± 31.6	31.3 ± 7.7	39.0 ± 12.7	31.0 ± 4.8
Azadirachtin	48.4	7.5 ± 49.2	30.0 ± 12.5	21.0 ± 10.6	15.0 ± 18.9
Azadirachtin + Pyrethrins	49.0 + 53.9	5.0 ± 27.1	20.0 ± 5.2	37.0 ± 25.3	20.0 ± 20.0
Spinosad	175.1	6.3 ± 33.2	42.5 ± 12.5	33.0 ± 15.2	29.0 ± 22.4
Potassium salts	2861.7	15.0 ± 30.1	22.5 ± 15.9	26.0 ± 13.3	15.0 ± 54.2
Potassium salts+ Spinosad	2721.1 + 6.8	13.8 ±22.3	23.8 ± 5.4	40.0 ± 19.3	27.0 ± 16.7
Pyrethrins	58.1	15.0 ± 87.1	23.8 ± 3.3	32.0 ± 6.8	6.0 ± 19.5
Burkholderia spp.	14,230.3	17.5 ± 28.5	25.0 ±11.1	25.0 ± 8.0	27.0 ± 23.1
Sabadilla Alkloids	33.6	26.3 ± 38.8	25.0 ± 17.0	21.0 ± 4.8	26.0 ± 15.2
P- Value from ANOVA		ns	ns	ns	ns

Insecticides were applied four times in 2014: 19, 25 Aug, 3, and 9 Sept.

Table 2.3 Field evaluation of organic insecticides for the control of BMSB in bell peppers and tomatoes, Kentland Research Farm, Blacksburg, VA 2015.

Treatment	Rate (g a.i./ha)	Average % of harvested fruit with stink bug damage ± SE			
		Pepper*		Tomato**	
		13-Aug	26-Aug	20-Aug	31-Aug
Water Control	0	47.0 ± 16.4	47.0 ± 19.7	65.0 ± 9.4	65.0 ± 7.0
Azadirachtin	48.4	22.0 ± 17.7	46 ± 2.2	58.0 ± 4.4	44.0 ± 5.9
Azadirachtin + Pyrethrins	49.0 + 53.9	19.0 ± 44.5	33.0 ± 22.0	60.0 ± 3.2	49.0 ± 12.0
Spinosad	175.1	31.0 ± 26.7	62.0 ± 18.9	35.0 ± 4.5	46.0 ± 17.4
Potassium salts	2861.7	19.0 ± 9.8	51.0 ± 7.2	59.0 ± 12.4	70.0 ± 8.6
Potassium salts+ Spinosad	2721.1 + 6.8	32.0 ± 9.8	56.0 ± 7.4	62.0 ± 6.6	55.0 ± 5.9
Pyrethrins	58.1	26.0 ± 25.6	40.0 ± 4.7	56.0 ± 14.3	57.0 ± 6.4
Burkholderia spp.	14,230.3	41.0 ± 22.7	56.0 ± 13.2	59.0 ± 8.4	58.0 ± 7.8
Sabadilla Alkloids	33.6	-	-	-	-
P- Value from ANOVA		ns	ns	ns	ns

*Peppers were treated five times: 27 July; 3, 10, 17, 18, 24 Aug

**Tomatoes were treated five times: 28 July; 4, 11, 18, 25 Aug

DISCUSSION

A number of insecticides including pyrethrins, azadirachtins, sabadilla, spinosad, and potassium salts of fatty acids demonstrated at least some toxicity to BMSB nymphs and adults in the lab bioassays. Using a treated glass surface bioassay, Lee et al. (2014) also demonstrated significant BMSB mortality with pyrethrins, spinosad, potassium salts, as well as *Burkholderia* sp.

Azadirachtins showed mixed results in my bioassays, but was not effective in the field. Even Azera which showed some promise in the lab is thought to have only been effective due to the pyrethrins. Lee et al. (2014) also showed that azadirachtin was ineffective against BMSB adults in the lab.

Sabadilla showed promise in the bioassays conducted in 2014, but because I was unable to get fresh product in 2015, and the 2014 Veratran D product likely lost its potency after a year, we do not feel confident about what we learned regarding BMSB efficacy. A new formulation of sabadilla is now commercially available (http://www.mgk.com/crop-protection/veratran-d/) and should perhaps be evaluated further for stink bug efficacy. Sabadilla has previously been shown to be effective against milkweed bugs, *Oncopeltus fasciatus* (Dallas) (Allen et al. 1945) and squash bug, *Anasa tristis* (DeGeer) (Walton 1946).

Spinosad was shown to be quite toxic to BMSB by Lee et al. (2014) and performed well in bioassays against other stink bugs (Kamminga et al. 2009). Unfortunately, this insecticide did not demonstrate significant activity against BMSB in my experiments.

Lee et al. (2014) showed that potassium salt residues were effective against BMSB after 4-5 days of exposure. My results showed that potassium salts combined with spinosad were highly effective against nymphs in topical assays, but potassium salts did not show much efficacy in contact assays, or in field trials.

My results showed little to no effect on BMSB nymphs or adults from *Burkholderia* sp. Carson et al. (2014) also found that foliar applications of *Burkholderia* sp. (Venerate) did not reduce stink bug and other heteropteran feeding damage to tomatoes in California.

In my lab bioassays, pyrethrins, or pyrethrins combined with azadirachtins, were the most consistently efficacious on BMSB nymphs and adults. Kamminga et al. (2009) also showed pyrethrins to have a high level of activity against stink bugs. Pyrethrins have also been shown to be effective at removing BMSB from wine grape clusters at harvest to prevent them from being crushed with the clusters and tainting the wine (Pfeiffer et al. 2010; Pfeiffer et al. 2012). However, in our field experiments, neither pyrethrins nor any of the other organically-approved insecticides significantly reduced stink bug feeding damage to either peppers or tomatoes. Organic insecticides generally break down more quickly under field conditions, and quickly lose their potency (Zehnder et al. 2007).

