# ABSTRACT

Title of Document: INDIGENOUS NATURAL ENEMIES OF THE INVASIVE BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE)

Ashley Lynn Jones, Master of Science, 2013

Directed By: Associate Professor Paula Shrewsbury, Department of Entomology

Brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive species native to Southeastern Asia. Since its arrival into the U.S., BMSB has become an economically important pest in many cropping systems, including woody ornamental plants. Here I have explored the potential impact of indigenous natural enemies on BMSB in woody ornamental nursery systems in Maryland. When sampling for indigenous natural enemies in 2012 and 2013, I found seven species of egg parasitoids attacking BMSB with especially high parasitism rates from *Anastatus reduvii*. Overall egg mortality averaged 58% and parasitism rates increased from32% in 2012 to 44% in 2013. When sampling for predators as biological control agents, predation was low overall. I found that *Arilus cristatus* consumed more BMSB than any other predator species tested though low abundances were observed in the field. I also found that the use of sentinel egg masses may underestimate rates of parasitism.

# INDIGENOUS NATURAL ENEMIES OF THE INVASIVE BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE)

By

Ashley Lynn Jones

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Advisory Committee:
Associate Professor Paula M. Shrewsbury, Chair
Associate Professor Cerruti R.R. Hooks
Professor Michael J. Raupp

# Dedication

This work is dedicated to all of my family and friends for their unwavering support throughout every endeavor of my life. I am so fortunate to have this amazing network of endless love and encouragement. To my Dad, who is my greatest hero, has always taught me all the right lessons about how to succeed and be happy in this world. He has always had my back and no words can ever really express my love and gratitude for all he has done for me. To my Mom, who defines love and support of her children, has never been shy to tell me how proud she is and even in hard times has never backed down in her efforts to aid me in my life path. I am so lucky and proud to have such a strong woman to look up to, I am forever grateful. To Crystal, who has shown me a kindness and compassion I have seen equal in no other, has taught me so many invaluable lessons on how I can only wish to carry myself. I will always look up to your sense of caring, love, and pride you seem to always find in others. To my sister, who probably taught me the most about life, has always loved and supported me. Through thick and thin, I will always love you. To my Aunt Tina and John, for always providing love and laughs when I needed them most. To my close friends, for not only helping me get through school, but showing me that it is possible to find family in unfamiliar places. To all of my family and friends, I could not have done it without you, I love you all.

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## Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive species native to regions of China, Japan, Korea, and Taiwan. In its native range, BMSB is considered a pest of tree fruits, vegetables, legumes, and ornamental trees. The highly polyphagous nature of this insect as well as its vast dispersal capabilities requires an integrated approach to management. Here I focus on the potential impact of indigenous natural enemies on BMSB in woody ornamental nurseries in Maryland. I sampled naturally laid (wild) BMSB egg masses for mortality and parasitism rates in 2012 and 2013. I found seven species of Hymenopteran egg parasitoids attacking BMSB eggs with high rates from one particular species, *Anastatus reduvii*. Overall egg mortality averaged 58% for both years and parasitism rates increased from 32% in 2012 to 44% in 2013. This increase suggests that native parasitoids may be responding to this novel host. The use of biological control management strategies in this system may be a viable option with emphasis on Hymenopteran egg parasitoids.

# Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae), is an introduced, invasive species first detected in the United States in 1996 in Allentown, PA (Hamilton, 2009). This species is native to regions in Japan, China, and Korea (Hoebeke & Carter, 2003). The arrival of this insect is thought to have been through container shipments from Asia to the Northeastern U.S. At first, the insects were misidentified as a native species and not until 2001 were they properly identified as the exotic species *H. halys* (Hoebeke & Carter, 2003). At this point the population in Allentown, PA was well established and had begun to spread. Now BMSB have been sighted in numerous states throughout the U.S. with highest population densities concentrated around the mid-Atlantic states (Jones & Lambdin, 2009).

The life cycle of BMSB has been examined in the mid-Atlantic region of the U.S. In Allentown, PA, BMSB have one generation per year and in WV, they have two generations per year (Nielsen et al., 2008a, Leskey et al., 2012c). BMSB overwintering adults begin emerging in early to mid-April (Hoebeke & Carter, 2003). The females lay pale green egg masses containing approximately 28 eggs often on the underside of host plant leaves in late May and continue throughout the summer season (late-August). BMSB develop through five nymphal instars prior to reaching full maturity (Nielsen & Hamilton, 2009a, Hamilton, 2009). First instar nymphs are black and orange in coloration and congregate around the egg mass. They will then disperse as they mature to various locations on host plants to feed. Later instars lose the orange coloration but begin to form distinct banding patterns on their legs and antennae (Hamilton, 2009). The adults have a marbled brown and white coloration with distinct black and white patterned abdominal edges. The adults also maintain the white banding on the antennae which is a diagnostic characteristic (Hoebeke & Carter, 2003). BMSB adults begin overwintering between late- September to late-October. There is variation in the life cycle depending on seasonal differences and geographic location (Leskey et al., 2012a).

BMSB are polyphagous insects which have the potential to do severe damage to many economically important plant commodities such as tree fruits and soybeans (Nielsen et al., 2008a, Wermelinger et al., 2008). In eastern Asia, BMSB have been reported as a pest on ornamentals, woody plants, fruit trees, vegetables, and legumes (Jones & Lambdin, 2009, Leskey et al. 2012a). Specifically in southern China, it damages economically important species including: *Diospyros kaki* L. (persimmon), *Glycine max* Merrill (soybean), *Malus domestica* L. (apple), *Morus* spp. (mulberry), *Prunus persica* Batsch (peach), *Prunus avium* L. (cherry), and *Pyrus pyrifolia* Nakai (pear) (Hoebeke & Carter, 2003). Ornamental trees and shrubs that are damaged include: *Syringa* spp. (lilac), *Paulownia tomentosa* Steud (paulownia), *Hibiscus* spp. (hibiscus), *Cryptomeria* spp. (Japanese cedar), *Buddleja davidii* Franch. (butterfly bush), *Pyracantha coccinea Roem.* (firethorn), *Lonicera* spp. (honeysuckle), and *Cupressus* spp. (cypress) (Wermelinger et al., 2008).

In the U.S. damage to host plants ranges from minor with no noticeable yield loss to severe with high losses (Leskey et al. 2012b). The wide availability of multiple hosts in combination with the highly polyphagous nature of this insect allow for an extended geographic range and activity in a wide range of cropping and natural systems. BMSB were initially reported as nuisance pests in man-made structures in 2008-2009 (Inkley 2012, Pfeiffer et al., 2012). In 2010, throughout the Mid-Atlantic, they caused significant damage to agricultural crops, vineyards, and orchards. Hosts included soybean, corn, peaches, stone fruit, apples, tomatoes, paulownia, and butterfly bush (Leskey et al. 2012a). Reports from 2010 indicate some growers losing entire crops of stone fruit from this pest (Leskey et al., 2012b).

Adult and nymphal stages of BMSB feed on various parts of the host plant including stems, leaves, fruits, and trunk. They tend to change hosts throughout the season from those with early-ripening fruits to hosts with late-ripening fruits (Leskey et al. 2012a). The feeding damage caused by BMSB can be severe and lead to unmarketable products. Fruit damage is generally described as dimpled or discolored and if severe enough may cause a spongy texture to the inner tissue (Hoebeke & Carter, 2003). This outer tissue may produce a ring of necrotic tissue surrounding the stylet puncture which varies in coloration. In peaches, pears, and cherries, the ring of necrotic tissue will often be white to tan in color whereas in apples it is generally brown (Nielsen & Hamilton, 2009b).

BMSB are reported to favor woody ornamental trees early and late in the season but have been observed to remain in ornamental trees throughout the entire season (Hoebeke & Carter, 2003, Martinson et al., 2013). BMSB have been reported to cause stippling and lesion associated discoloration to butterfly bush and paulownia leaves (Welty et al., 2008). Feeding on ornamental fruits has been observed by mid-summer as well as heavy trunk feeding in early fall (Martinson et al., 2013). Discolored bark, profuse sap flow, disfigured fruit, and wilting of plants were some forms of damage observed to trees such as: hawthorns, elms, maples, serviceberries, sycamores, and crabapples (Shrewsbury & Raupp, unpub.). With such a large host range and highly polyphagous nature, BMSB present a risk to ornamental trees though the impact is not yet well known.

In natural habitats BMSB are believed to overwinter in wooded areas with dead trees and rocky outcroppings (Leskey et al., 2012a). However, BMSB are also classified as nuisance pests as they overwinter in buildings as adults (Inkley 2012). They tend to aggregate in large numbers in man-made structures which can be burdensome especially when in homes. The search for overwintering sites begins in late September when they gather in groups on sides of buildings (Hamilton, 2009). They then enter buildings through loose siding and cracks around windows, vent outlets, and doors. Public concern has risen over this issue and generated much attention about potential control (Inkley 2012).

For an invasive pest with such a wide host range and vast distribution, a multifaceted management approach is necessary. Management strategies being examined include chemical, cultural, and biological measures. In 2010, no proper insecticide management strategy had been developed for BMSB and only one laboratory study demonstrated the susceptibility of BMSB to a select number of pesticides (Nielsen et al., 2008b). The number of insecticide applications by tree-fruit growers from 2010 to 2011 increased almost four fold (Leskey et al., 2012b). Later studies found several chemicals to be effective including: endosulfan, malathion, permethrin, fenpropathrin, dinotefuran, and methomyl (Leskey et al., 2012b). The effect of insecticides on adult BMSB mobility was also examined where pyrethroid insecticides were found to induce irregular movement and eventual incapacitation (Lee et al., 2013a).

Forms of cultural control have also been explored such as trap cropping (Holtz & Kamminga, 2010). Trap cropping is the use of plants placed within or around target crops that attract pests and reduce damage to the target crop (Hokkanen, 1991). The use of early maturing varieties of hosts has been shown effective against the green stink bug in soybeans (McPherson et al., 1988). A study in Japan showed a similar technique applied to BMSB in soybeans where early maturing varieties held higher numbers of BMSB than mid- and late maturing varieties (Osakabe & Honda, 2002). The potential to eliminate overwintering sites and alternative hosts would be very difficult for this specific pest due to its preference for man-made overwintering sites and broad host range (Holtz & Kamminga, 2010). The method of mechanically removing BMSB eggs and nymphs has been suggested (Cai et al., 2008), but mechanical removal or destruction would also pose challenges in broad scale production systems. Vacuuming, however, from overwintering structures does appear to be a viable management practice to reduce their nuisance potential. The use of pheromone baited traps has also been evaluated as a control and/or monitoring tool with emphasis on one chemical in particular, methyl (2E,4E,6Z)-decatrienoate, a well-known attractant of BMSB (Khrimian et al., 2008). This is an aggregation pheromone of the brown-winged green bug, *Plautia stali* (Hemiptera: Pentatomidae) that has been observed to effectively attract BMSB in high numbers but with seasonal variation (Khrimian et al., 2008, Aldrich et al., 2009, Nielsen et al., 2011, Leskey et al., 2012c, Lee et al., 2013b). The use of black light traps has also been observed as an effective monitoring tool of BMSB population size and spread (Nielsen et al., 2013).

The potential for biological control as part of a BMSB management strategy is promising and likely to provide long-term, sustainable suppression of BMSB. There are two main methods of biological control commonly implemented against exotic pest species: classical biological control and conservation biological control (Barbosa, 1998). Classical biological control is the importation of exotic natural enemies from the home range of the introduced pest where conservation biological control is the utilization of native and established natural enemies in the exotic pests new range (Barbosa, 1998). Classical biological control may take up to several years to implement whereas conservation biological control can begin immediately.

