

## ABSTRACT

Title of Thesis:	An assessment of host preference, reproductive suitability and feeding injury of the brown marmorated stink bug, <i>Halyomorpha halys</i> , on selected vegetables
Thesis directed by:	Emily S. Zobel, Master of Science, 2014 Associate Professor Cerruti R <sup>2</sup> Hooks Department of Entomology

The brown marmorated stink bug, *Halyomorpha halys* (Heteroptera), is an invasive insect from Asia that has become a major agricultural pest of field and vegetable crops in the Mid-Atlantic States. A field study was conducted to asses the seasonal abundance, host plant preference, reproductive suitability, and injury potential of *H. halys* on green bean, sweet corn, eggplant, okra and bell pepper. *H. halys* abundance, life stage phenology, and resulting feeding injury were monitored biweekly throughout the growing season. Overall seasonal abundance consisted of both overwintered adults and their F1 progeny. Sweet corn, okra and bell pepper had significantly higher abundances of *H. halys* compared to green bean, eggplant, and tomato. Eggplant, okra and bell pepper were the most suitable host plants for *H. halys* reproduction and development. Sweet corn, okra, bell pepper and tomato were very susceptible to feeding injury and experienced the highest injury rate per stink bug day. The implications of these findings with respect to sampling and management of *H. halys* in vegetable production are discussed.

An assessment of host preference, reproductive suitability and feeding injury of the brown  
marmorated stink bug, *Halyomorpha halys*, on selected vegetables

By Emily S. Zobel

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Advisory Committee:  
Associate Professor Cerruti R.R. Hooks, Chair  
Professor Emeritus Pedro Barbosa  
Professor Emeritus Galen P. Dively

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## **Dedication**

I dedicate this work to my family and friends. My parents, William and Vicki Zobel, who have never stopped believing in me and have always encouraged me to pursue my interests, even if it meant having insects in their freezer. My sister Erin Zobel-Allen, who is always there for me with a smile. My Niece and Nephew, the world is yours never be scared to venture out into it. To my friends, Brittany McLean, Seana Miller, Megan Johnson and Emily Everhart, who always have my back. My family and friends have made this possible and I am incredibly grateful.

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## Introduction

Agriculture is one of the leading industries in the United States, employing over 2 million people with a farm gate value of crops at more than 212 billion dollars (USDA 2012). The agricultural industry and associated profits are often impacted by abiotic elements such as wind, temperature, and humidity; and biotic factors such as disease; weed and insect pests. In 2007, over 78,000 metric tons of insecticides were applied to over 90 million acres of farmland in the United States to manage insect pests alone (USDA 2007, FAO 2012).

Many insect pests associated with cropping systems are polyphagous, and can colonize and develop on different host plants throughout the growing season. This ability to use multiple hosts throughout space and time in response to ever-changing cropping systems makes managing polyphagous pests a challenging undertaking (Kennedy & Storer 2000). Polyphagous insect pests often move to different host plants according to their life cycle requirements, host plant phenology, and food quality (Panizzi 1997). This behavior often results in a high concentration and buildup of a pest species on one host plant that subsequently leads to invasions of other hosts during the growing season (Kennedy & Storer 2000). Therefore, the successful management of polyphagous insect pests is contingent upon knowing which: plant species they utilized as hosts, pest stages feeds on various host plants, and phenological plant stages are preferred for feeding and reproduction.

**General stink bug biology.** Stink bugs are common polyphagous insects found in agricultural systems. Most herbivorous species feed on a wide variety of plants, including wild and domesticated plants such as ornamentals, fruits, cereal crops, woody

plants, and vegetables. Stink bugs feed on different plant parts including leaves, stems, seeds, flowers and fruit. However, they exhibit a preference for feeding on fruit and seeds (Schuh & Slater 1995, McPherson & McPherson 2000). Despite being categorized as generalists, most stink bugs demonstrate a preference for a particular plant species, families and developmental stage (Kennedy & Storer 2000). For example, the harlequin bug, *Murgantia histrionica* (Hahn), feeds on a variety of vegetables, field crops, weeds and fruit trees, but will do so only in the absence of its preferred taxon, *Brassica spp.* (McPherson & McPherson 2000).

Stink bugs have generally been considered minor pests that occasionally reach outbreak levels in agricultural systems. In many crop systems, they are mainly controlled by the use of broad-spectrum insecticides applied to control other arthropod pests. However, a reduction in the use of broad-spectrum insecticides partly due to the widespread adoption of transgenic crops has led to a rise in herbivorous stink bug populations (McPherson & McPherson 2000, Chouogule & Bonning 2012). This rise in local stink bugs coupled with the introduction and expansion of populations of the invasive brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stål) has elevated stink bug status to that of primary pests in many agricultural crops in the United States.

In 2005, stink bugs caused \$465,000 of damage in field corn and \$456,500 of damage in soybeans in Georgia alone (Buntin et al. 2005). In 2009, stink bugs were responsible for \$29 million worth of losses in U.S. cotton fields (Reay-Jones 2010, Williams 2010). In 2010, many mid-Atlantic growers reported major yield losses in soybean, sweet corn, peaches, peppers, tomatoes, and apples due to *H. halys*

(StopBMSB.org 2014). According to the United States Apple Association, *H. halys* caused \$37 million worth of injury to the 2010 apple crop (US Apple Association 2011).

**H. halys biology.** *H. halys* is a stink bug native to eastern China, Japan, Taiwan, and North and South Korea. It was identified in the United States officially in 2001 but is thought to have been introduced in Allentown, PA, from the Beijing, China in 1998 (Hoebeke & Carter 2003, Xu et al 2014). Since its introduction, *H. halys* has been detected in 41 states, the District of Columbia, and two Canadian provinces, with the largest population found in the Mid-Atlantic States (Leskey et al. 2012b, StopBMSB.org 2014). *H. halys* is a major nuisance and agricultural pest in the Mid-Atlantic region due to its behavior of overwintering inside structures constructed by humans including homes and feeding injury to commercial crops. It has 300 known host plants in its native range, where it is a pest of soybeans, other leguminous crops and tree fruit (Hoebeke & Carter 2003). There are currently 170 known host plants in North America, which include ornamentals, woody plants, tree and small fruits, row and vegetable crops (Leskey et al. 2012b, StopBMSB.org 2014).

*H. halys* has been reported to have one and two generations per year in Pennsylvania and West Virginia, respectively (Nielsen & Hamilton 2009, Leskey et al. 2012b). It overwinters as sexually immature adults, inside buildings or under the bark of dead, standing trees (Lee et al. 2014). Adults move into their overwintering habitats during late September and mid-October. When they emerge in late-March, adult females require an additional 148 degree days (DD) at a base temperature of 15°C (about 2 weeks) before they become sexually mature (Nielsen et al. 2008). Once sexually mature, females will mate, start laying eggs in late May and continue laying eggs throughout their

life. In a laboratory study, females laid an average of eight egg masses (~ 200 eggs) during their lifespan (Nielsen et al. 2008). Approximately 4 to 5 days are needed between oviposition events. Egg masses on average contain 28 eggs, which are laid on the underside of leaves. Eggs are barrel shaped and are initially light green changing to a cream color as they age. Eggs hatch in approximately 4 to 5 days. *H. halys* development requires 538 DD from egg to adult and an additional 148 DD for females begin to lay eggs. (Nielsen et al. 2008).