Differences in feeding injury severity were not recorded, so the possibility remains for there to be less severe feeding from some of the treatments. Future experiments may include a standard for damage size or number of marks to examine sub-lethal effects on BMSB populations in vegetable fields.

Currently, no effective cultural method exists for controlling BMSB. It is important to provide organic growers with information about the efficacy of all control options available to them. Unfortunately, there is no evidence to recommend any of the insecticides tested in this study for control of BMSB on crops in the field.

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Chapter 3: Efficacy of kaolin and essential oils at protecting fruiting vegetables from brown marmorated stink bug (Hemiptera: Pentatomidae)

ABSTRACT

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is a major pest of vegetable crops, fruit crops, and even ornamental plants in the mid-Atlantic States. Organic growers have limited chemical options to manage this pest, and are in need of better management options. Kaolinite [$Al_4Si_4O_{10}(OH)_8$] (Surround WP); a white, plate-shaped, aluminosilicate mineral that is sprayed on plants to alter the appearance, feel, and smell of a plant to an insect. Essential oils (Ecotec) are chemicals produced by plants which are repellent and even toxic to certain insects, and by mimicking octopamine these chemicals disrupt the insect's neurotransmitters. Choice test bioassays and field trials were conducted with two commercially available products to test their efficacy against BMSB adult and nymph (2nd – 4th instar) life stages in both the laboratory and the field settings. Kaolinite provided significant control of BMSB nymphs (p=0.03) and adults (p=0.01) in both choice test bioassays and in field trials. Essential oils did not provide any significant control of BMSB in choice test bioassays or in field trials. Further research is needed to determine if the efficacy of kaolinite holds up under heavy pest pressure.

INTRODUCTION

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is a highly polyphagous invasive pest that has become a major pest in tree fruit, fruiting vegetables, sweet corn, beans, small fruit, grapes, field corn, soybeans as well as some woody ornamental plants (Kuhar et al. 2012a, Leskey et al. 2012a). BMSB feeds by inserting its stylets into the fruit, stem, or leaves of a plant and draining fluids. Among vegetable crops, sweet corn, peppers, tomatoes, eggplant, and beans appear to suffer the greatest feeding damage from BMSB (Kuhar et al. 2012a, Rice et al. 2015). In certain areas of the mid-Atlantic U.S., control measures are essential to protect crops from economic damage (Leskey et al. 2012a). A number of insecticides including most registered pyrethroids, a few organophosphates and carbamates, and several neonicotinoids have been shown to be efficacious against BMSB (Nielsen 2008, Kuhar et al. 2012 b,c; 2013a,b,c; Leskey et al. 2012b, 2013). However, all of these insecticides are broad spectrum toxicants that can also be harmful to beneficial organisms such as arthropod predators, parasitoids, and pollinators (Leskey et al. 2012b). Moreover, secondary pest outbreaks of aphids, mites, or other insects can occur in tree fruit and vegetables from repeated use of some of these insecticides (Kuhar et al. 2011, Leskey et al. 2012a).

Control of BMSB is an even greater challenge for organic farmers, whose insecticide options are limited to a few naturally-derived chemicals that have demonstrated some activity in the lab (Kamminga et al. 2009, Lee et al. 2014), but typically have not performed well in the field at protecting vegetables from stink bug damage. Therefore, alternative management strategies are desired for sustainable and sound integrated pest management of BMSB. One potential strategy is the use of repellent or feeding deterrents to drive stink bugs away from cash crops. Kaolin is a commercially-available option for this strategy that has shown efficacy in other systems for other insects (Unruh et al. 2000, Puterka et al. 2003, Puterka and Glenn 2003). Kaolin [$Al_4Si_4O_{10}(OH)_8$] is a white, non-porous, non-swelling, low-abrasive, fine-grained, plate-shaped, aluminosilicate mineral or clay that is derived from weathered feldspar and quartz (Harben 1995). Raw kaolin is processed to remove traces of Fe_2O_3, TiO_2, and SiO_2 (Harben 1995), leaving behind a fine chemically inert powder that can safely be applied to plants to reduce heat stress, water loss, and sunscalding to fruit (Puterka 2000). Particle films of kaolin have also been shown to alter the appearance, tactile nature, and even smell and taste of a plant to insects (Puterka and Glenn 2003). Kaolin films may camouflage the plant by turning it white (Puterka et al. 2003; Puterka and Glenn, 2003), and limit an insect’s ability to move (Unruh et al. 2000) and grasp the plant surface (Puterka and Glenn 2003).

A group of naturally-derived insect repellent compounds are the terpenes and terpenoids, which are secondary metabolite compounds produced by certain plants to deter feeding by insect pests (Nerioa et al. 2010). These can render the plant distasteful or toxic to the animal feeding upon the plant. Plants that produce such compounds include rosemary, mint, and many other strongly scented plants that contain essential oils (Nerioa et al. 2010). Zhang et al. (2014) recently showed that several essential oils including rosemary and spearmint oil, as well as the individual compounds that comprise them, had repellent activity against BMSB in the lab. EcoTec (EcosSMART Technologies, Inc.) is a commercially-available blend of essential oils (10% rosemary oil, 2% peppermint oil) registered for agricultural applications. Herein, we evaluated the efficacy of kaolin particle film (Surround) and essential oils (EcoTec) to repel BMSB in lab choice tests and protect fruiting bell peppers in the field with weekly applications of the products.