BMSB has several reported natural enemies in its native region of eastern Asia. Studies performed within this native range show that egg parasitoids may be most effective as biological control agents (Zhang et al., 1993, Chu et al., 1997, Lanfen, 2007, Hou et al., 2009). These egg parasitoids include *Trissolcus halyomorphae* Yang, *Trissolcus flavipes* Thomson, *Trissolcus mitsukurii (*Ashmead), *Trissolcus plautiae* (Watanabe), *Trissolcus itoi* Ryu, *Telenomus mitsukurii* (Ashmead), *Telenomus nigripedius* Nakagawa, *Telenomus* sp. (Hymenoptera: Platygastridae), *Anastatus gastropachae* (Ashmead), *Anastatus* sp. (Hymenoptera: Eupelmidae), *Acroclisoides* sp. (Hymenoptera: Pteromalidae), *Ooencyrtus nezarae* Ishii, and *Ooencyrtus* sp. (Hymenoptera: Encyrtidae) (Kawada & Kitamura, 1992, Chu et al., 1997, Arakawa & Namura, 2002, Arakawa et al., 2004, Yu & Zhang, 2007, Lanfen, 2007, Lim et al., 2007, Hou et al., 2009, Lee et al., 2013b).

Of these reported egg parasitoids, *Te. mitsukurii* had a potential parasitism rate on BMSB eggs up to 84.7% (Chu et al., 1997), *Tr. flavipes* had a parasitism rate up to 63.3% (Zhang et al., 1993), *Tr. halyomorphae* had a parasitism rate up to 70% (Yang et al., 2009), and *Anastatus* sp. had a parasitism rate up to 77.2% (Hou et al., 2009). In addition, *Tr. mitsukurii* was observed to produce females with high fecundity after emergence from BMSB (Arakawa et al., 2004). Though there may be other types of natural enemies affecting BMSB populations in Asia, it appears that egg parasitoids are maintaining populations most effectively.

In the U.S. classical biological control is currently being explored with several species of *Trissolcus* (Leskey et al., 2012a). These include *Tr. mitsukurii, Tr. flavipes, Tr. itoi*, and *Tr. japonicas* (*halyomorphae* and *plautiae*) from Japan, China, and South Korea. These populations are currently maintained at the USDA ARS BIIR in Newark, DE for efficacy and host range testing (Hoelmer and Dieckhoff, personal communication). This specific approach often takes multiple years before initial releases of exotic natural enemies can begin. Therefore, other biological control measures also need to be explored.

Conservation biological control may be an effective and sustainable approach to pest management. An understanding and assessment of potential existing biological control agents is useful in designing and implementing a conservation biological control strategy (Tillman, 2011). Determining a baseline of indigenous and established natural enemy complexes in the non-native range of an exotic herbivore provides information on their possible direct impact on the pest and on the potential interactions with exotic natural enemies once released (Wallace & Hain, 2000).

In the U.S., indigenous egg parasitoids have been observed to parasitize BMSB egg masses. Those observed were *Anastatus reduvii* (Howard), *Anastatus pearsalli* Ashmead, *Anastatus mirabilis* (Walsh & Riley), *Trissolcus brochymenae* (Ashmead), *Trissolcus edessae* Fouts, Trissolcus *euschisti* (Ashmead), *Trissolcus thyantae* Ashmead, *Trissolcus utahensis* (Ashmead), *Telenomus podisi* Ashmead, *Telenomus utahensis* Ashmead, *Gryon obesum* Masner (Hymenoptera: Platygastridae), and *Ooencyrtus* sp. (Hoelmer and Dieckhoff, personal communication). *Trissolcus* species are egg parasitoids of hosts within the superfamily Pentatomoidea (Ryu & Hirashima, 1984, Okuda & Yeargan, 1988, Lanfen, 2007, Yang et al., 2009). *Anastatus* species are generalists egg parasitoids across several insect orders (Mendel et al., 1989, Hou et al., 2009). The rates of parasitism of these native egg parasitoids on invasive BMSB are currently unknown in naturally occurring eggs.

The overall objective of my studies is to determine mortality factors effecting BMSB eggs, identify the indigenous parasitoids associated with BMSB eggs, and quantify the impact these indigenous egg parasitoids impose on BMSB eggs. Studies will be conducted in nurseries producing ornamental woody trees and shrubs. Ornamental trees and nurseries were selected for these studies for several reasons. Ornamental plants are used by BMSB in both natural and managed environments. Within the U.S., woody ornamental production could be potentially at risk by BMSB. BMSB have also been observed feeding on numerous parts of woody ornamental trees including the fruit and foliage (Bergmann et al., unpub.) as well as the bark (Martinson et al., 2013). This may cause damage when trees are heavily infested by BMSB, decreasing the health and aesthetic value of trees. In addition, the woody ornamental tree (urban forest tree) industry has an estimated value exceeding $14 billion (Hall et al., 2006). Moreover, BMSB broadly use over 50 woody ornamental host plant species as feeding and/or oviposition hosts throughout the season (Bernon, 2004). The list of ornamental tree host species continues to grow (Bergmann et al., unpub.). Ornamental trees appear to be favored reproductive hosts with relatively high rates of BMSB oviposition (preliminary data).

The high rate of oviposition observed in many ornamental tree species allows an opportunity for surveying mortality factors of BMSB eggs and indigenous egg parasitoids in nurseries. If native parasitoids are attacking BMSB, recommendations on conservation biological control could be generated. These studies also provide baseline data on existing biological control which will be of interest when assessing impacts of exotic parasitoid releases.

## Methods

### *Study Sites and Experimental Design*

To assess BMSB egg mortality and obtain rates of parasitism by indigenous egg parasitoids in woody ornamental nurseries, three field sites within Maryland were chosen. The first site was Raemelton Farm in Adamstown, MD (Frederick County) and the other two sites were located at Ruppert Nurseries in Laytonsville, MD (Montgomery County). The two sites at Ruppert were separated by a distance of approximately 2 km. with no points of contact as well as roads, fields, residential property, and commercial property separating the sites. Three genera of trees, known to be used by BMSB for feeding and oviposition (personal observation), were chosen for monitoring at each nursery site (Table 1). Trees included *Acer rubrum* L. 'Franksred' (red maple), *Prunus serrulata* L. 'Kwanzan' (ornamental cherry), *Ulmus americana* L. 'Princeton' (American elm) and *Ulmus parvifolia* Jacq. 'Patriot' (Chinese elm). Two species of elms were used due to limited number of any one species at the multiple sites. The number of trees sampled of each species varied between nursery sites due to differences in abundance of each species at each nursery (Table 1). Each field site was sampled approximately twice weekly throughout the 2012 and 2013 BMSB activity period (late May – early September). Sampling dates were recorded.

### *Field Sampling of Trees for Eggs*

At every sampling event, all tree foliage up to a height of 2.7 m. was searched thoroughly for egg masses laid by BMSB. When located, the leaf containing the egg mass was marked with a piece of colored tape and the location of the egg mass within the tree was recorded. Egg masses were left in the field for one of two time periods: either 48 hours (post marking) or until eggs hatched. These two time frames were chosen to fully assess types of mortality that could vary temporally (48 hour time period for reducing the loss of parasitized eggs from predators or other factors and eggs left out until hatched were for monitoring predation). Egg masses on pieces of leaf were then collected from trees and placed in a 30 mL cup with lid for transportation to the laboratory for further analysis.

### *Assessment of Egg Fate*

Egg masses were brought back to the laboratory and placed in a 100mm x 15mm Petri dish (Thermo Fisher Scientific Incorporated). Petri dishes were then placed in a Percival incubator ($25^{o}C \pm 2^{o}C$, 16L: 8D) and monitored to determine their fate: BMSB hatch (egg survival); parasitoid emergence; or other sources of mortality including predation. If BMSB nymphs emerged they were removed within 24 hours. If BMSB nymphs did not emerge, egg masses remained in the incubator and were monitored for potential parasitoid emergence. Parasitoids that emerged were removed and placed in 70% ethanol with corresponding egg mass label for later identification. Egg parasitoids were identified according to “Hymenoptera Parasitoids associated with Pentatomidae in North America” (Dieckhoff et al., 2012). The number of parasitoids emerging (per egg mass), species, and sex were recorded. If BMSB or parasitoids did not emerge, eggs were further assessed to determine other potential causes of mortality.

Egg fate and mortality factors were assigned to individual eggs with the use of a stereo microscope. Egg fate categories included: “hatched” where BMSB emerged from the egg with no apparent mortality (Fig. 1a), “sucked” where there were three or more stylet sheaths protruding from the egg indicating a sucking predator had attacked the egg (Fig. 1b), “chewed” where eggs had been clearly fed upon by an organism with chewing mouthparts (Fig. 1c), “parasitized” where there was a distinct dark colored oviposition scar protruding slightly from the egg or parasitoid emergence had occurred (Fig. 1d,e), and “unascribed” where a direct cause of mortality could not properly be diagnosed. This “unascribed” category was subdivided into four subcategories: "non-hatch" where the eggs appeared unharmed and remained the usual mint green to white in coloration, "indented" where the eggs were clearly indented with no stylet sheath and remained normal in coloration (Fig. 1f), "missing" where the egg mass imprint clearly showed there were eggs missing, and "discolored" where the eggs were any color besides mint green to white including grey, brown, orange, and black (Fig. 1g). If there was no emergence of BMSB or parasitoids, eggs were then dissected for signs of parasitism.

### *Statistical Analysis*

The occurrence of eggs over time was standardized and expressed as the sums of eggs per $m^3$ of foliage pooled across all tree species and sites per week. Week was defined as all sampling conducted within a 7 day period and consisted of 1-2 visits to each site. Percent egg mortality was obtained per week ((sum of all eggs with no BMSB emergence in a week / sum of all eggs in a week) x 100). Similar calculations were used to estimate percent egg parasitism. Weeks are presented as May-4 which is the fourth week in May, June-1 which is the first week of June and so forth. A linear regression analysis was run on both mortality and parasitism over time for each year (Proc Reg, SAS Institute 2002-2008).

To obtain total egg mortality per year separated into individual mortality factors, percent egg mortality was calculated per week for each mortality factor ((sum of all eggs with no BMSB emergence due to a mortality factor in a week / sum of all eggs in a week) x 100). The resulting percentages were used to obtain the mean and standard error reported for the year. To compare the total egg mortality per factor between years, the percentages of egg mortality per week were run using a Two sample T-test at $P \leq 0.05$ (Proc Mixed, SAS Institute 2002-2008). A comparison of parasitoid sex ratios between years was made using $X^2$ test. All analyses were done using SAS version 9.2 (SAS Institute 2002-2008).

# Results

Throughout this study the total number of eggs collected was 56,197 (2,105 egg masses). Of this total, 24,124 eggs (897 egg masses) were collected in 2012 and 32,073 eggs (1,208 egg masses) collected in 2013. In 2012 and 2013, there was a trend for greater abundance of eggs in mid-June and mid-July (Figure 2). Percent egg mortality from all factors in 2012 ranged from 24.08 - 88.44% and increased significantly over time (y=4.25x + 24.63, $R^2$= 0.8719, df=1, $P < 0.001$) (Figure 3). In 2013, percent egg mortality ranged from 40.21 - 99.21% and increased significantly over time (y=2.72x + 38.29, $R^2$= 0.6209, df=1, $P$= 0.0008) (Figure 3). Percent egg parasitism in 2012 ranged from 7.08 - 50.00% and increased over time (y=2.53x + 12.53, $R^2$= 0.6282, df=1, $P$=0.0007). In 2013, percent egg parasitism ranged from 29.18 - 80.95% and increased over time (y=2.11x + 27.99, $R^2$= 0.4746, df=1, $P$= 0.006) (Figure 4). Parasitism of egg masses (one or more eggs were parasitized within the egg mass) was 47.05% in 2012 and 52.48% in 2013 (Table 2).