*H. halys* has five nymphal stages. The first instar nymphs are dark brown and orange in color and remain on the hatched egg mass until they develop to second instars. During the first instar stage, nymphs feed on the surface of the egg to acquire the necessary gut symbionts needed for proper development and survival (Taylor et al. 2014). Older nymphs are identified by white bands on their legs and antennae. Adults look similar to native brown stink bugs, *Euschistus servus* (Say), and spined soldier bugs, *Podisus maculiventris* (Say), however, *H. halys* can be identified by its brown-gray abdomen, rounded “shoulders,” and distinctive white bands on its legs and antennae (Hoebke & Carter 2003).

Adult and nymphal stages feed by inserting their stylets into the plant tissue. They then release a “gel” and watery saliva into the plant. The “gel” saliva is used to form a hard salivary sheath which helps prevent the loss of plant juices during feeding (Miles 1972, Peiffer & Felton 2014). Watery saliva contains digestive enzymes, which liquefies the plant tissue and allows the stink bug to ingest its food (Miles 1972). Enzymes and other components found in stink bug saliva are responsible for the tissue injury which affects plant health and can cause flower and fruit abortion (Peiffer & Felton 2014). In

addition, the feeding injury causes dimples, discoloration, and spongy texture on the fruit, which reduces crop quality and marketability.

**H. halys management.** Since *H. halys* is relatively new to North America, initially there were no established IPM programs to manage it. When populations reached outbreak levels in 2010, many growers used multiple applications of broad spectrum insecticides to minimize economic losses due to their feeding (Lee et al. 2013). The repeated use of broad-spectrum insecticides led to secondary pest outbreaks of mites, scale insects, and aphids due to the loss of natural enemies (Leskey et al 2012a). This reliance on multiple applications of insecticides caused many growers to abandon IPM programs that were established for other arthropod pests (Leskey et al. 2012a).

Managing *H. halys* can be difficult because of its polyphagous feeding habit and ability to disperse throughout a landscape. *H. halys*, similar to other stink bugs, have higher populations along field margins, mainly next to woods or other host crops (Reay-Jones 2010, Kevin et al. in Press). Studies showed that adults can fly > 2 km in a day and fifth instars can move ~ 4.6 m/hr, however this distance will vary based on ground temperature and terrain (Lee, unpublished data). This mobility allows *H. halys* to readily colonize different host crops during the growing season, often resulting in rapid re-invasion after chemical control. This behavior suggests that a multifaceted area-wide management approach is required to control this pest.

Research is currently being conducted to investigate the use of cultural practices and biological control to manage *H. halys*. Cultural practices such as trap cropping and growing early maturing varieties can be effective in small farm situations (Leskey et al. 2012b). Pheromone traps baited with methyl (2E,4E,6Z) -decatrienoate and black light

traps are effective monitoring tools but have not been effective as a management tool (Khrimian et al. 2008, Nielsen et al. 2013). The U.S. Department of Agriculture is currently exploring classical biological control options with several species of Asian parasitoids (Leskey et al. 2012b, Durham & O'Brien 2013). Surveys of U.S. native enemies of stink bugs have also provided evidence of predation and parasitism on *H. halys*, but their presence and effectiveness varies among plant habitats (Leskey et al. 2012b).

*H. halys* can feed on 170 known North American host plants, including many cultivated crops. Thus, crop loss assessment and risk evaluation are a necessary prerequisite to the development and optimization of an IPM program. Specifically, it is important to know which host plants: are preferred, serve as reproductive hosts, and are of high economic risk due to *H. halys* feeding injury. Though *H. halys* is known to feed on vegetable crops in its native region (Fukuoka et al 2002, Hoebeke & Carter 2003, Lee et al. 2014), limited research has been conducted on its feeding habits in vegetable systems in the United States. The objectives of this study was to examine the colonization, population dynamics, host preference, reproductive suitability of various vegetable crop species and feeding injury potential of naturally occurring *H. halys* populations in these crops. Stink bug life stages and associated feeding injury were monitored on several vegetable crops during the growing season to address these objectives.

## Materials and Methods

**Experimental site.** The experimental site was a 0.16 h field at the University of Maryland Central Maryland Research and Education Center, Clarksville facility,

Clarksville, MD. The immediate habitats surrounding the experimental site consisted of corn fields on the south and east sides, a highway and non-cropped grassy areas on the north side, and small trees followed by a woodlot on the west side. Six common vegetable crops were selected as host plants and grown according to recommended commercial practices for Maryland, during the 2013 growing season. Test crops included green beans (*Phaseolus vulgaris*, variety ‘Provider’), okra (*Abelmoschus esculentus*, variety ‘Clemson Spineless’), eggplant (*Solanum melongena*, variety ‘Nadia’), bell pepper (*Capsicum annuum*, variety ‘Paladin’), tomato (*Solanum lycopersicum*, variety ‘Rocky top’) and sweet corn (*Zea mays saccharata*, variety ‘BC 805’), representing crop species in the Solanaceae, Malvaceae, Fabaceae and Poaceae. Seeds were purchased from Johnny’s Selected Seeds (Fairfield, Maine) and Syngenta Seeds (Greensboro, NC).

Test crops were established on black plastic mulch in plots arranged in a Latin square design with six replications of each vegetable type. Plots were blocked as columns in a north-south direction and blocked as rows in an east-west direction. Each plot consisted of three rows spaced 1.5 m apart and 9 m long. Green beans and sweet corn were planted in double rows over each plastic bed, while the remaining vegetables were planted in single rows. The different planting arrangements represented the plant density recommended for each crop. Green bean and sweet corn are normally planted in narrower rows and on bare-ground. Individual plots were separated by 1.5 m of black plastic mulch or bare soil.

**Plot Establishment.** Okra, green beans and sweet corn were seeded directly into black plastic mulch on May 22, 2013. Two seeds were placed in each hole and were thinned to one plant once plants emerged. Four week old greenhouse grown tomato,

eggplant and bell pepper plants were transplanted by hand in the black plastic mulch on the same date. Approximately 240 mL of transplant water with soluble fertilizer 20-15-20 (N-P-K) was applied around each transplant. Eggplant, okra, and tomato plants were transplanted 61 cm apart, while pepper were planted 46 cm apart. Double rows of green bean and sweet corn were spaced 61 and 89 cm apart, respectively, on each black plastic row. Green bean and sweet corn seedlings were spaced 15 and 30.5 cm apart within each row, respectively. Plants that did not germinate or survive were re-seeded or transplanted again on June 3, 2013.

To avoid lodging, pepper plants were supported by a single string along each side attached to short stakes, whereas the tomato plants were trellised with multiple strings on taller stakes to allow plants to grow straight up. Plots were drip irrigated 4-6 hours biweekly from June to end of August and kept free of weeds by weekly hoeing and hand removal. All crops were maintained through the entire growing season, except for green bean and sweet corn. For green bean, plants were removed following the final harvest on July 25, 2013, and a second crop was direct-seeded. For sweet corn, stalks were manually cut down on August 12, 2013 after the final harvest to simulate the crop destruction practices used in commercial production.