MATERIALS AND METHODS

Laboratory choice tests

The experimental arenas were 56 × 56 × 56 cm fine mesh insect rearing and observation cages with vinyl windows (BioQuip Products, Rancho Dominguez, CA). Two 9-cm Petri dish halves were placed on opposite corners of the cage. Freshly-picked *Paulownia tomentosa* (Thunb.) Steud. (leaves were trimmed to fit the Petri dish and were treated with either kaolin, essential oils, or left untreated prior to placement in the arena. A cherry tomato (NatureSweet GLORYS) was placed in the center of the leaf to provide another potential food source to draw the stink bug onto the treated surface. Tomatoes received the same treatment as the leaf they were placed on. Kaolin (Surround) was applied in a 50-liter trash bag (Great Value, Tall kitchen bags w/ drawstring), trimmed leaves were placed inside with 120-60 g of dry kaolin powder. The bag was shaken thoroughly to coat the leaves. Excess powder was shaken gently from the leaves before they were removed from the bag. Tomatoes were placed in a quart container with Surround and shaken gently until they were coated. A mixture of essential oils (Ecotec) was applied with a hand sprayer to leaves and tomatoes until run off and shaken gently to remove excess. Controls were untreated leaves and tomatoes.

Prior to the start of each experiment, 60 insects were separated into groups of ten in pint containers. Pint containers were gently tapped to release insects into a corner of the arena so they started the same distance from each treatment. Insect position within the arena were noted at 20 minutes, 40 minutes, 60 minutes, 2 hours, 4 hours, 6 hours, 8 hours, and 24 hours after the start of the experiment. Only insects observed on the test surface were counted, all others were assumed to be elsewhere within the arena. This experiment wascompleted three times (n=3) for both adult and nymph (2nd-4th instar) stages. A total of 360 individuals were used for this experiment. All treatments were analyzed using a paired t-test.

Field efficacy trials

Aristotle bell peppers were planted at Virginia Tech Kentland Research Farm (Blacksburg, VA). Plots were arranged in a randomized complete block design with 4 replicates (n=4), each plot was four rows. Individual plots were 4 rows x 6 m. long. Control and kaolinite treated plots were separated from essential oil treated blocks by a buffer block. This was to minimize any impacts essential oil treatments may have had on the other treatments. A carbon dioxide backpack sprayer with a 3-nozzle drop boom was used to apply the treatments. Treatments were applied 1 time weekly for each experiment. During 2014, 50 pepper fruit were harvested from each plot on Aug 29 and Sep 22 then examined for insect damage. In 2015, only 40 peppers were harvested per plot due to reduced production on Aug 12, 21, and 28. Percentage fruit damage data was analyzed using ANOVA and Fisher's Protected LSD to separate means.

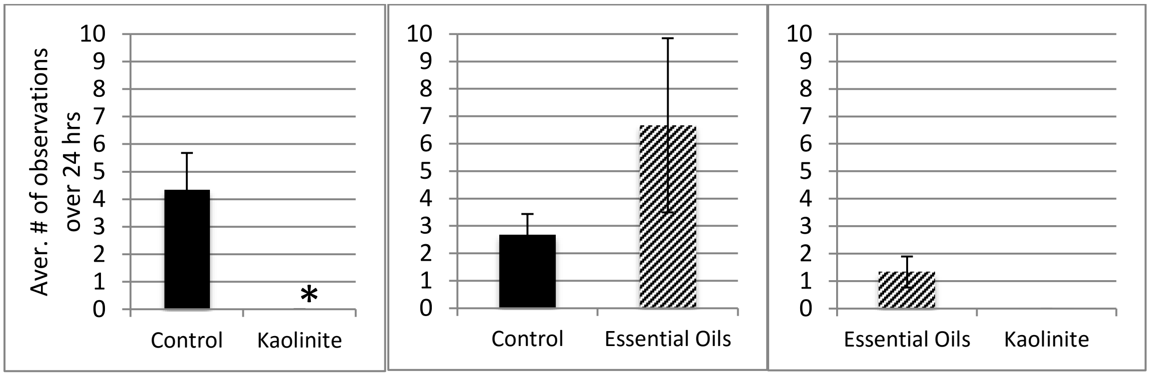
RESULTS

Kaolinite provided significant control of BMSB and nymphs (p=0.03) and adults (p=0.01) in choice test bioassays when compared to the untreated control (Figure 3.1). Nymphs did not interact with the kaolinite treated leaf, and adults had an average of < 1interaction with the kaolinite treated leaf over 24 hours. Kaolinite also provided consistent control of BMSB in field trials with all five harvests across two field seasons yielding significantly less damage on subsamples from plots treated with surround (Fig. 3.2; 3.3). Kaolinite applications resulted in an average 76% reduction in injury across all five harvests, with as much as 90% at the second harvest.

Essential oils did not provide any significant control of BMSB in choice test bioassays. Treating leaves and tomatoes with essential oils did not prevent BMSB adults and nymphs from feeding or interacting with the treated surface, and nymphs interacted with the essential oil treated surface more than the untreated surface. In field trials essential oils only showed significantly less injury than the control in two of the five harvests. It averaged a 27% percent reduction in damage across all five harvests, with a high of 65% at the second harvest, but was more damaged than the control at the fourth harvest (Fig 3.2; 3.3).

Pest pressure was relatively low in 2014, with controls averaging between 21% and 12% damage (Fig. 3.2). Pest pressure was moderate in 2015 with all controls averaging >30% damage (Fig. 3.3).