In 2012, parasitism was the greatest cause of mortality and averaged 31.83 ± 0.94%, chewing predation averaged 7.70 ± 2.36%, sucking predation averaged 2.24 ± 0.44%, and unascribed mortality averaged 15.39 ± 3.76% (Figure 5). In 2013, parasitism was again the greatest cause of mortality and averaged 43.83 ± 0.68%, chewing predation averaged 4.84 ± 0.96%, sucking predation averaged 2.27 ± 0.28%, and unascribed mortality averaged 7.78 ± 3.43% (Figure 5). Parasitism rates significantly increased from 2012 to 2013 (t=2.36, df=26, *P*= 0.03) (Figure 5). From 2012 to 2013, chewing predation (t= -1.12, df=26, *P*=0.27) and sucking predation (t=0.06, df=26, *P*=0.96) did not significantly differ (Figure 5). Unascribed mortality rates significantly decreased from 2012 to 2013 (t= -6.55, df=26, *P*<0.001) (Figure 5).

The number of eclosed parasitoid adults observed from egg masses in this study totaled 15,357 (5,638 in 2012 and 9,719 in 2013). The species observed included *An. reduvii*, *An. pearsalli*, *An. mirabilis*, *Tr. brochymenae*, *Tr. euschisti*, *Te. podisi*, and *Ooencyrtus* sp. (Figure 6a and 6b). Females were identified to species, males were pooled by genera. Of the total parasitoids observed in 2012: 61.17% were *An. reduvii* females, 3.87% were *An. pearsalli* females, 0.82% were *An. mirabilis* females, 32.12% were *Anastatus* males (most were associated with *An. reduvii* females), 1.14% were *Tr. brochymenae* females, 0.37% were *Tr. euschisti* females, 0.16% were *Trissolcus* males, 0.04% were *Te. podisi* females, 0.04% were *Telenomus* males, and 0.28% were *Ooencyrtus* sp. (Figure 6a). Of the total parasitoids observed in 2013: 79.12% were *An. reduvii* females, 1.98% were *An. pearsalli* females, 0.63% were *An. mirabilis* females, 16.77% were *Anastatus* males (most were associated with *An. reduvii* females), 0.96% were *Tr. brochymenae* females, 0.16% were *Tr. euschisti* females, 0.14% were *Trissolcus* males, 0.19% were *Te. podisi* females, and 0.05% were *Telenomus* males. No *Ooencyrtus* sp. were observed in 2013 (Figure 6b). The sex ratio of observed parasitoids (male: female) was 1: 2.05 in 2012 and 1: 4.86 in

2013. There was a significant relationship between sex and year of grouped *Anastatus* species with more females produced in 2013 than 2012 ($\chi^2$=494.21, df=1, $P$<0.001).

## Discussion

Surveying and evaluating the effect of indigenous natural enemies on an invasive pest species is a vital first step in biological control (Wallace & Hain, 2000). Once the natural enemy complex and their relative effect on this pest are determined, further progress may be made to establish biological control programs. The objective of this study was to determine what types of mortality affect BMSB eggs in woody ornamental nurseries with a main focus on egg parasitism and determine what parasitoid species attack BMSB eggs. In this two year study, I found seven species of indigenous Hymenopteran egg parasitoids attacking BMSB eggs and an overall high rate of total egg mortality.

BMSB females lay approximately 244 eggs over a lifetime with an average of 28 eggs per egg mass (Nielsen et al., 2008a). The ability to monitor BMSB seasonal abundance of eggs gives an adequate prediction of the potential population size and variation of BMSB populations within our study sites. The temporal abundance of eggs provides information on the voltinism of the insect. I found an increased abundance of eggs in mid-June and mid- July suggesting there were two generations of BMSB in Maryland. This information also supports the observation of two generations per year of BMSB in the mid-Atlantic region reported by Leskey et al. (2012c). Overall egg mortality was similar in both 2012 and 2013, with increasing rates of mortality as the season progressed. Parasitism was the main source of egg mortality. Overall parasitism rates increased from an average of 31.8% in the 2012 season to an average of 43.8% the 2013 season.

One parasitoid species, the Eupelmid *An. reduvii* was responsible for approximately 91% of all observed parasitism in this study. In total, three species in the genus *Anastatus* accounted for a combined 98% of all BMSB egg parasitism. *Anastatus* species have a history of successful biological control. Beginning in the 1960's, *Anastatus japonicas* Ashmead has been utilized for control of the lychee stink bug, *Tessaratoma papillosa* Drury (Hemiptera: Tessaratomidae) in China. The rearing and release of *An. japonicas* resulted in egg parasitism rates of over 90% (Lanfen, 2007). *Anastatus* sp. released in Beijing for control of pine moth averaged egg parasitism rates of 23.77% (Hou et al., 2009). *Anastatus* sp. have been shown to be effective in reducing populations of two fruit spotting bugs *Amblypelta nitida* and *Amblypelta lutescens lutescens* (Hemiptera: Coreidae) (Fay & De Faveri, 1997). Release techniques and efficacy are currently being evaluated for *Anastatus* sp. for *A. nitida* and *A.l. lutescens* (Danne et al., 2013). A native Asian *Anastatus* species was also tested against BMSB in Beijing with parasitism rates of 64.7% for the first generation and 52.6% for the second generation (Hou et al., 2009).

Parasitism has been previously reported for two of three *Anastatus* spp. found in this study. In one study, *An. reduvii* regularly parasitized egg masses of *Anisota peigleri* (Lepidoptera: Saturniidae) at low rates (average 30% of an affected egg mass) (Serrano & Foltz, 2003). *An. pearsalli* parasitized the eggs of *Leptoglossus occidentalis* (Hemiptera: Coreidae) at low rates (12.1% of two egg masses) (Maltese et al., 2012). Here I report parasitism rates of *An. reduvii* and *An. pearsalli* parasitizing BMSB eggs at rates of approximately 91% and 5% respectively.

Elevated abundance and high levels of parasitism in ornamental plant nurseries may be related to the preference of *Anastatus* spp. for arboreal habitats. *Anastatus* has been recorded parasitizing *Tessaratoma papillosa* Drury a Hemipteran pest of lychee and longan as well as other fruit trees. In addition, *Anastatus* parasitize eggs of pine moths which are also arboreal pests of pine trees, *Amblypelta nitida* and *Amblypelta l. lutescens* are pests of fruit and nut trees, *Anisota peigleri* are pests of oak trees, and *Leptoglossus occidentalis* are pests of coniferous trees (Lanfen, 2007, Hou et al., 2009, Danne et al., 2013, Serrano & Foltz, 2003, Maltese et al., 2012).

*Anastatus* species showed an interesting change in sex ratios. Collectively, the three *Anastatus* species had a sex ratio (male: female) of 1: 2.05 in 2012. This shifted to a sex ratio of 1: 4.86 in 2013. This shift in sex ratio from males to females may be due to an increase in host resource (more BMSB eggs). Many ovipositing parasitoids respond to the traces of other females by increasing the proportion of male progeny (Hamilton, 1967, Liljesthrom et al., 2013). It may be possible that male production decreased due to greater resource availability likely reducing the likelihood of female parasitoid interaction.

*Trissolcus* species have been a large focus of BMSB egg parasitoid research (Arakawa & Namura, 2002, Arakawa et al., 2004, Yang et al., 2009, Lanfen & Zhongqi, 2010, Talamas et al., 2013). *Trissolcus* species are specific to the superfamily Pentatomoidea and have many economically important hosts (Yang et al., 2009). Due to this specificity, there has been an emphasis on the testing and evaluating *Trissolcus* species for biological control. In Brazil, *Tr. basalis* was released for the control of stink bug populations in soybeans and a reduction in stink bug density of 58% was reported. This parasitoid was particularly effective against *Nezara viridula*, *Piezodorus guildinii*, and *Euschistus heros* (CorreaFerreira & Moscardi, 1996). In our study system, *Tr. brochymenae* and *Tr. euschisti* had a combined parasitism rate of 1.4% on BMSB eggs. This low rate of parasitism alone is unlikely to reduce BMSB populations. It appears as though *Anastatus* spp. and more specifically, *An. reduvii*, are more effective native egg parasitoids of BMSB than native *Trissolcus* spp. in woody ornamental nurseries. Further studies of population dynamics between native and exotic parasitoids will be needed if exotic *Trissolcus* parasitoids are released as a biological control for BMSB.

We know what indigenous egg parasitoids attack BMSB eggs and what proportion of eggs experience mortality in ornamental nurseries. With an overall average egg mortality of 57.2% in 2012 and 58.7% in 2013 and a parasitism rate which increased from 31.8% in 2012 to 43.8% in 2013, the prospect for biological control of BMSB in ornamental nurseries is promising.

Table 1: Total number of trees sampled at each of the three nursery sites by species.

| | **Number of Trees Sampled** | | | |
|---|---|---|---|---|
| **Site** | *Acer rubrum* | *Prunus serrulata* | *Ulmus americana* | *Ulmus parvifolia* |
| **1** | 19 | 47 | 73 | - |
| **2** | 28 | 20 | - | 31 |
| **3** | 87 | 70 | 100 | - |

Table 2: Total percentage of egg masses parasitized per year (one or more eggs were parasitized within the egg mass).

| Year | Total egg masses parasitized (%) |
|---|---|
| **2012** | 47.05 |
| **2013** | 52.48 |

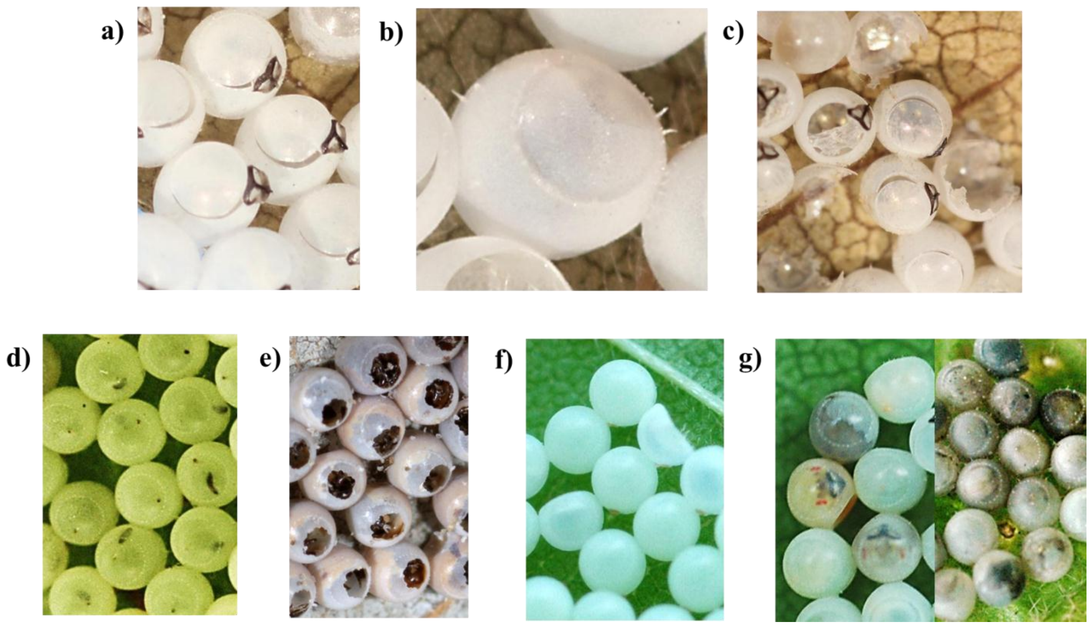


Figure 1:  Examples of egg mortality factors a) eggs where BMSB nymphs emerged (hatched) – no mortality b) Predation by haustellate insects with stylet sheaths protruding from egg c) Predation by mandibulate insects with jagged holes present on eggs d) Parasitized eggs with brown oviposition scars and discoloration e) Eggs with parasitoid emergence – exit holes have been chewed from discolored eggs f) Indented eggs where mortality is unknown g) Unascribed mortality – eggs with varying discoloration and no signs of predation or parasitism.