**Data collection.** Stink bug abundance within the middle row of each plot was assessed by a team of four technicians examining plants and a single person recording data. Sampling started at 35 days after planting (last week of June) and continued biweekly for 8 weeks, after which weekly sampling was conducted for another 7 weeks until 134 days after planting (beginning of October). At each sampling date, two pairs of technicians, positioned on opposite sides of the row, thoroughly examined the stems,

leaves and fruiting structures of each plant and counted the number of *H. halys* and native stink bugs observed. When stink bug counts were high, multiple tally counters were used. Data were recorded by stink bug species on the number of unhatched egg masses, hatched egg masses with first instar nymphs, small nymphs (2<sup>nd</sup> and 3<sup>rd</sup> instar), large nymphs (4<sup>th</sup> and 5<sup>th</sup> instars) and adults.

Phenological stage of each crop was recorded weekly. Plant growth was categorized as early vegetative, bud stage, open flower, immature fruit, and marketable fruit for all vegetables, except sweet corn. For sweet corn, plant stages were whorl, green tassel, silking, early kernel development, and harvestable ears. Weekly harvesting of each vegetable crop commenced when plants produced the first marketable fruit. All marketable fruit was removed from the three rows of each plot, although the fruit from the center row was kept separate and visually inspected for stink bug injury. Data were recorded on the total number of harvestable fruit and the number of stink bug injured fruit. Due to the large number of green beans harvested, the total number was estimated by dividing the total weight of green beans by the average weight per pod (based on the weight of a subsample of 100 green beans). However, all bean pods were inspected for stink bug injury. Stink bug injury to sweet corn was assessed twice when ears reached peak fresh market maturity. All marketable ears from the center row were husked open to record the number of injured kernels and the percentage of the ear area with undeveloped kernels.

**Statistical Analysis.** Stink bug abundance data were converted to density per m<sup>2</sup> for analysis in order to standardize across crops. Although native stink bug species were found, the analysis focused on *H. halys* and specific ANOVA models (SAS Release 9.3,

SAS Institute Inc., Cary, NC) were used to address each of the following questions. First, are there spatial differences in *H. halys* infestation patterns over the cropping season? In this analysis, the average weekly total number of nymphs and adults pooled over all crops was averaged by row and column arranged in the Latin Square design. Data across rows and columns were analyzed by mixed model ANOVA, with row, column and week as fixed factors and crop as a random factor. To take into account the changing colonization patterns throughout the growing season, three different time sets were analyzed; the whole season, and the first half of the season and the second half of the season. Means and standard deviations were plotted to examine colonization patterns in relation to the surrounding habitats.

Second, are there differences in seasonal abundance and age structure of the *H. halys* population among crops and in relation to the phenological stages of each crop? To summarize stink bug population dynamics in each crop, means and standard deviations were computed for each life stage and plotted over sampling dates and in relation to the crop phenological stages. Mixed model ANOVAs were performed to test for significant host plant preferences at different dates and crop phenological stages. Densities of *H. halys* stages were averaged over five phenological stages (early vegetative/whorl, bud stage/green tassel, flowering/silking, early fruit development, marketable fruit/ears). Data for this analysis represented the overall density per m<sup>2</sup> during the entire phenological stage of the cropping period (which included multiple sampling dates). Vegetable crop and phenological stage were treated as fixed factors and the repeated measures option was used to adjust for autocorrelation effects among phenological stages. Row and column (Latin Square) were treated as random factors.

Thirdly, are there differences in the suitability of each vegetable crop as a reproductive host plant of *H. halys*? To account for both population density and duration of infestation, cumulative stink bug days for each *H. halys* stage were calculated according to the procedures outlined by Ruppel, (1983) and Ragsdale (2006). Stink bug days were derived by averaging the number of stink bugs per m<sup>2</sup> recorded over two sequential sampling dates and multiplying by the number of days between the sampling dates. Values of stink bug days were then accumulated over the entire cropping season. Specific one-way ANOVA models were performed to test for significant differences among vegetable crops in the proportion of the total stink bug days consisting of small nymphs, large nymphs and adult *H. halys*. Crop was treated as a fixed factor, and row and column were treated as random factors. The relative proportions were used to reflect reproductive suitability of each vegetable crop. Crops with significantly higher proportions of adult stink bug days and lower proportions of nymph stink bug days were considered to be less suitable as a reproductive host. In all ANOVA analyses described above, *H. halys* abundance data were square root or log transformed to meet statistical assumptions of normality and homogeneity of variance, but the untransformed means are presented. LSMEANS with Tukey–Kramer adjustment was used to test for differences among combinations the fixed factors. Treatment effects were considered significant when  $P < 0.05$ .

Finally, to examine the relationship between *H. halys* densities and marketable yield, linear regression models (SAS Proc Reg procedure) were used to estimate the injury rate per insect for each vegetable crop. Analyses were performed on the cumulative percentage of injured marketable fruits and regressed on the corresponding

cumulative stink bug days for all stages at each harvest date. The slope of the regression line for each crop reflected the injury rate per stink bug unit.

## Results and Discussion

### Colonization patterns.

Stink bug adults began colonizing test plots the first week of July and reached peak numbers during late July and again during the first two weeks of September. *H. halys* accounted for 99.0% of the total stink bugs observed, whereas the remainders were native species, primarily the *E. servus*. Most early colonizers were overwintered adults because only 296 DD (low developmental temperature = 15°C) had accumulated at the study location, since mid-May when *H. halys* adults were consistently captured in black light traps (personal communication, GP. Dively). A study by Nielsen et al. (2008) reported that *H. halys* development requires 538 DD from egg to adult and an additional 148 DD for females to begin laying eggs, thus there was not enough temperature accumulation for *H. halys* to complete a generation from egg laying to adults by the end of June. Overwintered adults did not colonize the plots until early July, so adults were either late to emerge from overwintering sites or immigrated from all directions after feeding on other host plants in the surrounding area.

*H. halys* adults overwinter under tree bark and in buildings (Lee et al. 2014), and the population goes through the first generation primarily on tree hosts, so one would predict that the western side of the field site, closer to the woods and buildings, should have higher densities. However, statistically there were no significant differences among mean weekly adult densities across rows or columns during the first half of the growing season. Nymph and adult populations increased during the second half of the growing

season (sampling dates after Aug 10) and showed significant patterns in overall density pooled over crops when analyzed across columns. Crops within the southern column, which was closest to a corn field, had higher numbers of adults compared to the other columns of plots (Table 1). This column also had significantly higher numbers of nymphs than the northern column, which was closest to a highway ( $F_{5,188} = 3.12$ ,  $P=0.01$ ). This spatial pattern is not surprising, since stink bugs are known to have higher populations along the borders between host plants (Reay-Jones 2010, Tillman 2010). However, the higher densities observed in plots on the south side, particularly in okra and pepper, were due to the immigration of adults and nymphs from a neighboring corn field, which was at the less preferred hard dough stage. Furthermore, the fact that disproportionately higher numbers of large nymphs relative to small nymphs were present during the second half of the growing season suggests that many fourth and fifth instars emigrated from the corn field.