Nymphs



Adults

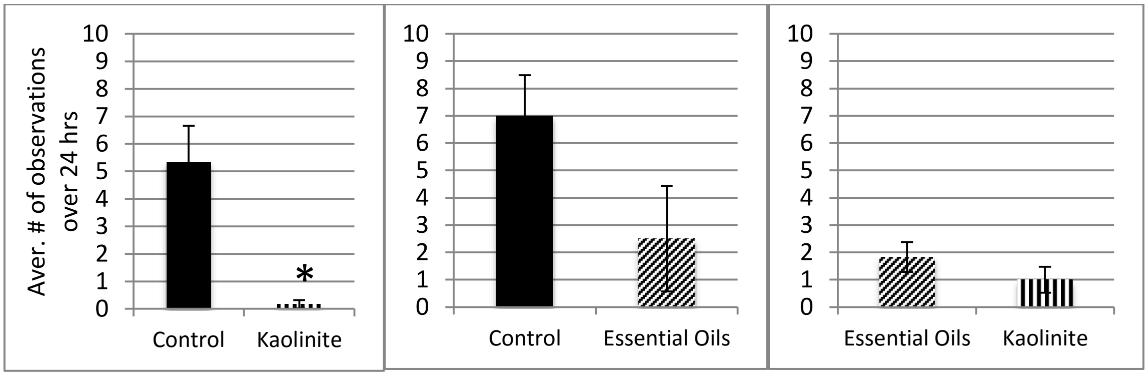


Figure 3.1. Summary of results from choice test bioassays comparing kaolinite and essential oils against nymph (2nd-4th instar) and adult life stages of brown marmorated stink bug in peppers, at Kentland Research Farm, Blacksburg, VA 2014. * indicates treatments significantly different according to T test.

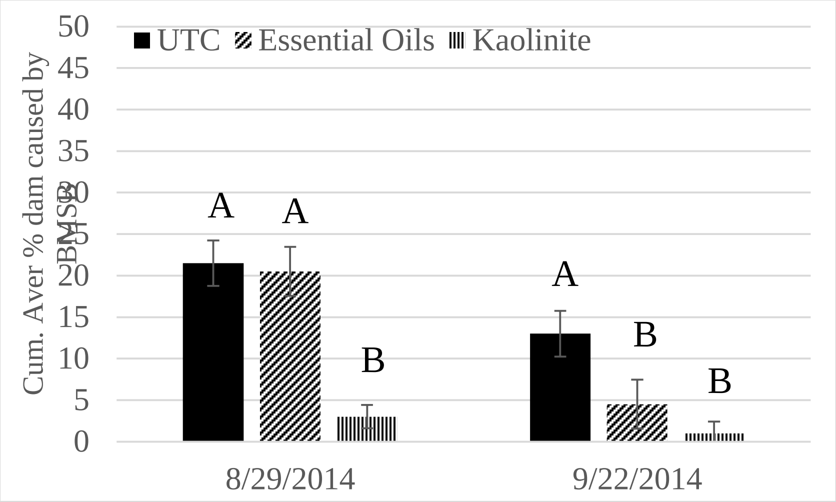


Figure 3.2. Results from field evaluation of kaolinite and essential oils for the control of brown marmorated stink bugs in bell peppers, Kentland Research Farm, Blacksburg, VA 2014. Bars within an assessment time with a letter in common are not significantly different according to Fisher’s protected LSD. Treatments were applied five times in 2014: 22, 29 Aug and 5, 12, 19 September.

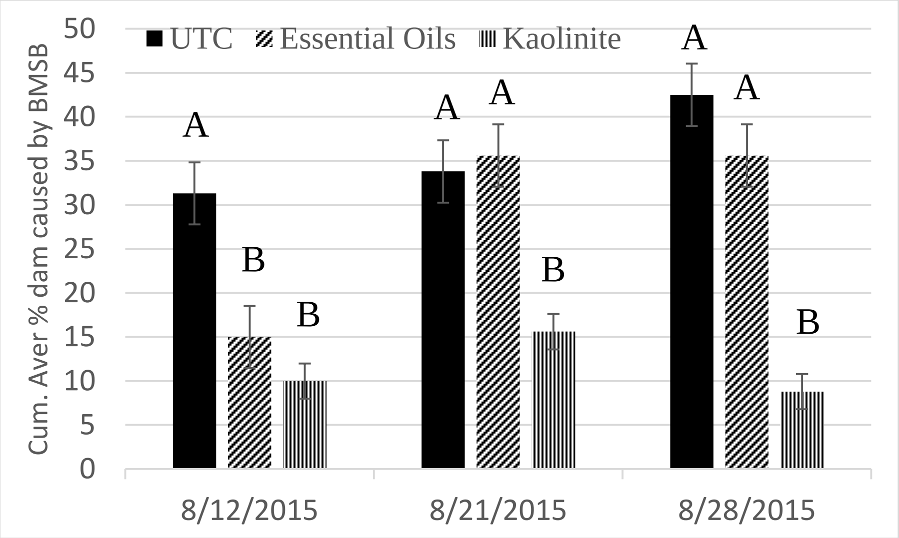


Fig 3.3. Results from field evaluation of kaolinite and essential oils for the control of brown marmorated stink bugs in bell peppers, Kentland Research Farm, Blacksburg, VA 2015. Bars within an assessment time with a letter in common are not significantly different according to

Fisher's protected LSD. Treatments were applied four times in 2015: 31 July and 7, 14, 21 August.

DISCUSSION

Arthropods rely on touch, taste, sight, and smell to locate and accept host plants (Miller and Strickler 1984). The purpose of particle film technology in insect control is to change the behavior of an insect pest either before or after the insect makes contact with the plant. Particle films of kaolin alter the appearance, tactile nature, and possibly even the taste and smell of a plant to insects (Puterka and Glenn 2003). Particle film may camouflage the plant by turning it white (Puterka et al. 2003, Puterka and Glenn 2003), or limit an insect's ability to move (Unruh et al. 2000) and grasp the plant surface (Puterka and Glenn 2003).

Our laboratory experiments demonstrated that BMSB spent very little time on foliage and food that was treated with a kaolin film. Nymphs were never observed on the kaolin-treated foliage or fruit during the choice tests, and adults that did interact with the kaolin were often not on it long. Furthermore, in the field, applications of kaolin to bell peppers significantly reduced stink bug feeding damage to fruit. Thus, the use of kaolin appears to be a viable pest management option for BMSB on vegetables. Moreover, as the use of kaolin has other horticultural and pest management advantages, it may be a viable option for fruiting vegetable cropping systems. For instance, peppers can suffer high losses to sun scalding injury (Díaz-Pérez 2014), and kaolin has been shown to reduce that problem in tree fruit and other crops (Glenn and Puterka 2005).