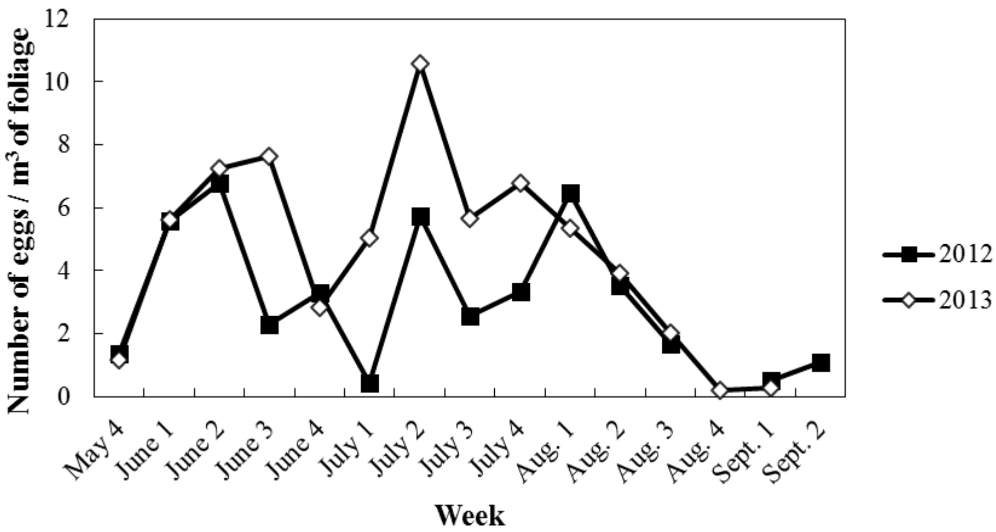


Figure 2: Abundance of BMSB eggs per $m^3$ of foliage over time in 2012 and 2013. Egg abundance was the total number of eggs observed on all trees at all sites every week. One week was the total number of eggs observed in a seven day period.

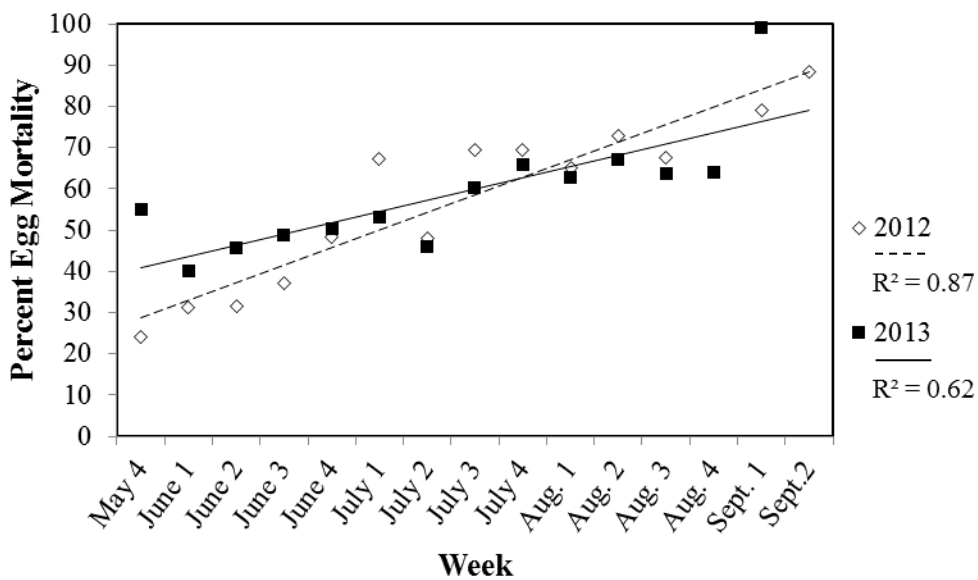


Figure 3: Percent egg mortality over time in 2012 and 2013. Percent egg mortality was the total # eggs without BMSB emergence / total # eggs for a given week. One week was the total number of eggs observed during in a seven day period. In 2012, the linear regression of percent egg mortality (y) on time (x) was $y = 4.25x + 24.64$, df=1, $P< 0.001$. In 2013, the linear regression of percent egg mortality (y) on time (x) was $y = 2.72x + 38.29$, df=1, $P= 0.0008$.

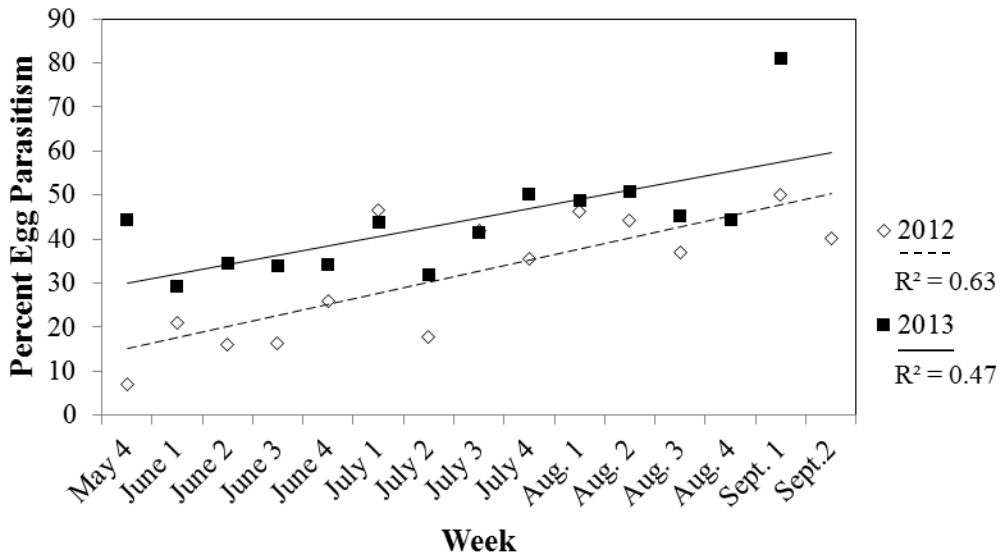


Figure 4: Percent egg parasitism over time in 2012 and 2013. Percent egg parasitism was the total # eggs parasitized / total # eggs for a given week. One week was the total number of eggs observed during in a seven day period. In 2012, the linear regression of percent egg mortality (y) on time (x) was y = 2.52x + 12.53, df=1, *P*= 0.0007. In 2013, the linear regression of percent egg mortality (y) on time (x) was y = 2.11x + 27.99, df=1, *P*= 0.006.

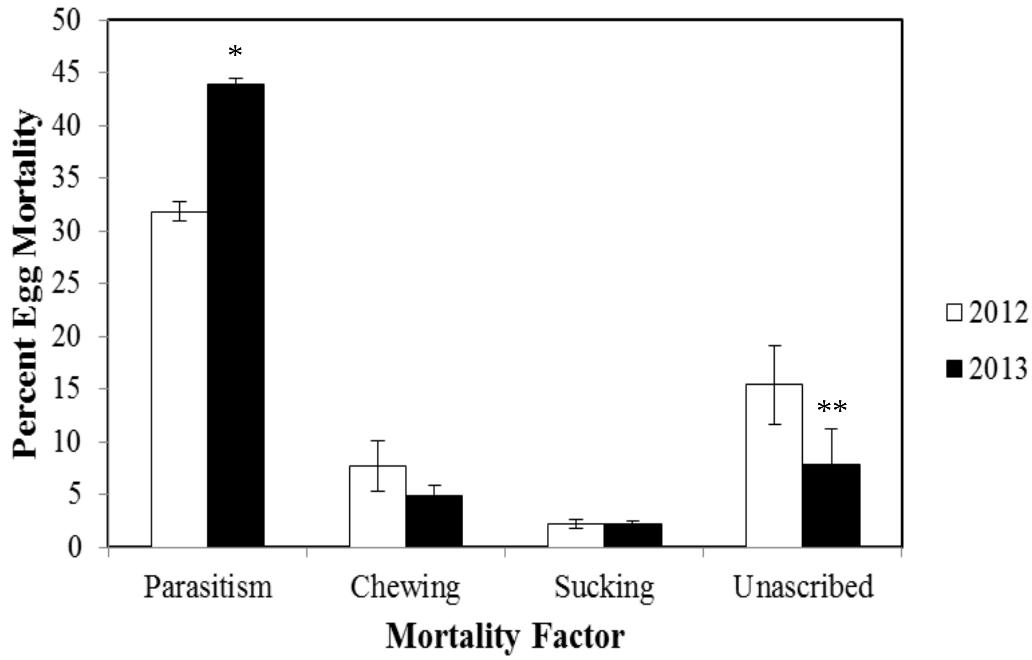


Figure 5: Mean percent egg mortality for each category of mortality pooled over all tree species and sites by week. Bars represent means and lines represent standard errors. * denotes a significant difference between years at $P \leq 0.05$. ** denotes a significant difference between years at $P \leq 0.001$.

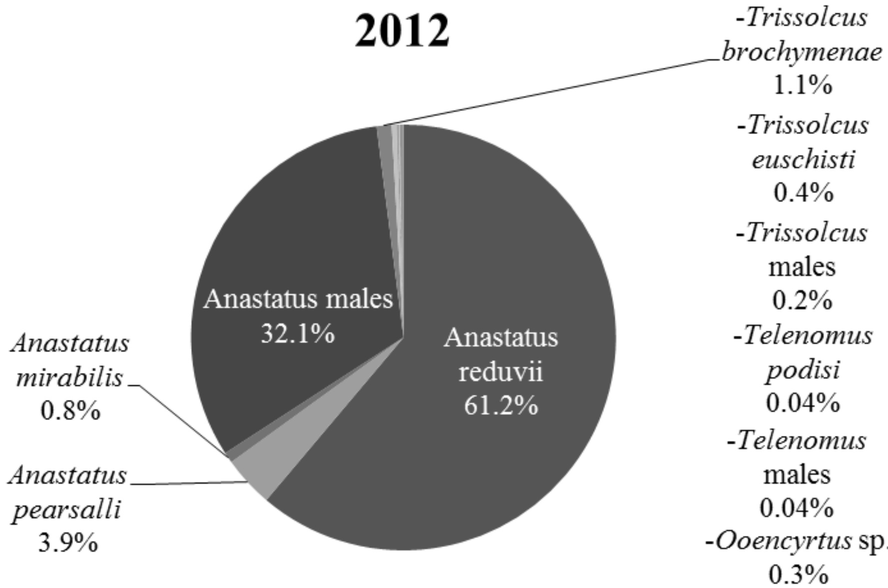


Figure 6a: Species of native egg parasitoids observed from parasitized BMSB eggs in 2012. Percentages calculated from total number of parasitoids eclosed from sampled eggs. Males were grouped by genera; females were identified to species.

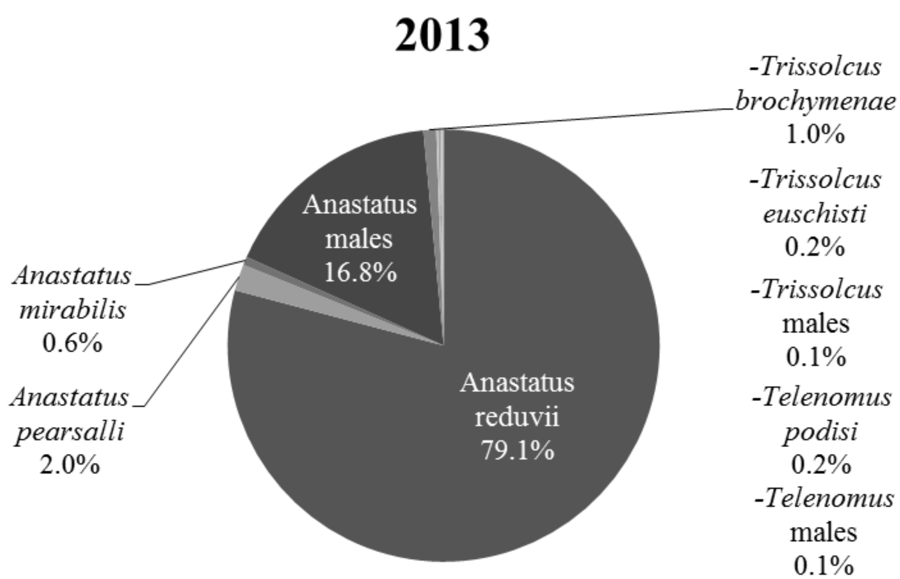


Figure 6b: Species of native egg parasitoids observed from parasitized BMSB eggs in 2013. Percentages calculated from total number of parasitoids eclosed from sampled eggs. Males were grouped by genera; females were identified to species.