### **Population dynamics and fruit injury.**

**Green bean.** Adult stink bugs were first detected in the early season planting of green bean on June 28 (38 days after planting) during the flowering stage (Figure 1). Although *H. halys* was the predominant species, about 7% of the stink bug observed were native species. The *H. halys* populations peaked about two weeks later at an average level of 0.55 adults/m<sup>2</sup>. Low levels of egg masses found during July (less than 0.05/m<sup>2</sup>) resulted in barely detectable levels of small and large nymphs on July 5 and July 15 (45 and 55 days after planting), respectively. The phenology and population structure of *H. halys* life stages were significantly different in the late season planting of green beans compared to the first planting. Adults were initially found on August 12 (18 days after

planting) during the vegetative stage (Figure 2). *H. halys* made up 95% of the stink bugs observed and their densities reached an average level of 0.27 adults/m<sup>2</sup> on September 20. The number of small and large nymphs reached a peak 0.12/m<sup>2</sup> on September 6 and 0.23/m<sup>2</sup> on September 20, respectively. While the timing and proportions of the life stages indicates that *H. halys* was able to successfully reproduce on the late planting, no egg masses were found in this planting. Furthermore, the relative higher numbers of large nymphs suggest these life stages immigrated from surrounding vegetable plots. In contrast, the early season planting had relatively few nymphs compared to adults, which may be attributed to the reproductive status of the adults. Most of the adults colonizing the green bean plots during early July had overwintered as adults and thus may have been post-reproductive.

In both green bean plantings, marketable pods were harvested about 50 days after planting and showed evidence of feeding injury that appeared as a sunken yellow-white cloud, often with a brown spot in the center where they had inserted their stylets (Figure 3a). An overall 21,126 beans were harvested, of which only 1.8%  $\pm$  0.24 (SEM) of the pods showed stink bug feeding injury (Table 2). Though the percentage of injured bean pods reported by Kuhar et al. (2012a) was higher at 10-15%, this represented the lowest injury level of the six vegetable crops based on the proportion of the total harvestable crop. This low frequency of injury is thought to be a result of lower *H. halys* densities and the disproportionately higher numbers of fruiting bodies produced by green bean plants compared to the other crops. Moreover, green beans are indeterminate in their fruiting development and thus produce a surplus of young pods that may not reach harvestable size. Thus, much of the stinkbug feeding may have been inflicted on the

flower buds and immature pods. For this reason, the study did not take into consideration the loss of yield due to aborted pods or physiological stress caused by stink bugs feeding directly on the stems, leaves and fruiting bodies. For instance, it is known that *H. halys* nymphs readily feed on green bean plants without pods in laboratory colonies.

Nevertheless, there was a significant relationship between the cumulative percentage of injured pods and the cumulative stink bug days over the course of each green bean crop. Pooled over all life stages, stink bug days accounted for 35.2% of the variation in the percentage of injured pods, with a significant regression slope of 0.098 ( $R^2=0.35$ ,  $F_{(1,22)}=11.96$ ,  $P = 0.002$ ) (Figure 4). The slope of this relationship can be interpreted as the relative injury rate, meaning that each stink bug day resulted in about one 10<sup>th</sup> of a percentage of harvestable pods injured (Table 2). This rate was the lowest of all six vegetable crops.

**Okra.** More than 99% of the stink bugs observed in okra were *H. halys* and the first adults were found on July 2 (41 days after planting) during the bud stage (Figure 5). There were two distinct peaks in the adult density, the first occurred during late July which mainly consisted of overwintered adults and possibly a few colonizing F1 adults produced on early-season host plants surrounding the study site. The second peak occurred during the first two weeks of September and were presumably F1 adults produced from the generation of nymphs on okra and immigrating from other host plants within and surrounding the study site. The timing of the peaks suggest that these adults were offspring from the overwintered adults. This is likely as temperature accumulations between the two periods (June 22 to August 30) amounted to 572 DD, thus exceeding the required 538 DD needed for total development (egg to immature adult).

Of the six vegetable crops, okra attracted and supported the highest number of stink bug adults and nymphs over the extended period of its indeterminate fruiting cycle. *H. halys* populations reached mean levels of 1.8 and 6.8 adults/m<sup>2</sup> during the early and late peak periods, respectively. Egg masses were consistently found in okra from late July through most of August at levels ranging from 0.03 to 0.06/m<sup>2</sup>. Small and large nymphs peaked in early September, at which time densities averaged 1.3 small and 2.8 large nymphs/m<sup>2</sup>. Taken altogether, the population dynamics of these life stages strongly suggest that okra was a highly preferred host plant for feeding, and reproduction.

Okra provided an abundance of fruiting structures for *H. halys* to feed upon. Stink bugs were frequently observed on fruiting terminals and feeding on flower buds, flowers and immature pods. In China, similar feeding behavior were reported for *H. halys* on Chinese hibiscus (*Hibiscus rosa-sinensis*) (Hoffmann 1931, Hoebeke & Carter 2003) and in the United States by brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula*, and green stink bug, *Acrosternum hilare*, on cotton plants (Willrich et al. 2004). This suggests that the immature fruiting bodies were the preferred feeding sites for *H. halys*, particularly the nymphs. Such feeding has been reported to cause flower abortion, delayed development and reduced germination in various crops (Stephensons 1981, Willrich et al. 2004). Although no data were recorded on this type of injury, the subsequent damage was evident on the marketable-sized pods, which was characterized by wart-like bumps and pod curving, also known as “cow-horned” (Kemble et al. 1995) (Figure 3b).

Marketable pods were harvested starting on July 15 (55 days after planting) and continued weekly throughout most of September. Of the 5,035 pods harvested, 33.3% ±

2.77 (SEM) showed injury symptoms from stink bug feeding (Table 2), of which 68% were “cow-horned” and 32% exhibited wart-like bumps only. Although the fruit quality standards for okra are largely based on pod size, color and fiber content, the distorted and curled pods would clearly be considered unmarketable but it is questionable whether the wart-like bumps would affect fruit quality depending on the market outlet. Furthermore, there was no consistent evidence of internal tissue deformity associated with the cow horned or wart-like symptoms. Assuming both types of injury represent marketable loss, the percentage losses in this study were similar to the 40% yield loss reported in Kuhar et al. (2012b-d). Based on these injury symptoms alone, there was a significant relationship between the cumulative percentage of injured pods and the cumulative stink bug days over the entire okra harvest period. Pooled over all feeding stages, stink bug days accounted for 94.8% of the variation in the percentage of injured pods, with a significant regression slope (or relative injury rate) of 0.132 ( $R^2=0.95$ ,  $F_{(1,64)} = 1176.71, P < 0.001$ ) (Figure 6). Surprisingly, despite having higher and more sustained infestations of stink bugs than the other vegetables, the injury rate of okra per stink bug stage was one of the lowest. This was attributed to the large numbers of okra pods produced per plant, the quick developmental time of the fruit, and the compensatory ability of the plant.

**Eggplant.** This host plant produced fruiting structures about one week ahead of the other vegetable crops and thus attracted the earliest *H. halys* adults during the bud stage on June 28 (38 days after transplanting ) (Figure 7). Native species were also present but represented only 3.1% of the population. *H. halys* adult activity showed two distinct periods on July 22 and August 30, with peak densities averaging 0.17 and 0.19 adults/m<sup>2</sup>, respectively. Similar to stink bug abundance patterns in okra, the temperature

accumulations between peaks allowed for a generation of stink bug recruitment in eggplant. Egg laying was evident during the first period of adult activity, with egg mass levels ranging up to  $0.05/m^2$ , but very few eggs were found during August and September. Small and large nymphs were first found on July 11 and 25, respectively, and both stages exhibited abundance patterns associated with each period of adult activity (Figure 7). Densities of small and large nymphs reached peak levels of 0.07 and  $0.17/m^2$  on August 12 and 30, respectively.