Zhang et al. (2014) examined the potential of several essential oils as spatial repellents against BMSB, and tested the primary components to determine what drives the repellent activity. α-terpineol and β-caryophyllene from rosemary (*Rosmarinus officinalis* L.) (Logrado et al. 2013) were both listed as strongly active, and 1,8-Cineole from peppermint (*Mentha* × *piperita* L.) (Kumar and Patra 2012) was listed as weakly active. Essential oils data is inconsistent and makes it difficult to determine significance. While there was some significance in field trials, that was only in two of the five harvests. Results for essential oils in choice tests were not significantly different. Nymphs on average interacted with the essential oil treated leaves and tomatoes more frequently than the nymphs interacted with the untreated leaves and tomatoes.

Kaolin has the potential to be a key piece to pest management programs. However it can be difficult to remove from some produce with a wash line (e.g. around plant stems), and consumers would need to be educated that this white inert residue is not a dangerous pesticide and can be easily wiped off by hand. This would provide growers an alternative control option that may can be combined with existing control strategies like insecticide sprays or implemented separately to help prevent damage to crops at all stages. Since our results only show the efficacy of kaolinite under mild to moderate pest pressure, further research is needed to determine if the efficacy of kaolinite holds up under heavy pest pressure.

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Appendix: Diagnosing injury and effects of brown marmorated stink bug feeding on various vegetables

INTRODUCTION

Stink bugs feed by inserting their stylets into the stems, leaves, buds, fruit, or pods of their host plants. Pentatomidae typically use a lacerate and flush method of feeding, where they use their stylets to lacerate an area of cells and then break down the lacerated cells with saliva (Miles 1972; Velicova et al. 2010). The enzymes, injected into the feeding site along with their saliva, liquefy the plant tissue and then they draw the liquefied plant tissue back up through the stylets (McPherson and McPherson 2000, Peiffer and Felton 2014). Feeding injury reduces the quality and marketability of the fruit. Feeding may also cause early fruit set or abortion of the fruit if the injury is severe enough (Nielsen and Hamilton 2009; Kuhar et al. 2012b). Brown marmorated stink bugs (BMSB) have been known to transmit bacteria or yeast infections such as *Eremothecium coryli* to plants when feeding as a way of indirectly damaging the plant (Brust and Rane 2013).

Crop losses of >50% have commonly been caused by heavy infestation of BMSB in fields. It appears that vegetables like sweet corn, okra, and pepper are highly preferred as host plants in vegetable cropping systems and suitable for reproduction. (Kuhar et al. 2012b) Tomatoes do not appear to be good for reproduction, but can suffer heavy damage. BMSB attacks most vegetable crops that are present from late July to October in the Mid-Atlantic region (Kuhar et al. 2012a).

BMSB move into fields late in the season to feed, and they can cause serious injury to a variety of vegetable crops. In crops like beans (*Phaseolus* spp.) and okra feeding injury may cause scarred, faded sunken areas, and deformed pods. In crops like tomatoes and peppers injury looks like faded or sunken areas, these are fleshy fruits and much of the damage is internal. The injury may result in discoloration that is visible (Rice et al. 2014).

Although we have observed 2nd-5th instars and adults of BMSB feeding on a wide variety of vegetables, there has been very few reports documenting the effects of feeding by different stages of the bug and at different stages of fruit development. My objective was to photograph and document different types of feeding injury by BMSB on vegetables.

METHODS

Mature Fruit

Mature bell peppers, tomatoes, and beans were used for this experiment. Fresh produce was washed, inspected for blemishes, and photographed prior to exposure to insects. Exposed areas were marked on the fruit with a permanent marker. Five to six insects of the adult or nymph life stage were placed in plastic quart containers. Fruit were exposed to insects through mesh at the top of the container for 24 hours. Peppers because of their shape had to be placed into the quart containers. Vegetables were removed after 24 hours and photographed.

Bagged bugs on plants in the field

Untreated bell peppers, tomatoes, eggplant, yellow squash, and bean plants with early stage developing fruit were selected from the field. Selected developing vegetables were photographed before being bagged. Insects were bagged (2 adults, 4-5 nymphs 2nd to 4th) over the developing fruit and were given 72 hours to feed under field conditions. Bags and insects were removed after 72 hours, and developing structures were inspected for any indication of feeding damage and photographed. Vegetables were checked every 3-4 days for changes in growth and photographs were taken if change was noted. If there was no sign of feeding injury developing fruit were left in the field after last photograph was taken. In addition to bagging insects, a control was used to ensure bags did not negatively impact fruit development.

Effect of BMSB feeding on asparagus growth

We conducted field experiment on growing asparagus at Kentland Research Farm near Blacksburg Virginia. We bagged 2-3 adult insects on asparagus spears using mesh bags for 48 hours and compared to control of bag covered asparagus with no BMSB. Spears were measured before bags were placed on the spears. After 48 hours spears were cut at ground level and taken back to the lab to be measured and photographed. We used 20 spears in all and recorded the growth of each spear over 48 hours.

RESULTS AND DISCUSSION

Lab Feeding Trial

Mature peppers (Fig. A.1 A), tomatoes (Fig A.2 A), and beans (Fig. A.3 A) exposed to 2-3 adult BMSB for 24 hours showed no obvious injury. Mature peppers (Fig A.1 B) exposed to 4-5 nymphs for 24 hours showed no obvious injury, however mature tomatoes (Fig. A.2 B) and beans (Fig. A.3 B) exposed to 4-5 nymphs for 24 hours showed extensive feeding injury. Extending the feeding period to 48 hours would likely improve the results, and allow for a comparison of adult and nymph feeding injury.