# Chapter 2: Survey and evaluation of endemic predators of the brown marmorated stink bug, *Halyomorpha halys*, in woody ornamental nurseries of the mid-Atlantic.

## Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive species native to regions of Southeastern Asia. In its native range, BMSB is considered a pest of tree fruits, legumes, vegetables, and ornamental trees. The wide host range of this pest and the vast dispersal capabilities require the use of many forms of management. Here I focus on the potential for biological control of BMSB in woody ornamental nurseries in Maryland. Field surveys of BMSB and predator densities revealed low abundances of predator populations. When conducting feeding trials to elucidate the relative impact of different predator species, I found that wheel bugs, *Arilus cristatus*, consumed significantly more BMSB than any other predator species tested. Overall, endemic predators provide some potential for biological control of BMSB, but their overall impact is unknown.

## Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae) is an invasive species native to China, Japan, Korea, and Taiwan (Hoebeke & Carter, 2003). The first appearance of the BMSB in the U.S. can be traced back to Allentown, PA in 1996 (Hamilton, 2009). BMSB is now established throughout the mid-Atlantic states (Leskey et al., 2012a). BMSB are polyphagous in nature and their hosts include many economically important plant commodities such as soybeans and tree fruits (Leskey et al ., 2012a, Nielsen et al., 2008a, Wermelinger et al., 2008). In their native range, BMSB are known pests of ornamentals, woody plants, vegetables, fruit trees, and legume crops (Jones & Lambdin, 2009).

BMSB can cause severe feeding damage and has the potential to destroy entire crops at high densities (Leskey et al., 2012b). Management strategies for BMSB are difficult to develop due to their broad host range and high mobility. Conventional chemical controls are especially challenging to administer due to BMSB's wide distribution, mobility, and tendency to continually immigrate into crop fields over time (Lanfen, 2007). The current emphasis for reductions in pesticide use in food production, create opportunities for biological control, a sustainable form of pest management for many invasive pests.

Biological control is the use of living natural enemies to control pest species (Barbosa, 1998). Conservation biological control is the preservation or protection of endemic natural enemies already present within a system (Barbosa, 1998). The use of conservation biological control could be effective against BMSB in large production systems. Identifying endemic natural enemies that attack BMSB and evaluating their levels of impact is the first step to developing and implementing a conservation biological control program. Evaluation of natural enemies is important as it provides insight to the value and weaknesses of the indigenous natural enemy complex (Wallace & Hain, 2000).

The use of egg parasitoids as biological control agents against BMSB has been a large research focus (Arakawa & Namura, 2002, Arakawa et al., 2004, Yang et al., 2009, Lanfen & Zhongqi, 2010, Talamas et al., 2013) (See Jones Chap. 1). However, predators may also be a vital suppressor of BMSB populations (Biddinger et al., 2012). In their native range, BMSB have several predators. These include: *Orius* sp. (Hemiptera: Anthocoridae), *Arma chinensis* (Fallou) (Hemiptera: Pentatomidae), *Misumena tricuspidata* (Araneida: Thomisidae), *Astochia virgatipes* Coquillett (Diptera: Asilidae), and *Isyndus obscurus* (Dallas) (Hemiptera: Reduviidae) (Lanfen, 2007, Kawada & Kitamura, 1992, Lee et al., 2013b). However, the impacts of these predators on BMSB populations are not well described.

Only anecdotal accounts of the predator complexes attacking BMSB within cropping and natural systems in the mid-Atlantic region are reported to date, including predators found in ornamental nursery systems. Therefore, the objective of this study was to determine what predators were associated with and potentially attacking BMSB in woody ornamental nurseries. Field surveys and laboratory feeding trials provided information on predator abundance and attack rates. With this information, we can identify predators with high potential as biological control agents and place specific attention on their conservation within production nurseries.

# Methods

## *Field Sites*

To obtain information on BMSB predators and their abundances in woody ornamental nurseries, three field sites within Maryland were chosen. The first site was Raemelton Farm in Adamstown, MD (Frederick County) and the other two sites were located at Ruppert Nurseries in Laytonsville, MD (Montgomery County). The two sites at Ruppert were separated by a distance of approximately 2 km with mixed use landscapes including roads, fields, residential property, and commercial property interspersed. The Raemelton site was visited biweekly from late July to late October 2011. Approximately 150 trees were monitored during each visit at this site in 2011. The Raemelton and Ruppert sites were visited biweekly throughout the 2012 growing season from late June to mid-August. Approximately 180 trees total were monitored at each visit in 2012.

## *BMSB and Predator Field Density*

To monitor BMSB and predator abundance, two, three minute timed visual counts were administered for each tree at each monitoring visit (three minutes for BMSB density and three minutes for predator density). All foliage, fruit, and bark on trunk and branches were examined for the presence of BMSB and predators. For BMSB, the number of all active stages observed on each tree was recorded and categorized by life stage: early nymphs ($1^{st}$ and $2^{nd}$ instars), late nymphs ($3^{rd}$, $4^{th}$, and $5^{th}$ instars), and adults. All predators observed were counted, identified to species in the case of insects and species or family in the case of spiders, and their life stage was recorded.

### *Laboratory Feeding Trials*

The most abundant predators observed in the nurseries and predators readily available commercially were selected for feeding trials. Commercially available predators were included as a presage for augmentative biological control, the practice of introducing biological control agents into an environment through inoculative releases (Barbosa, 1998). Feeding trials were designed to answer two questions: do predators in nurseries and commercially available predators feed upon BMSB and do predators differ in their rate of consumption?

Predators collected from the field that were used in feeding trials included: Adult and nymphal ($3^{rd}$ and $4^{th}$ instars) wheel bugs, *Arilus cristatus* L. (Hemiptera: Reduviidae); multicolored Asian lady beetle adults, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae); rough stink bug adults, *Brochymena quadripustulata* (Fabricius) (Hemiptera: Pentatomidae); daring jumping spider adults, *Phidippus audax* Hentz and woodland jumping spider adults, *Thiodina* sp. (Araneae: Salticidae). Purchased predators were ordered through Green Methods.com (Beneficial Insectary, Inc., Redding CA), a commercial distributor of natural enemies, and included: green lacewing larvae ($3^{rd}$ instar), *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae); minute pirate bug adults, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae); and convergent lady beetles, *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae).

Feeding trials were administered in a completely randomized design. All predators tested were starved for 48 hours with access to water via moist sponge prior to the feeding trial. Predators were then placed individually into 15 x 15 x 9cm screen cages and provided an unlimited food resource of varying life stages of BMSB for 48 hours. Not all predators received every life stage of BMSB due to difficulty in synchronizing BMSB life stages with predator life stages. Cages with no predators served as controls. The number or replicates, life stage of prey provided, and number of prey provided in each trial can be found in Table 3. All cages were held a Percival incubator (25$^{o}$C ± 2$^{o}$C, 16L: 8D). Every 24 h, consumption rates of predators (# prey eaten/ 1 d) were recorded for the 48 h duration of the trial.

### *Statistical Analysis*

For field surveys, average counts of BMSB and predators were established by taking the sum of all BMSB or predators counted in one week and dividing by the number of trees sampled that week. A week was defined as the sampling effort within a 7 day period and presented as month and week number sampled (ex. July-4 is the fourth week in July). A linear regression was run on both BMSB and predator densities over time for each year to determine if densities changed over time and if predator density might be responding to BMSB densities. Feeding trial data were corrected for control mortality using Abbott's formula:

$$P= (P'-C) / (1-C)$$

Where P= Abbott's corrected proportion, P'= observed proportion responding to treatment test, and C= proportion responding to control test (Abbott, 1925). The corrected totals were compared by each prey life stage using a one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparison at $P \leq 0.05$. All analyses were done using SAS version 9.2 (SAS Institute 2002-2008).

# Results

## *BMSB and Predator Field Density*

In 2011, 194 predators and 1,409 BMSB were observed. In 2012, 379 predators and 883 BMSB were observed. Predators included: wheel bugs, *A. cristatus* (adults and nymphs); lacewings, *Chrysoperla* sp. (larvae); spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae); praying mantises, *Tenodera sinensis* and *Stagmomantis carolina* (Mantodea: Mantidae); jumping spider species (Araneae: Salticidae); crab spider species (Araneae: Thomisidae); and orb-weaver spider species (Araneae: Araneidae). Predators and their abundances are presented in Table 4.

In 2011, BMSB decreased dramatically in abundance over time (y= -0.88x + 5.16, $R^2$=0.71, df=1, *P*= 0.02) (Figure 7a). Predators observed in 2011 also decreased dramatically in abundance over time (y= -0.06x + 0.43, $R^2$= 0.75, df=1, *P*=0.01) (Figure 7b). In 2012, no significant trend was observed in the abundance of BMSB over the course of the season (y= 0.04x + 1.26, $R^2$= 0.011, df=1, *P*=0.83) (Figure 8a). A similar lack of temporal pattern was seen in predators observed in 2012 (y=0.02x + 0.65, $R^2$= 0.014, df=1, *P*=0.80) (Figure 8b).

## *Laboratory Feeding Trials*

When given adult BMSB as a prey item, adult *A. cristatus* consumed 0.75 ± 0.05 per day, *P. audax* and *Thiodina* sp. (combined results due to low numbers of each jumping spider species) consumed 0.22 ± 0.11 per day, and adult *B. quadripustulata* consumed no BMSB. When compared, *A. cristatus* and *P. audax* /

*Thiodina* sp. had significantly different consumption rates (t=4.24, df=30, $P<0.001$). *A. cristatus* and *B. quadripustulata* also had significantly different consumption rates (t=4.80, df=30, $P<0.001$) (Figure 9). When given 2$^{nd}$ and 3$^{rd}$ instar BMSB as prey items, *A. cristatus* (nymphs) consumed 2.56 ± 0.20 per day and *H. convergens* consumed 0.05 ± 0.18 per day. *Arilus cristatus* consumed significantly more nymphs than *H. convergens* (t=9.48, df=16, $P<0.001$) (Figure 10).

When provided 1$^{st}$ instar BMSB as prey, *A. cristatus* (nymphs) consumed 4.0 ± 0.44 per day, *C. rufilabris* consumed 1.54 ± 0.30 per day, *O. insidiosus* consumed 0.22 ± 0.41 per day, *H. convergens* consumed 0.37 ± 0.37 per day, and *H. axyridis* consumed 0.67 ± 0.39 per day. *A. cristatus* consumed significantly more 1$^{st}$ instar BMSB than all other predators: *H. axyridis* (t=5.76, df=44, $P<0.001$), *C. rufilabris* (t=4.71, df=44, $P<0.001$), *O. insidiosus* (t=6.36, df=44, $P<0.001$), and *H. convergens* (t=6.42, df=44, $P<0.001$). *C. rufilabris* consumed significantly more 1$^{st}$ instar BMSB than *H. convergens* and *O. insidiosus* (t=2.46, df=44, $P<0.05$) and (t=2.60, df=44, $P<0.05$) respectively (Figure 11). No predators consumed BMSB eggs.