Similar to okra, eggplant growth had a relatively long flowering pattern that provided fruiting structures as feeding sites from July through to mid-September. Immature fruit was first noted on July 8 (48 days after transplanting) and the basal fruit was harvestable by July 18. Adults and nymphs were found primarily around flowers, buds and young fruit, where their feeding activity probably affected fruit set. However, the effect of this indirect injury on yield loss could not be determined. The only evidence of stink bug feeding injury was characterized as small dimples on the surface of the eggplant fruit, without any associated exterior discoloration. These dimpled areas were believed to be sites where the feeding stylets were inserted through the skin of the eggplant. Thus, they were difficult to distinguish from other surface defects, such as scars and pitted areas. In fact, most fruit displaying these feeding sites were of normal size, shape and color of marketable eggplant. However, when the skin around the dimpled area was peeled back, the interior of the fruit often exhibited a tan to brown discoloration (Figure 3c). Of the 838 eggplant fruit harvested, only  $5.07\% \pm 1.08$  (SEM) showed definitive signs of feeding injury (Table 2), though this is likely an underestimation of the fruit damage due to the cryptic nature of the feeding injury. A study by Fukuoka et al.

(2002) reported stink bug injury levels on eggplants up to 90%, but this difference in crop losses are probably due to differences in the infestation level, eggplant variety and growing conditions. The regression analysis showed that the abundance and duration of stink bug activity expressed as cumulative stink bug days accounted for 62.2% of the variation in the percentage of injured fruit, with a significant regression slope (or relative injury rate) of 0.326 ( $R^2=0.62$ ,  $F_{(1,52)}=85.68$ ,  $P < 0.001$ ) (Figure 8). In contrast with okra, the injury rate per stink bug to eggplant was higher, even though the population density was an order of magnitude lower. This difference is likely due to significantly lower numbers of fruit per plant and the fact that the fruit provided a larger surface area for feeding and required a much longer time to reach harvestable size.

**Bell Pepper.** Nearly 100% of the stink bugs in bell peppers were *H. halys* and the first adults were detected on June 28 (38 days after transplanting) during the open flower stage (Figure 9). The seasonal activity of adults exhibited two distinct periods during the reproductive stages of plant growth, similar in the timing of activity peaks observed in okra and eggplant. Adult densities reached peak mean levels of 2.2 and 3.4 adult/m<sup>2</sup> on July 25 and August 30, respectively. Egg masses were present throughout most of July and early August but numbers were low (generally <0.04/m<sup>2</sup>) and inconsistent among sampling dates. Small and large nymphs were initially found on July 8 and July 22 (Figure 9), and nymph populations peaked on August 30, with an average of 0.77 small and 1.0 large nymphs/m<sup>2</sup>, respectively.

Pepper plants are indeterminate plants, so they continued to grow taller as new leaves and lateral side shoots develop from the stem axils. Flowers also develop continuously as long as mature fruit is removed. Thus, various stages of flowering and

fruit set were present when *H. halys* stages were active on the plants. However, the majority of adults and nymphs were observed on immature and mature fruit. Marketable sized crown peppers were first harvested on July 18 (58 days after transplanting) and weekly harvests removed a total of 1,134 peppers (Table 2). Of the total harvest, 39.7% ± 4.19 (SEM) of the bell peppers exhibited stink bug feeding injury, which was characterized as pale-yellow cloudy areas on the fruit surface, associated with whitish, spongy tissue beneath the affected area (Figure 3d). Injury caused by multiple feeding events was often aggregated around the shoulder of the pepper. Although the affected areas were superficial and cosmetic in nature, they represented defects that detracted from the appearance and market quality of the pepper. Furthermore, injury often resulted to a break in the fleshy wall of pepper during storage, which increased the incidence of rot organisms. Because *H. halys* preferred mature fruit, the cumulative stink bug days accounted for 84.8% of the variation in the percentage of injured peppers. Similar to eggplant, this relationship generated a significant regression slope or injury rate per stink bug to pepper of 0.32 ( $R^2=0.85$ ,  $F_{(1,52)}=289.38$ ,  $P < 0.001$ ) (Figure 10).

**Tomato.** Numerous studies reported stink bugs as economic pests of fresh market and processing tomatoes (Kennedy et al. 1983, Lye & Story 1988, Zalom et al. 1997, McPherson and McPherson 2000, Nault & Speese 2002, Brust 2008). Results of this study also showed significant injury to tomato fruit caused by stink bugs; however, the population dynamics, species composition, and relative injury rate were significantly different from those of the other vegetable crops. Fourteen percent of the stink bugs encountered in tomato were native species, primarily *E. servus*, which was a higher percentage than the other vegetable crops. Secondly, the overall population density of

stink bugs during the fruiting period was the lowest of the six vegetable crops tested. *H. halys* adults were late to colonize the tomato plots, being first detected on July 18 (58 days after transplanting), when flowers and immature fruit were present on the plants (Figure 11). Adult densities were very low until August 30 when numbers increased and reached peak levels of 0.22 adults/m<sup>2</sup> on September 24. Very few egg masses were detected on tomato plants and consistent nymphal activity was also not evident until later in August, reaching peak densities of 0.05 small and 0.26 large nymphs/m<sup>2</sup>, respectively. Numbers of nymphs per plot were highly variable and the phenology of the nymphal stages relative to adult activity did not indicate a clearly defined reproducing generation. In fact, large nymphs were more numerous than adults on August 30, which suggests that many nymphs may have immigrated into the tomato plots from neighboring vegetables.

Weekly harvests of marketable fruit began on August 1 and continued through most of September. A total of 2,021 red and blush tomatoes were harvested over the fruiting cycle, of which  $33.9\% \pm 2.97$  (SEM) exhibited signs of stink bug feeding injury that in most cases reduced the marketing quality of the tomato (Table 2). The injury was characterized as light-yellow discolored blemishes (so-called “cloud spots”), often with distinguishable pinpricks seen within the affected area where the feeding stylets were inserted, and associated with spongy or corky tissue below the surface (Figure 3e). The tissue surrounding the spongy mass appeared normal. Other studies have reported similar feeding injury characteristics on tomato caused by several native stink bug species (Lye & Story 1988, Lye et al. 1988, Zalom et al. 1997), and have also shown that feeding can lead to premature ripening and smaller fruit (Lye et al. 1988). It is also known that stink bugs including *H. halys* introduce yeast and other pathogens into the tomato by their

mouthparts, which results in eventual fruit decay and reduced storage life (Zalom et al. 1997).