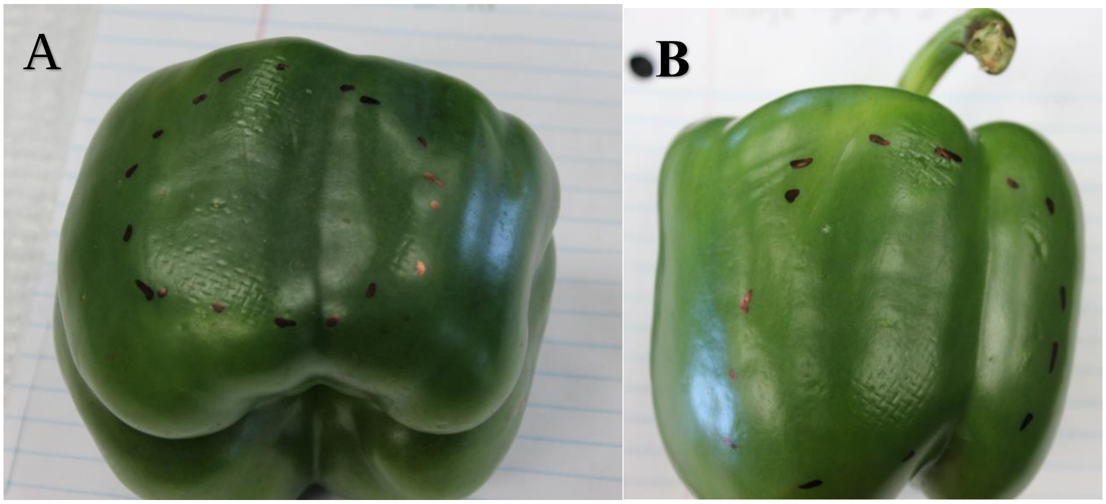

Fig. A.1. Mature bell peppers after 24 hours of exposure to adult (A) and nymph (B) BMSB.

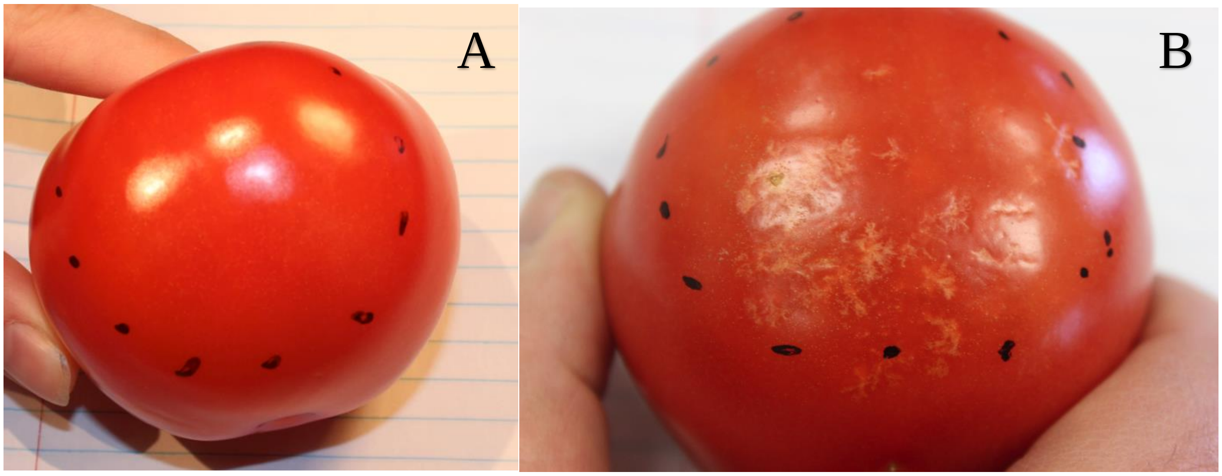

Fig. A.2. Mature tomatoes after 24 hours of exposure to adult (A) and nymph (B) BMSB.

Fig. A.3. Mature beans after 24 hours of exposure to adult (A) and nymph (B) BMSB.

<u>Bagged bugs on plants in the field</u>

Untreated bell peppers showed no obvious injury after 72 hours of exposure to adult and nymph BMSB. Adults and nymphs were found dead in the bags after the 72 hour feeding period. Fruit were examined at 2 and 5 days (Fig. A.4) after the initial feeding period and no injury had developed. Fruit were left in the field after the final photographs were taken.

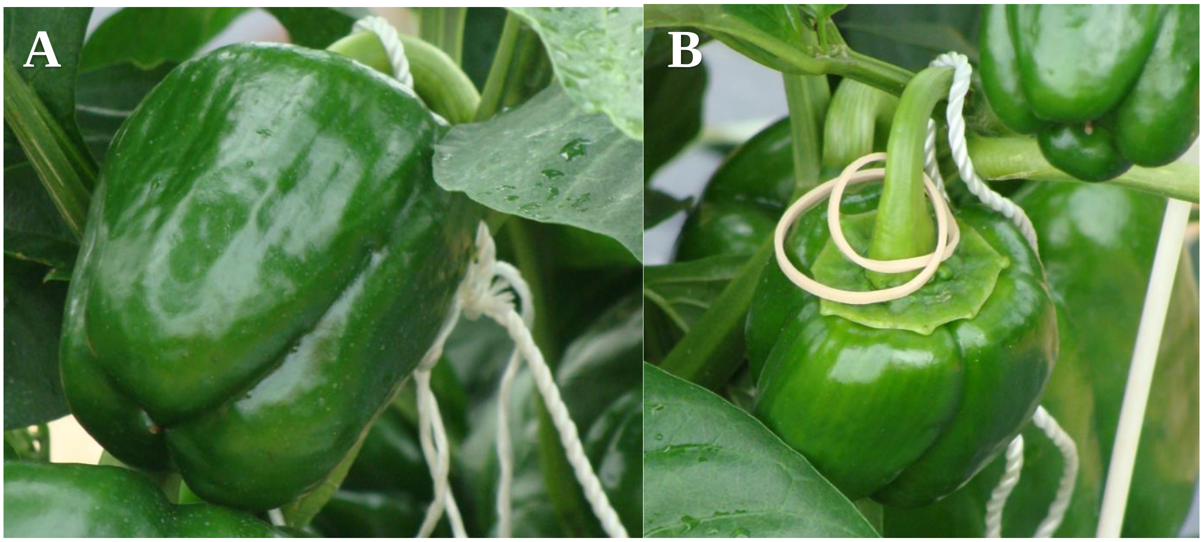

Fig. A.4. Bell peppers 5 days after a 72 hour exposure to BMSB adults (A) and nymphs (B).