## Discussion

Laboratory feeding trials found that adult and nymphal stages of *A. cristatus*, consumed BMSB active stages. Compared with other predators, *A. cristatus* consumed significantly more adults, 1$^{st}$ instars, and 2$^{nd}$ and 3$^{rd}$ instars of BMSB. *Arilus cristatus* also reached the second greatest abundances in the field, second only to spiders. *Arilus cristatus* could be viewed as an important predator of BMSB in woody ornamental nurseries. *Arilis cristatus* and BMSB have overlapping habitats in the field as they are both commonly found in trees (Martinson et al., 2013, Hagerty & McPherson, 2000). Late in the season, BMSB can be found feeding through tree bark as either adults or late nymphs (Martinson et al., 2013) and adult *A. cristatus* can be found searching for oviposition sites (Hagerty & McPherson, 2000). This overlap in habitat preferences and the effectiveness of this predator combined may contribute to population reductions of BMSB.

*Chrysoperla rufilabris* consumed higher rates of $1^{st}$ instar nymphs than either *O. insidiosus* or *H. convergens*. These predators represent potential biological control agents which can be ordered from commercial sources and used in biological control programs. If a particular habitat contains high numbers of $1^{st}$ instar BMSB nymphs, the release of *C. rufilabris* may be beneficial in control efforts.

The majority of predators observed in the field were species of spiders and *A. cristatus*. When assessing the effectiveness of biological control agents, host specificity has been observed as an effective trait for successful control (Kimberling, 2004). Spiders and wheel bugs are generalist predators and although they are not host specific, they may still be important biological control agents (Murdoch et al., 1985). The diversity in diet of generalist predators has been observed to significantly enhance predator survival and fecundity (Harwood et al., 2009). Fluctuations in population densities of generalist predators may also be observed yearly due to their polyphagy and ability to respond to changing prey densities (Symondson et al., 2002). This may explain the varied rates in which our predator numbers have changed over time. Overall, patterns in BMSB and predator abundance suggest that predator abundance may be tracking prey abundance but further studies need to be conducted to directly test this potential response. Though likely useful in an overall biological control strategy as part of a suite of natural enemies, relying on endemic predators alone in woody ornamental nurseries may not be the most effective route for biological control of BMSB.

Table 3: Experimental conditions for predator feeding trials with brown marmorated stink bugs as prey.

| Predator | Prey life stage | # Prey provided | # Reps | # Controls |
|---|---|---|---|---|
| ***A. cristatus* (adult)** | Adult | 5 | 25 | 9 |
| ***A. cristatus* (nymph)** | Egg | 27-28 | 5 | 1 |
| | 1st Instar | 15 | 7 | 3 |
| | 2nd and 3rd Instar | 10 | 8 | 3 |
| ***B. quadripustulata*** | Adult | 2 | 3 | 3 |
| ***C. rufilabris*** | Egg | 27-28 | 3 | 2 |
| | 1st Instar | 15 | 15 | 5 |
| ***H. axyridis*** | Egg | 27-28 | 1 | 1 |
| | 1st Instar | 15 | 9 | 3 |
| ***H. convergens*** | Egg | 27-28 | 3 | 2 |
| | 1st Instar | 15 | 10 | 3 |
| | 2nd and 3rd Instar | 5 | 10 | 3 |
| ***O. insidiosus*** | Egg | 27-28 | 2 | 2 |
| | 1st Instar | 15 | 8 | 3 |
| ***P. audax* and *Thiodina* sp** | Adult | 2 | 5 | 3 |

Table 4: Abundance (number per tree) of predators observed through weeks 1-7 in 2011 and 2012.

| **Predator** | **Number observed per tree per week in 2011** | | | | | | | **Number observed per tree per week in 2012** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **Total** |
| Wheel bugs (nymphs and adults) | 0.046 | 0.033 | 0.007 | 0.027 | 0.020 | 0.007 | 0.007 | 0.517 | 0.008 | 0.100 | 0 | 0.067 | 0.025 | 0.067 | **0.043** |
| Lacewing larvae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.033 | 0.083 | 0 | 0.217 | 0 | 0.025 | 0.033 | **0.027** |
| Spined soldier bugs | 0 | 0.007 | 0 | 0.007 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0.001** |
| Praying mantids (nymphs and adults) | 0 | 0.013 | 0.027 | 0.027 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.017 | **0.007** |
| Jumping spiders | 0.207 | 0.140 | 0.060 | 0.007 | 0.080 | 0.033 | 0.007 | 0.267 | 0.067 | 0.083 | 0.075 | 0.167 | 0.058 | 0.167 | **0.083** |
| Crab spiders | 0.023 | 0 | 0.007 | 0.007 | 0.020 | 0 | 0.007 | 0.183 | 0.042 | 0.117 | 0.017 | 0.050 | 0 | 0.133 | **0.028** |
| Orb weaver spiders | 0.126 | 0.113 | 0.233 | 0.007 | 0.053 | 0.020 | 0.087 | 0.150 | 0.050 | 0.333 | 0.292 | 0.733 | 0.208 | 0.767 | **0.172** |

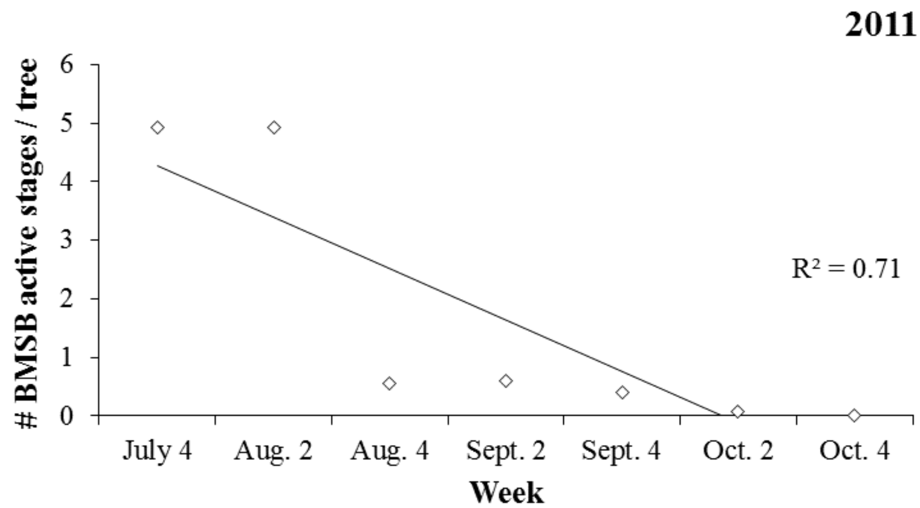


Figure 7a: BMSB abundance (number per tree per week) in 2011. The linear regression line equation is y = -0.88x + 5.16 (df=1, *P*= 0.017) where x is the week and y is the abundance of BMSB.

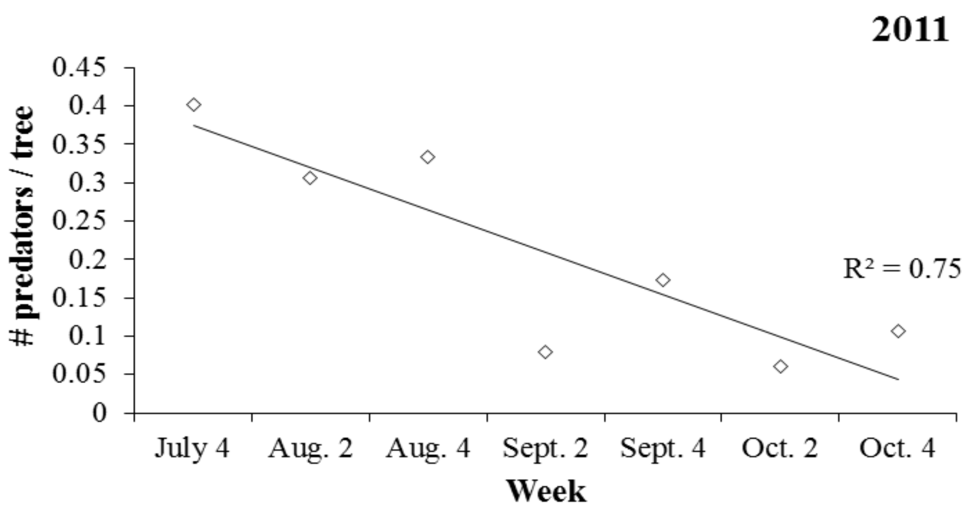


Figure 7b: Predator abundance (number per tree per week) in 2011. The linear regression line equation is y = -0.06x + 0.43 (df=1, *P*= 0.012) where x is the week and y is the abundance of predators.

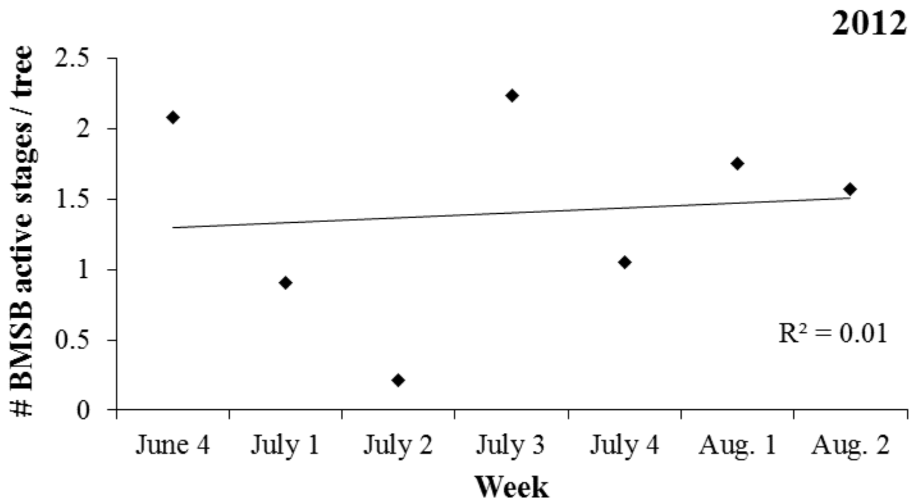


Figure 8a: BMSB abundance (number per tree per week) in 2012. The linear regression line equation is y = 0.03x + 1.26 (df=1, *P*= 0.824) where x is the week and y is the abundance of BMSB.

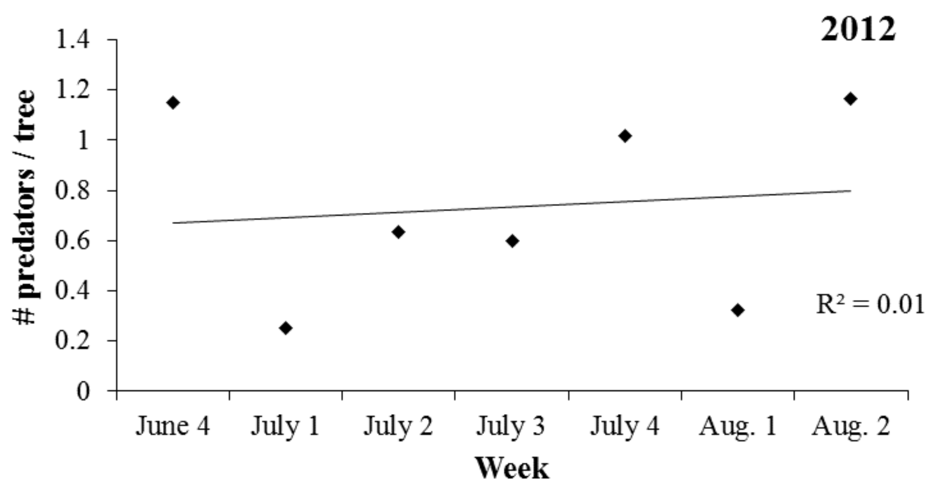


Figure 8b: Predator abundance (number per tree per week) in 2012. The linear regression line equation is y = 0.02x + 0.65 (df=1, *P*= 0.801) where x is the week and y is the abundance of predators.