Interestingly, tomato had the highest relative injury rate compared to the other vegetable crops (Table 2), yet the abundance and duration of stink bug activity during the fruiting cycle was the lowest in terms of cumulative stink bug days. The relationship between the percentage of injured fruit and cumulative stink bug days shown in Figure 12 revealed a significant slope of 1.65 ( $R^2=0.23$ ,  $F_{(1,40)} = 12.02$ ,  $P = 0.001$ ). However, stink bug abundance accounted for only 23.1% of the variation in fruit injury and the strength of the relationship was based primarily on three outlier data points. Without these points, the relationship would likely show a weak association between injury and stink bug days. Other researchers have reported tomato injury levels that were higher than expected based on the number of stink bugs present (personal communication T. Kuhar). Stink bugs in general are nocturnal feeders (Shearer et al. 1996, Krupke et al. 2006) so it is possible that *H. halys* and native species moved into the tomato plots during the night to feed and then moved to neighboring plots during the day when sampling was conducted. Moreover, the relative low level of reproduction in tomato supports this idea and suggests that tomatoes are a preferred feeding site but not a suitable host for colonization and oviposition.

**Sweet Corn.** Although not considered major pests, several species of stink bugs have been reported to cause economic damage to field corn (Negron & Riley 1987, McPherson et al 2000, Ni 2010, Tillman 2011). *Nezara viridula* and *E. servus* are predominant stink bug species found in field corn in the southeastern United States. Higher populations have been reported along adjacent crop edges and dispersal between

corn and other crops, such as cotton, peanuts, soybeans, and wheat, is common throughout the growing season (Tillman 2011, Reisig et al. 2013). Early and late corn plantings can serve as a reproductive host for *N. viridula* and *E. servus*, however late plantings are more favorable to nymphal development than early plantings, leading to a higher adult population in late plantings (Tillman 2010, 2011). Stink bug feeding on the vegetative stages of corn results in severe wilting, plant mortality, delayed flowering, ear loss, and reduction in ear length and weight (Clower 1958, Negron & Riley 1987). Ni et al. (2010) found that 3 or more adult *E. servus* feeding on corn for 9 days during the tasseling stage increased the number of discolored kernels and reduced the ear and kernel weight, however feeding during the later development stages had little effect on yield.

There are few studies of stink bug damage to sweet corn. Results of this study clearly showed that *H. halys* adults had a strong affinity to sweet corn as a host plant. Adult stink bugs were first detected during the early green tassel stage on July 2 (41 days after planting). Although they were observed feeding on the green tassels emerging from the whorl, there was no evidence of direct injury or any noticeable impact on tassel development and pollen shed. A major influx of colonizers (99% were *H. halys*) occurred over the preceding three weeks, coinciding with anthesis, silking and ear development (Figure 13). At this time, the population density reached mean levels up to 6.33 adults/m<sup>2</sup>, and the majority of adults were observed on the ears, feeding on developing kernels by inserting their stylets through the husk leaves. *H. halys* utilized sweet corn as a reproductive host as evident by a relatively consistent presence of egg masses ranging in density from 0.01 to 0.10/m<sup>2</sup> during the entire period of adult activity. Despite the fact that the oviposition rate was the highest overall compared to the other

vegetables (based on average densities when adults were present), relatively low densities of nymphs were observed during late July or early August, just several weeks prior to harvest maturity. Densities of small and large nymphs peaked at mean levels of 0.31 and 0.38 nymphs/ m<sup>2</sup>, respectively. Due to the short period of attractiveness and the timing of nymphal development, the progeny of *H. halys* reproduction in sweet corn did not complete a full generation. Based on unexplained increases in nymph populations in adjacent vegetable crops, it was obvious that many nymphs and adults were forced to move out of the sweet corn plots after harvest.

Two separate harvests were conducted during mid-August when the primary ears reached fresh market maturity and then plots were terminated according to commercial management practices. A total of 383 marketable ears were removed from the sweet corn plots and husked open to examine for kernel injury. Of the six vegetable crops, sweet corn had the highest incidence of *H. halys* injury, with 97.0% ± 1.42 (SEM) of ears showing symptoms of feeding injury on the kernels. Other studies have reported similar levels of ear damage ranging from 70 to 100% (Fukuoka et al. 2002, Kuhar et al. 2012a). Stink bug feeding resulted in injured or aborted kernels depending on when the feeding occurred relative to kernel development. Injured kernels were partially or fully developed and showed a sunken area with a chalky white pericarp surrounded by darker yellow or brownish discolored margins (Figure 3f). Aborted kernels did not fully develop and often had brown spots on the ear cob corresponding to areas of missing kernels. This type of damage was probably the result of earlier stink bug feeding on the ovaries or it is possible that extensive feeding on the tassel could have affected pollen shed and thus reduced fertilization of ovaries. The severity of kernel injury varied widely

from the ear to ear and averaged  $12.5 \pm 0.83$  SEM injured kernels per ear. Nineteen % of the affected ears also had aborted kernels that accounted for 20% of the kernel area missing. Percentage of injured ears and number of injured kernels per ear were positively related to the cumulative stink bug days (Figure 14), which accounted for 37.3% and 33.6% of the variation in the injury measurements, ( $R^2=0.37$ ,  $F_{(1,10)} = 5.95$ ,  $P = 0.03$ ;  $R^2=0.33$ ,  $F_{(1,10)} = 5.06$ ,  $P = 0.04$ ) respectively. The slope or relative injury rate of 0.711 for the percentage of injured ears was the second highest (Table 2) and was significantly different from those of green bean, okra, eggplant and bell pepper.

This high injury rate per stink bug was attributed to the relative low numbers of ears per  $m^2$  compared to the other vegetable crops and the fact that just one stink bug feeding on an ear can render it as an injured ear. There was no significant relationship between the percentage of aborted missing kernels per ear and cumulative stink bug days, suggesting that perhaps other factors may have contributed to the incompletely filled ears.

### **Preference for host plant and phenological growth stages.**

*H. halys* is a polyphagous herbivore that utilizes at least 170 cultivated or uncultivated plants for food and reproduction (Leskey, et al. 2012b, BMSB IMP Working Group, 2013). Because stink bugs in general can go through multiple generations during the growing season and they prefer fruiting structures of plants (Schuh & Slater 1995, McPherson & McPherson 2000), host switching from one temporally available food plant with suitable feeding sites to another is common. This appears to represent the feeding behavior of *H. halys* in this study. Adult *H. halys* were attracted to and colonized all six vegetable crops but their population abundance and duration of activity were significantly different depending on when each crop produced suitable fruiting structures for food.

Adults initially colonized the first planting of green beans during early July because it was the only host plant at the time to provide fruiting structures and developing beans (Figure 15). The adult population was relatively low overall in green beans based on the total cumulated stink bug days of 67.04. However, once the green tassels emerged in mid-July, sweet corn became the preferred host plant and supported the highest population of adults (591.50 cumulated stink bug days) until the plots were harvested and terminated in early August. Okra and bell pepper plants also produced fruiting structures that attracted adults during late July and early August but the total cumulative stink bug days during this period in both crops were less than half that of sweet corn. As mentioned earlier, these early colonizers may have been overwintered adults but the majority of invading adults during late July were probably F1 progeny of the overwintered population based on degree-day accumulations required for development. Adult activity in all vegetable plots dropped to relatively low levels during mid-August, even in the more preferred host plants that had attractive fruiting structures. This drop in activity reflected the end of the overwintered generation of adults and the beginning of the next F1 generation, although it is unclear whether these were discrete generations or partially attributed to immigrants from outside the study site.