Untreated tomatoes showed no obvious injury after 72 hours of exposure to adult and nymph BMSB. Adults were found alive, but nymphs were found dead in the bags after the 72 hour feeding period. Fruit were examined at 2 and 5 days (Fig. A.5) after the initial feeding period and no feeding injury had developed. Tomatoes developed a necrotic spot 2 days after the stink bugs were removed, this resulted in the fruit rotting and falling off the plant.

Fig. A.5. Tomatoes 5 days after a 72 hour exposure to BMSB adults (A) and nymphs (B).

Untreated Eggplants showed no obvious injury after 72 hours of exposure to adult and nymph BMSB. Adults were found alive, but nymphs were found dead in the bags after the 72 hour feeding period. Fruit were examined at 2 and 5 days (Fig. A.6) after the initial feeding period and no feeding injury had developed. Eggplants fully developed and no additional injury appeared.

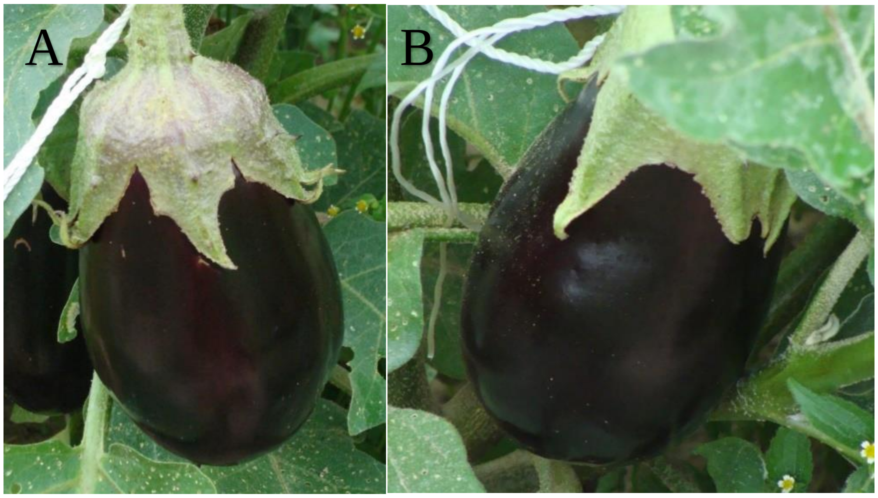

Fig. A.6. Eggplant 5 days after a 72 hour exposure to BMSB adults (A) and nymphs (B).

Untreated squash showed no obvious injury after 72 hours of exposure to adult and nymph BMSB. Many of the adult and nymph BMSB went missing from the bags as the fruit grew beyond the capacity of the bag, and any nymphs we did find were dead. Fruit were examined at 2, 5, and 10 days (Fig. A.7) after the initial feeding period and no feeding injury had developed. Squash fully developed and no additional injury appeared.

Fig A. 7. Yellow squash 10 days after a 72 hour exposure to BMSB adults (A) and nymphs (B).

Untreated beans showed some injury after 72 hours of exposure to adult BMSB, and some deformity took place as the bean grew (Fig A.8 A). Beans did not show any injury from nymph BMSB (Fig A.8 B) Fruit were examined at 2, 5, 7, and 12 days after the initial feeding period and most showed no feeding injury.

Fig. A. 8. Beans 12 days after a 72 hour exposure to BMSB adults (A) and nymphs (B).