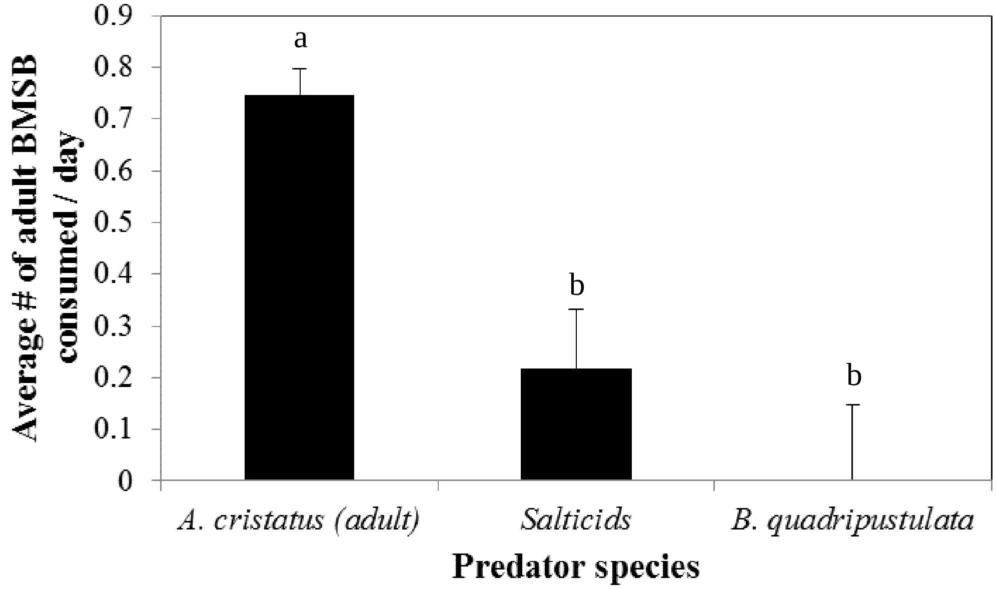


Figure 9: Average number of adult BMSB consumed per day by *Arilus cristatus* (wheel bugs), *Phidippus audax* and *Thiodina* sp. (Salticids), and *Brochymena quadripustulata* (rough stink bugs). Bars are the average numbers of adult BMSB consumed per day and vertical lines are standard errors. Bars that share a common letter indicate that the consumption rate of BMSB did not differ between predators ($P$>0.05, Tukey's comparison).

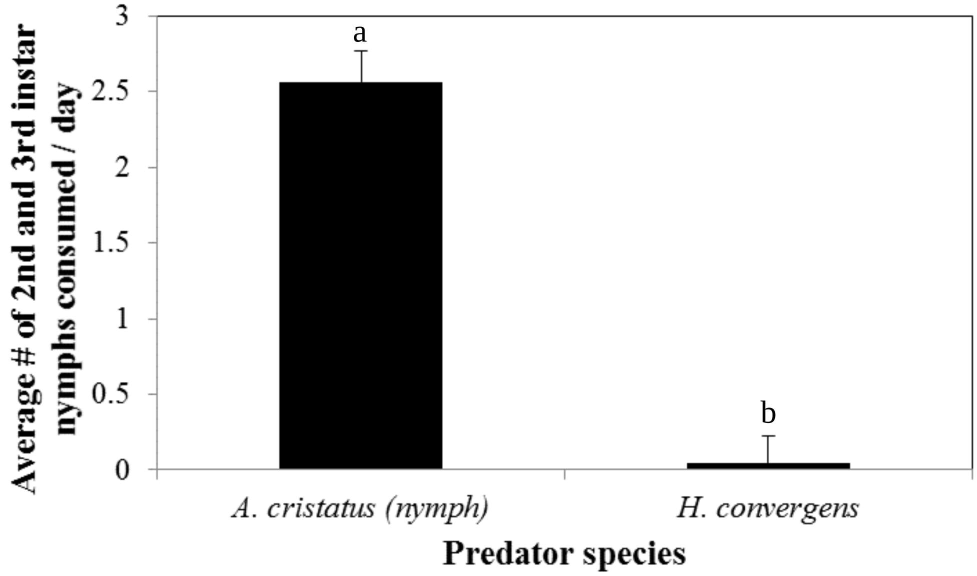


Figure 10: Average number of 2$^{nd}$ and 3$^{rd}$ instar BMSB nymphs consumed per day by *Arilus cristatus* (wheel bugs) and *Hippodamia convergens* (convergent lady beetles). Bars are the average numbers of 2$^{nd}$ and 3$^{rd}$ instar BMSB nymphs consumed per day and vertical lines are standard errors. Bars that share a common letter indicate that the consumption rate of BMSB did not differ between predators ($P$>0.05, Tukey's comparison).

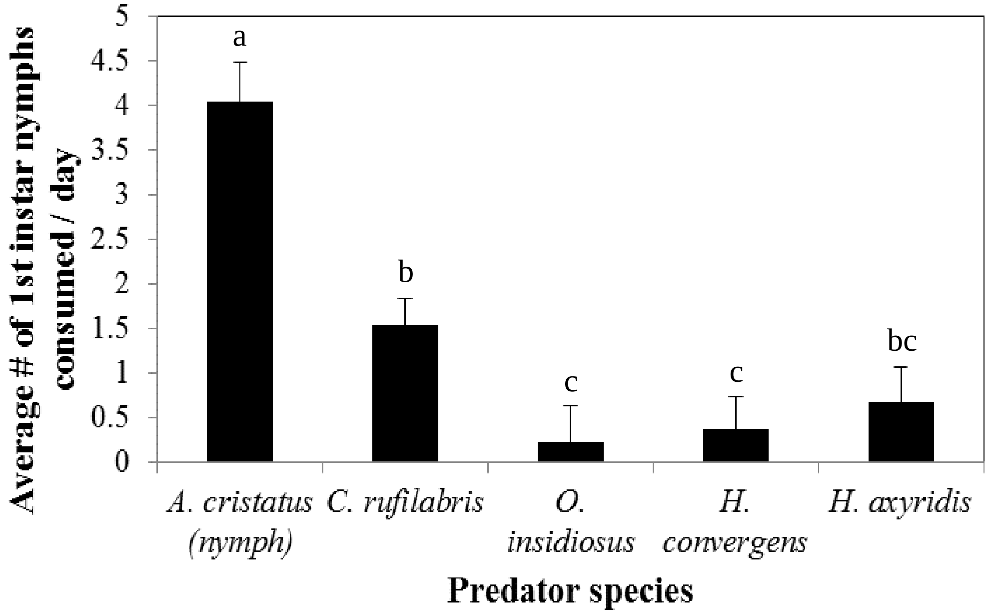


Figure 11: Average number of 1st instar BMSB nymphs consumed per day by *Arilus cristatus* (wheel bugs), *Chrysoperla rufilabris* (green lacewings), *Orius insidious* (minute pirate bugs), *Hippodamia convergens* (convergent lady beetles), and *Harmonia axyridis* (multicolored Asian lady beeltes) . Bars are the average numbers of 1st instar BMSB nymphs consumed per day and vertical lines are standard errors. Bars that share a common letter indicate that the consumption rate of BMSB did not differ between predators (*P*>0.05, Tukey's comparison).

# Chapter 3: Comparison of sentinel and naturally laid egg masses for assessing parasitism of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae).

## Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive species native to regions of Southeastern Asia. Considered a pest in its native range, BMSB has become an economically significant pest since its arrival into the U. S. BMSB are highly polyphagous and have a vast dispersal capability requiring the use of an integrated form of pest management. Biological control has potential to be one form of management for BMSB in different managed and natural systems and Hymenopteran egg parasitoids show great promise as a component of biological control for this pest. Accurate assessment of parasitism rates under field conditions is critical for determining baseline parasitism rates of native egg parasitoids and the effectiveness of parasitoid release strategies. We compared the use of sentinel (laboratory reared) and wild (naturally-laid) egg masses to estimate rates of parasitism when exposed to endemic parasitoids in woody production nurseries. Sentinel egg masses had significantly (13 times) less parasitism than wild egg masses. The use of sentinel egg masses may dramatically underestimate actual rates of parasitism and provide inaccurate estimates of egg parasitoid activity.

# Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae) is an invasive species that was first detected in the United States in 1996 (Hamilton, 2009). The native range of BMSB is eastern Asia in portions of China, Japan, Korea, and Taiwan (Hoebeke & Carter, 2003). In eastern Asia, BMSB is considered a pests of many fruit trees, vegetables, legumes, and ornamentals (Jones & Lambdin, 2009). BMSB have a highly polyphagous nature and the ability to disperse great distances (Nielsen et al., 2013, Lee et al., 2013c). Since their arrival in the Unites States, BMSB have demonstrated similar behavior and are now considered pests in many of our cropping systems and have been detected in 40 states within the U.S. (Leskey et al., 2012a).

Management strategies for BMSB will integrate many tactics such as chemical, cultural, mechanical, genetic, and biological control. Due to the ability of BMSB to utilize many host species in varying managed and natural systems, and their high dispersal capability, some tactics may not provide satisfactory control (Lanfen, 2007). Biological control may be a promising management approach for BMSB. Specifically, conservation and classical biological control are currently being explored as options for managing BMSB in many managed and natural systems towards suppressing BMSB. Conservation biological control is the preservation and utilization of endemic natural enemies within a system for control of a pest species (Barbosa, 1998). Classical biological control is the importation and eventual release of exotic natural enemies from the home range into the introduced range of the pest (Barbosa, 1998). Biological control can be highly sustainable but initially requires testing to determine if natural enemies are attacking a particular pest (Wallace & Hain, 2000).

In their native range, egg parasitoids of BMSB appear to be most effective in suppressing BMSB populations (Zhang et al., 1993, Chu et al., 1997, Lanfen, 2007, Hou et al., 2009). Thirteen species of parasitoids across 5 genera attack BMSB eggs (Kawada & Kitamura, 1992, Chu et al., 1997, Arakawa & Namura, 2002, Arakawa et al., 2004, Yu & Zhang, 2007, Lanfen, 2007, Lim et al., 2007, Hou et al., 2009, Lee et al., 2013b). The most promising species include: *Telenomus mitsukurii* (Ashmead) (Hymenoptera: Platygastridae) with a potential parasitism rate of BMSB eggs up to 84.7% (Chu et al., 1997), *Trissolcus halyomorphae* Yang (Hymenoptera: Platygastridae) with a parasitism rate up to 70% (Yang et al., 2009), *Trissolcus flavipes* Thomson with a parasitism rate up to 63.3% (Zhang et al., 1993), and *Anastatus* sp. (Hymenoptera: Eupelmidae) with a parasitism rate up to 77.2% (Hou et al., 2009).

In the United States, exotic parasitoids of BMSB are in quarantine but none have been released to date (Leskey et al., 2012a). Preliminary studies found several species of native egg parasitoids attacking BMSB. Those observed were *Anastatus reduvii* (Howard), *Anastatus pearsalli* Ashmead, *Anastatus mirabilis* (Walsh & Riley), *Trissolcus brochymenae* (Ashmead), *Trissolcus edessae* Fouts, *Trissolcus euschisti* (Ashmead), *Trissolcus thyantae* Ashmead, *Trissolcus utahensis* (Ashmead), *Telenomus podisi* Ashmead, *Telenomus utahensis* Ashmead, *Gryon obesum* Masner (Hymenoptera: Platygastridae), and *Ooencyrtus* sp. (Hymenoptera: Encyrtidae) (Hoelmer and Dieckhoff, personal communication, see Jones Chpt 1). Before exotic parasitoids are released, it is important to identify indigenous parasitoids and gain an accurate estimate of any suppressive effects they may have on BMSB.