After sweet corn was harvested, okra and bell pepper became the preferred host plants for *H. halys*, supporting the highest populations of adults until the study ended in early October. Due to the long fruiting periods of both crops, sustained adult activity occurred for more than a month and amounted to total adult cumulative stink bug days of 1,374.82 and 712.30 for okra and bell pepper, respectively. The abundance and duration of adult activity in these host plants exceeded all others including sweet corn. However,

if sequential plantings of sweet corn had been present, which is a common commercial practice (Tillman 2010, 2011) *H. halys* adults and nymphs may have move to it instead of okra and bell peppers, lessen their late season abundance. In terms of overall seasonal abundance, green bean, eggplant and tomato were the least attractive to *H. halys*, with cumulative adult stink bug days totaling to 67.03, 54.48 and 30.70, respectively.

The findings based on peak densities and cumulative stink bug days provide strong evidence that sweet corn, okra and bell pepper are preferred vegetable hosts for *H. halys* adults. This invasive species has been reported as a major pest of sweet corn and bell pepper in its native range (Fukuoka et al. 2002, Lee et al. 2013) and in the United States (Kuhar et al. 2012a-d). For okra, there is little information about *H. halys* as an economic pest of this crop in its native range; however it has been reported to cause injury on other *Malvaceae* plants in China (Hoebeke & Carter 2003) and is known to feed on okra in the United States (Kuhar et al. 2012a). *H. halys* is an important pest of eggplant in Japan (Fukuoka et al. 2002), yet the infestation was relatively low in this study and is questionable whether the feeding injury would be considered economic. It should be noted however that *H. halys* attraction to eggplant could be much greater if the crop is grown in isolation from more preferred host plants. Similarly, *H. halys* densities in tomato were the lowest in spite of the injury observed and the fact that native stink bugs are common pests of tomatoes in the United States (Kennedy et al. 1983, Lye & Story 1988, Zalom et al. 1997, McPherson & McPherson 2000, Nault & Speese 2002, Brust 2008). Results are inconclusive regarding the actual impact of *H. halys* feeding on this crop but it seems that tomato is probably the least preferred as a host plant. However, like eggplant, infestations may be higher if tomato was the only host plant

available. For instance, *H. halys* was reported to have caused severe yield loss on commercial tomato fields in the Mid-Atlantic States in 2011 (StopBMSB.org, 2014).

Although host plant preference varies substantially, *H. halys* showed a strong affinity to the reproductive stages of plant growth. Table 3 summarizes the average density of adults pooled over sampling dates during five developmental stages of each vegetable crop. With the exception of sweet corn, the highest densities occurred during fruiting when immature or mature marketable fruit were present. This was expected since *H. halys*, as well as other stink bug species, are known to exhibit a strong preference for feeding on fruits and seeds (Schuh & Slater 1995, McPherson & McPherson 2000). Interestingly, sweet corn attracted significantly higher numbers of adults during the vegetative and flowering stages compared to the other crops, even though suitable immature fruit were available on bell pepper, okra and eggplant at the same time. One plausible explanation is that adults were attracted to sweet corn plants because they were significantly taller than the other plants. There is some agreement among researchers that *H. halys* adults tend to colonize taller host plants (BMSB IMP Working Group 2013). Similar behavior has been observed by *Bathycoelia natalicola* Distant, the two-spotted stink bug, in macadamia orchards (Schoeman 2014). This would also be a plausible explanation for why okra became the preferred host plant after sweet corn.

### **Reproductive suitability of host plants.**

Phytophagous insects use particular host plants for food to sustain growth, survival and reproduction of offspring; however, the observed adult preference for certain host plants does not always result in successful performance of offspring (Panizzi & Slansky 1991, Velasco & Walter 1992, Clarke & Walter 1993). In this study, a crop with

eggs and nymphal instars showing densities that reflected the temporal development of consecutive life stages was considered a suitable reproductive host plant (Clarke & Walter 1993, Panizzi 1997, Panizzi 2000). *H. halys* females oviposited on the six vegetable crops, however the majority of egg masses were found in okra (31%), sweet corn (22%), and eggplant (20%). Sixty percent of all egg masses were found during the month of July, which was primarily the result of oviposition by the overwintered adults. The ratio of egg masses to the number of adults at any given sampling date was unusually low, when one considers that 8 to 18 egg masses are produced per female adult (Kawada 1982, Nielsen 2008). Egg masses were hard to find on leaves, compared to nymphs and adults, so the low numbers may be in part due to sampling error. Also, egg predation could have removed many egg masses because studies have shown relatively high levels of egg predation as well egg masses that are missing after they have been found and subsequently observed later (personal communication C. Hooks).

A better criterion for successful reproduction is the number of nymphs produced on the host plant relative to the number of adults. To express this in terms of overall seasonal abundance, Table 4 summarizes the mean proportions of the total stink bug days attributed to the nymph and adult stages found in each vegetable crop. Sweet corn had the lowest proportions of stink bug days attributed to the nymphal stages, which suggests that sweet corn was less suitable for reproduction. This is likely due to its short period of available food for feeding before ears were harvested. Tillman (2010) found low densities of nymphs of native stink bugs in early plantings of field corn. However, this study also reported higher densities earlier in the crop development stages of later plantings, which provided enough time during the entire crop cycle for full development

of the stink bug stages. This suggests that sequential plantings of sweet corn in the same field, which is a common commercial practice, may allow nymphs to move between plantings in order to complete development. Furthermore, it is not uncommon for growers to leave sweet corn plantings standing after harvest which would result in a more suitable reproductive host plant for *H. halys*.

Eggplant, green bean, okra and bell pepper had the highest proportions of stink bug days for small and large nymphs, and the seasonal abundance of the nymphs reflected the expected trend of progressive development over time. Taken altogether, the results indicate that these vegetable crops were suitable host plants for *H. halys* reproduction and development. Tomato had similar proportions of life stages but these values (particularly for large nymphs) were probably overestimated due to the expected immigration of nymphs into tomato plots from adjacent host plants and the possible diurnal movement in and out of plots by adults. In any case, based on egg mass numbers alone and relatively few small nymphs, tomato was probably the least suitable for *H. halys* reproduction.

## Summary

This study provides the first detailed account of the seasonal abundance, host plant preference, reproductive suitability, and injury potential of *H. halys* on crops of green bean, sweet corn, eggplant, okra and bell pepper. The overall seasonal abundance of *H. halys* in these vegetable crops consisted of both overwintered adults and their F1 progeny which encompassed almost two generations over the growing season. Overwintered adults first colonized green beans and sweet corn at the end of June and reached peak population densities during July, particularly in sweet corn, okra and bell

pepper which attracted the highest abundance of stink bugs. First generation nymphal progeny from the overwintered adults resulted in significantly larger populations of F1 adults that steadily build up during the latter half of August, producing a second generation of nymphal stages through most of September. *H. halys* represented 99% of the stink bugs observed, except for green bean and tomato plots which had significant numbers of native *E. servus*.