Effect of BMSB feeding on asparagus growth

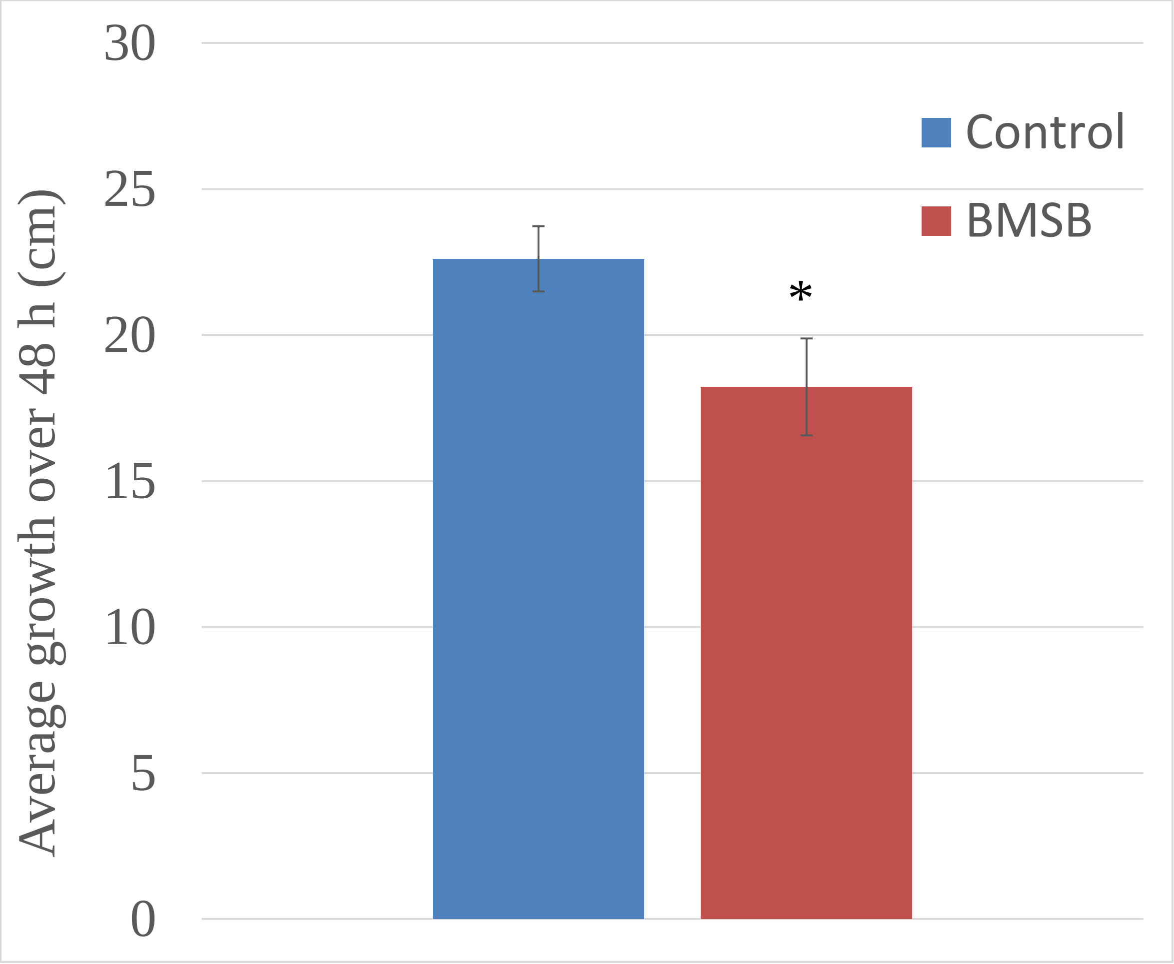

Figure A.9. Average growth of asparagus growth (cm) over 48 hours with adult BMSB present. *Significantly different according to Fisher's protected LSD.

Our data shows that BMSB can have a significant impact on asparagus growth (Fig. A.9), and we observed them feeding primarily near the apical meristem of the asparagus spear. Under heavy feeding pressure it could be hypothesized that deformity, stunting the growth, and possibly death of the spear could occur.

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Diagnosing stink bug injury to vegetables

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In the mid-Atlantic U.S. vegetable crops are attacked by several different stink bug species (1). The primary pest species include: the invasive brown marmorated stink bug (BMSB), *Halyomorpha halys*, which has become the dominant species in most landscapes (2), brown stink bug, *Euschistus servus* Say, which is the most common species attacking tomatoes; green stink bug, *Chinavia hilaris* Say (3); and harlequin bug, *Murgantia histrionica*, which is primarilly a pest of brassica vegetables only (4). All stink bugs are piercing sucking feeders that insert their stylets into the fruit, pods, buds, leaves, and stems of plants. Their injury can manifest itself in different ways. For instance, feeding on the fruit of peppers (Fig. 1) and tomatoes (Fig. 2) will produce characteristic white or yellow scars on the skin where the feeding stylets were inserted into the fruit, or sunken in areas from the internal fruit tissue collapsing below (Fig. 3). In corn, the feeding stylets of BMSB nymphs and adults are inserted through the husk and pierce the tender kernels, which may cause them to become aborted, collapsed or discolored (Figs. 4 & 5). Feeding injury to beans may result in scarred, faded out sunken areas (Fig. 6), as well as deformed pods (Fig. 7), which also occurs in okra (Fig. 8). The primary pest of brassica crops such as collards, broccoli, cabbage, and kale is harlequin bug. Both adults and nymphs of this species feed on aboveground plant tissues, leaving characteristic white blotches on the leaves (Fig. 9), which can turn necrotic and wilt under heavy pest pressure (Fig. 10).

Fig. 1. BMSB nymph feeding on pepper. (Photo by A. Morehead)

Fig. 2. Brown stink bug feeding on tomato. (Photo by A. Morehead)

Fig. 3. Damaged white spongy tissue below where BMSB stylets were inserted. (Photo by G. Dively, U. MD).

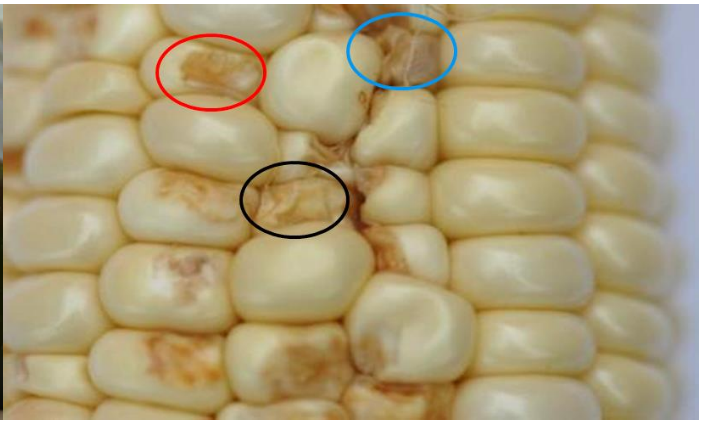

Fig. 4. BMSB feeding injury on corn kernels (Photo by W. Cissel, Univ. Delaware).

Fig. 5. BMSB feeding injury on corn kernels. (Photo by T. Kuhar)

Fig. 6. Severe BMSB feeding injury on snap beans. (Photo by H. Doughty, Virginia Tech)

Fig. 7. Resulting deformity from BMSB feeding on developing bean pods. (Photo by A. Morehead)

Fig. 8. Resulting deformity from BMSB feeding on developing okra pods. (Photo by G. Dively, U. MD)

Fig. 9. Harlequin bug feeding injury on collards. (Photo by T. Dimeglio)

Fig. 10. Severe harlequin bug feeding injury. (Photo by T. Dimeglio)

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