To determine parasitism rates and identify parasitoid species complexes, two methodologies are commonly utilized. The first involves surveying naturally laid "wild" eggs deposited in the environment by the target pest and the second involves the use of "sentinel" eggs obtained from a laboratory colony that are placed into the environment and surveyed for parasitoid activity (Jones et al., 2001, Wright et al., 2001, Mansfield & Mills, 2002, Ehler, 2002, Koppel et al., 2009, Tillman, 2010, Tillman, 2011, Maltese et al., 2012, Vieira et al., 2013). These two methodologies have been used simultaneously to obtain information on the identity and activity of Pentatomid egg parasitoids (Jones et al., 2001, Ehler, 2002, Koppel et al., 2009, Tillman, 2010, Tillman, 2011, Vieira et al., 2013). Preliminary field studies in woody ornamental nurseries of BMSB egg parasitoids suggested that there were differences in parasitism rates estimated by the two methods – sentinel vs. wild egg masses. Validation of the use of sentinel eggs is important to accurately measure the impact of both indigenous parasitoids and exotic parasitoids released in a classical biological control effort. The objective of this study was to compare the parasitism rates observed in wild eggs and sentinel eggs of BMSB under field conditions in an ornamental nursery environment.

## Methods

### *Study Sites*

To assess BMSB rates of parasitism by indigenous egg parasitoids amongst wild and sentinel eggs, three field sites within Maryland were chosen. These nurseries produce field grown woody ornamental trees and shrubs. The first site was Raemelton Farm in Adamstown, MD (Frederick County) and the other two sites were located at Ruppert Nurseries in Laytonsville, MD (Montgomery County). The two sites at Ruppert were separated by a distance of approximately 2 km. with no points of contact as well as roads, fields, residential property, and commercial property separating the sites. Three genera of trees, known to be used by BMSB for feeding and oviposition, were chosen for monitoring at each nursery site (Table 5). Trees included *Acer rubrum* L. 'Franksred' (red maple), *Prunus serrulata* L. 'Kwanzan' (ornamental cherry), *Ulmus americana* L. 'Princeton' (American elm) and *Ulmus parvifolia* Jacq. 'Patriot' (Chinese elm). Two species of elms were used due to limited number of any one species at the multiple sites. The number of trees sampled of each species varied between nursery sites due to differences in abundance of each species at each nursery (Table 5). Studies were conducted in 2012 and 2013.

### *Sentinel Egg Mass Preparation*

Sentinel egg masses were collected from a BMSB colony housed at the University of Maryland (Dively lab), College Park, MD, USA. Newly laid egg masses were removed from colony cages 2-6 hours before placement in the field. Sentinel egg masses were always aged less than 24 hours as eggs older than approximately 60 hours are less susceptible to parasitism (Lanfen, 2007). Every 24 hours colony cages were searched and all eggs within the cage were removed so the age of eggs used in the study was always less than 24 h.

Once removed from the cages, the egg masses were detached from the leaf surface. This was carefully completed with the use of BioQuip Featherweight Forceps (wide tip) and water (when necessary). Water was applied to the edges of the egg mass to reduce adhesion of eggs to the leaf surface. By sliding one side of the forceps gently under the egg mass, the entire mass was removed. Care was important to avoid puncturing the bottom of the eggs. The egg mass was then placed on filter paper to remove excess water.

One Therm-O-Web, Inc. (Wheeling, IL) 0.5” double sided mounting square was placed in the center of a piece of filter paper cut into an approximately 4 x 3cm rectangle. This piece of filter paper was then labeled with appropriate information and the freshly removed egg mass was affixed to the adhesive square. When affixing the egg mass, it was oriented with the operculum side up as it would occur in the wild. A small quantity of sterilized fine sand was then placed around the egg mass to cover any exposed adhesive and prevent parasitoids from getting stuck. This process was repeated for every egg mass retrieved from the colony to be placed in the field as a sentinel (Figure 12).

### *Wild Egg Mass Searching*

At every sampling, all tree foliage up to a height of 2.7 m. was searched thoroughly for BMSB egg masses. The particular set of trees for this study were searched every 48 h, so the approximate age of the wild egg masses was known to be less than 48 h. When located, the leaf housing the egg mass was marked with a piece of colorful tape and the location of the egg mass within the tree was recorded.

### *2012 Sentinel Egg Mass Output and Pairing with Wild Egg Masses*

In 2012, sentinels were placed into field sites from the beginning of June to the beginning of July. Studies were conducted at these times due to availability of sentinel egg masses which were obtained from the BMSB colony. Sentinel egg masses aged ≤ 24 h were paired with wild egg masses aged ≤ 48 h (maximum age difference of 48 h) within a tree block (a group of trees of the same species within a site) (Table 5). Sentinels were pinned to the underside of leaves to mimic natural conditions (Figure 12). Once paired, egg masses were left in the field for the same amount of time at one of two time periods: 48 h or until BMSB emerge (approximately 7 days).

### *2013 Sentinel Egg Mass Output and Pairing with Wild Egg Masses*

In 2013, sentinels were placed into field sites from the end of July to mid-August. Study trees were examined every other day to detect new egg masses. Sentinel egg masses aged ≤ 24 h were paired with wild egg masses aged ≤ 48 h (maximum age difference of 48 h) on the same tree. Sentinels were placed in the mirrored location of the wild egg mass on the opposing side of the tree crown. The sentinels were also pinned to the underside of leaves to mimic natural conditions (Figure 12). Once paired, egg masses were left in the field for the same amount of time at one of two time periods, either 48 h or until BMSB emerged (approximately 7 days).

### *Egg Assessment for Parasitism*

Egg masses were brought back to the lab and placed in a 100mm x 15mm Petri dish (Thermo Fisher Scientific Incorporated). They were then placed in a Percival incubator (25$^{o}$C ± 2$^{o}$C, 16L: 8D) for monitoring of BMSB emergence and parasitism. BMSB nymphs were removed within 24 hours of hatching. If BMSB nymphs did not emerge, egg masses were incubated for parasitoid emergence. Parasitoids were removed and placed in 70% ethanol as they emerged. The number of parasitized and non-parasitized egg masses, defined as an egg mass in which at least one egg produced a parasitoid and numbers of individual eggs parasitized was recorded for each pair of egg masses for each year.

### *Statistical Analysis*

Differences in percent parasitism of sentinel and wild (treatment) egg mass pairs (replicate) for egg mass and for individual eggs pooled over the two years was determined using a paired t-test (SAS Institute 2002-2008).

## Results

In the 2012 field season, 46 sentinel egg masses were paired with 46 wild egg masses. Similarly, in the 2013 field season, 46 sentinel egg masses were paired with 46 wild egg masses. In total, 92 sentinel/wild egg mass pairings were made over the course of two years. Eggs left in the field for both 48 h and until BMSB emergence were pooled as there was no significant difference in rates of parasitism ($t = 0.13$, $df = 90$, $P = 0.90$). The average rate of parasitism of all sentinel and wild egg masses in the study were 4.35 ± 2.14% and 52.17 ± 5.24 % respectively. The average rate of parasitism of all individual sentinel and wild eggs in the study were 2.7 ± 1.44% and 41.83 ± 4.59 % respectively. Total number of egg masses and individual egg counts of both sentinel and wild type eggs, and percent of egg masses parasitized and individual eggs parasitized pooled over the two years are located in Table 6. The mean of sentinel egg parasitism was significantly lower than the mean of wild egg parasitism (t= -7.73, df= 91, $P<0.001$). Similarly, mean parasitism of sentinel egg masses was significantly lower than the mean parasitism of wild egg masses (t= -7.73, df= 91, $P<0.001$).

## Discussion

Although sentinel egg masses are useful in obtaining information on BMSB egg parasitoid complexes, the use of sentinel egg masses may grossly underestimate rates of parasitism and provide an inaccurate measure of a parasitoid's true ability to impact BMSB populations. The difference in egg parasitism between paired wild egg masses and wild eggs, and sentinel egg masses and sentinel eggs in woody ornamental nurseries in Maryland provides evidence that sentinels may not adequately estimate parasitism. To this point, rates of parasitism of wild egg masses were about 13 times greater than that of sentinel egg masses.

The environment in which wild and sentinel egg masses originate may play an important role in parasitoid behavior and rates of parasitism. Insect parasitoids use semiochemical cues either directly or indirectly related to the host to mediate searching behavior (Lewis & Martin, 1990, Vet & Groenewold, 1990, Vet & Dicke, 1992). The specific chemical cues detected may vary by parasitoid and parasitoid /

host species. One study found that *Trissolcus basalis* responds to absorbed contact kairomones of the host in epicuticular waxes on the leaf surface (Colazza et al., 2009). A study on *Anastatus japonicas* Ashmead found that adult females may initiate distant searching behavior when they contact semiochemicals from scent gland exudates of Tessaratomidae, a family of true bugs (Yufang & Dexiang, 2000, Lanfen, 2007).

If particular semiochemicals required to initiate searching behavior are lacking in sentinel eggs, then these eggs may not be located by the parasitoid and used for oviposition. This may have been the case in our system as wild eggs laid in the field might contain important cues provided by the ovipositing adult BMSB or oviposition host plant. This supposition is supported by significantly higher parasitism rates of wild eggs compared with those placed in the field from a laboratory setting. The absence of an adult female BMSB and thus absence of potential direct host semiochemicals could have attributed to this difference.

One potential factor which could have affected the results ispotential differences in age of the sentinel and wild egg masses. Wild egg masses could have been in the field for no more than 48 h prior to sentinel egg placement. Sentinel eggs were less than 24 hours in age. The maximum potential age difference between wild and sentinel egg masses is 48 h. This difference in age should be negligible due to both egg sources overlapping in the field during their 60 h period of susceptibility to parasitism (Lanfen 2007). This age difference alone could not explain the significant difference in parasitism rates between sentinel and wild egg masses.

These results may not be applicable outside of ornamental plant nurseries or for other parasitoid – host systems.  However, this data does shed light into the need for testing the reliability of sentinel egg masses in estimating parasitism rates and the effects of egg parasitoids on prey populations.  Further studies should focus on the particular semiochemicals indigenous parasitoids use when searching for a host.

Table 5: Number of trees within each block by species per site.

| | **Number of Trees in Each Block*** | | | |
|---|---|---|---|---|
| **Site** | *Acer rubrum* | *Prunus serrulata* | *Ulmus americana* | *Ulmus parvifolia* |
| **1** | 19 | 47 | 73 | - |
| **2** | 28 | 20 | - | 31 |
| **3** | 87 | 70 | 100 | - |

*Block = a group of trees of the same species within a site.

Table 6: Comparison of wild vs. sentinel egg abundance and parasitism of egg masses and individual eggs.

| **Egg mass type** | **No. egg masses** | **No. eggs** | **% parasitized egg masses *** | **% parasitized eggs *** |
|---|---|---|---|---|
| **Wild** | 92 | 2476 | 52.2 a | 41.8 a |
| **Sentinel** | 92 | 2368 | 4.3 b | 2.7 b |

*means within columns with different letters are significantly different (t-test, alpha=0.05)

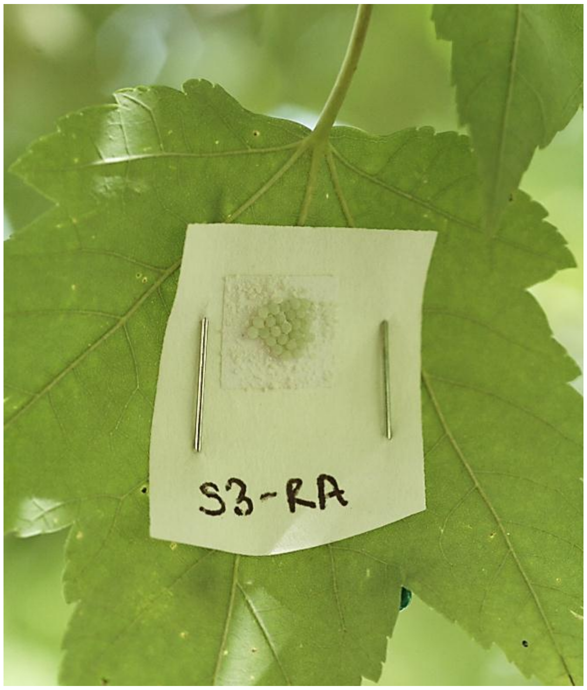


Figure 12: Example of mounted sentinel egg mass placed into field.  Note the pins used hold the filter paper in place without impeding natural enemy movement.

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