Results clearly showed that *H. halys* strongly preferred host plants with reproductive structures for feeding and was more abundant and capable of reproducing on vegetable crops that had extended periods of fruiting. The timing and duration of the fruiting period of each crop had a strong influence on the overall seasonal abundance and reproductive capability of *H. halys*. One exception was sweet corn which attracted a relatively high density of *H. halys* adults during the green tassel stage prior to ear development. However, this response was probably partly due to the taller canopy height of the plants compared to the other crops. This physical characteristic may have influenced host plant selection of other crops such as okra which reached several meters in height. After the sweet corn plots were harvested and removed, there was evidence of inter-plot movements by adult stink bugs and older nymphs to nearby plots of other host plants. Okra and bell pepper were preferred host plants of *H. halys* for the majority of the growing season because these indeterminate plants had attractive fruiting structures available from mid-July through September. Green bean, eggplant and tomato were the least preferred host plants in terms of overall abundance. Although the production of vegetable crops in this study was intended to represent a typical small farm system, relative host plant preferences may be different if planting times and periods of fruiting

varied in different mixtures of vegetable crops. This study did not have sequential plantings of sweet corn and green beans, which is a common commercial practice and may result in different mid and late season abundances than those reported here. Also, less preferred host plant in this study, such as eggplant, may experience higher populations and feeding injury by *H. halys* if it is the only host plant available.

Results showed significant differences in the reproductive suitability of the host plants as evident by the relative proportion of nymphal stages to adults. Overwintered *H. halys* adults oviposited on all vegetable crops, but the densities of egg masses were relatively low compared to the number of adults present. Therefore, any ovipositional preference by *H. halys* to particular host plants was unclear based on the egg mass densities. Although sweet corn was a preferred host plant for adult feeding, the kernel development stages as the favorite feeding sites were not available long enough to accommodate extended nymphal development to adults. A similar situation was true for the short crop cycle of green bean, although this host plant would otherwise be considered a suitable reproductive host. Eggplant, okra and bell pepper were unquestionably the most suitable host plants for *H. halys* reproduction and development. They supported the highest proportions of nymphs with seasonal abundance trends indicating progressive development to adult eclosion.

The study demonstrated that the yield loss potential due to feeding injury by *H. halys* varied significantly among vegetable host plants. Sweet corn, eggplant, bell pepper and tomato were very susceptible to feeding injury and experienced the highest injury rate per stink bug. This was partly due to the length of time that individual fruit were exposed to stink bugs but mainly the result of higher and more prolonged infestations of

adults and nymphs. The exception was the high rate of tomato fruit injury that was incongruent with the lowest infestation of *H. halys* observed on tomato compared to the other crops. This unsolved association needs further studies to fully understand the feeding behavior of *H. halys* and native stink bugs on tomato, particularly if the injury is caused by nocturnal feeding of stink bugs moving in and out of plots (which mean that daytime sampling underestimated adult density). The harvest data on eggplant also did not clearly characterize the fruit injury and the impact of *H. halys* feeding on fruit quality and marketable yield. Finally, this study did not address the possible loss of yield due to abortion or physiological stress caused by stink bug feeding on immature fruiting structures. Thus, additional studies involving treated and untreated plots of vegetables that are naturally infested with *H. halys* are required to quantify the full impact of this invasive species on fruit set and fruit quality.

Several findings of this study have implications to the sampling and management of *H. halys* in vegetable production. First, the challenges of managing this highly mobile polyphagous stink bug were well demonstrated by the changes in colonization patterns and the inter-plot movement of adults and nymphs among vegetable crops, triggered by crop destruction and changes in available fruiting structures as crops matured. Secondly, knowledge of the seasonal phenology and abundance of *H. halys* stages infesting the different vegetable crops will help pest managers schedule scouting visits to assess infestation levels and apply timely control measures. For instance, since the efficacy of many insecticides depends on targeting applications against the early nymphal stages, knowing when these stages are present is very useful information. Knowledge of the host plant preferences can be used also to decide when and where to plant certain vegetable

crops to minimize *H. halys* colonization and avoid the movement of stink bugs between host plants. Finally, although the economic injury level for *H. halys* infestations has not been developed for any vegetable crop, characterization of the feeding injury and estimates of the relative injury rates determined in this study are prerequisites for the development of treatment thresholds for control action.

In summary, this study focused only on the basic questions about host plant selection and crop loss assessment of *H. halys* feeding on vegetable crops. Future research is needed to better unravel why certain crops and their associated phenological growth stages are preferred by *H. halys* over others and whether their feeding preference is based solely on the nutritional status of feeding structures or on other factors, such as the physical and chemical characteristics of plants. This information will lead to better predictions of the vulnerability of vegetable host plants in time and space.

**Table 1. Mean density ( $\pm$  SEM) of adult and nymph brown marmorated stink bug populations pooled across all vegetable crops within each replicate column<sup>1</sup> of a Latin square design of the experimental field site during the second half<sup>2</sup> of the growing season. Clarksville, MD. 2013.**

Column	Mean brown marmorated stink bug per m <sup>2</sup> <sup>3</sup>	
	Nymphs (2 <sup>nd</sup> – 5 <sup>th</sup> ) instars	Adults
1	0.221 $\pm$ 0.057 (b)	0.878 $\pm$ 0.211 (a)
2	0.438 $\pm$ 0.177 (ab)	1.066 $\pm$ 0.286 (a)
3	0.180 $\pm$ 0.059 (a)	1.064 $\pm$ 0.285 (a)
4	0.278 $\pm$ 0.084 (ab)	1.287 $\pm$ 0.364 (a)
5	0.326 $\pm$ 0.116 (ab)	1.085 $\pm$ 0.310 (a)
6	0.924 $\pm$ 0.384 (a)	1.448 $\pm$ 0.381 (a)

<sup>1</sup>Columns were laid out in a south to north direction across rows of black plastic mulch.

Each column contained one plot of each vegetable crop (sweet corn, green bean, eggplant, tomato, bell pepper and okra). <sup>2</sup> Second half of the growing season was from August 10 onward. <sup>3</sup>Means within a column with the same letter in parenthesis are not significantly different ( $P=0.05$ ).

**Table 2. Total number of harvested fruit, the mean percentage ( $\pm$  SEM) of injured fruit due to stink bug feeding, and the relative injury rate<sup>1</sup> of six vegetable crops during the growing season. Clarksville, MD. 2013.**

Vegetable Crop	# Harvested Fruit	Mean % injured fruit ( $\pm$ SEM)	Injury Rate <sup>2</sup>
Green bean	21,126	1.80 $\pm$ 0.24	0.098 (bcd)
Okra	5,035	33.25 $\pm$ 277	0.132 (d)
Eggplant	838	5.07 $\pm$ 1.08	0.326 (bcd)
Bell pepper	1,434	39.70 $\pm$ 4.19	0.318 (c)
Tomato	2,021	33.91 $\pm$ 2.97	1.652 (a)
Sweet corn	383	96.99 $\pm$ 1.42	0.711 (b)

<sup>1</sup> Injury rate calculated by comparing the cumulative stink bug days with the cumulative percentage of injured fruit. <sup>2</sup> Means within a column with the same letter in parenthesis are not significantly different ( $P=0.05$ )

**Table 3. Overall mean density per m<sup>2</sup> ( $\pm$  SEM) of adult brown marmorated stink bugs during different crop developmental stage of six vegetable crops. Clarksville, MD. 2013.**

Vegetable Crop	Plant Development Stage <sup>1</sup>				
	Vegetative stages	Flower buds & Green tassel <sup>2</sup>	Open flower & Silking <sup>2</sup>	Immature fruit	Marketable fruit
Sweet corn	0.051 $\pm$ 0.015 (f)	1.656 $\pm$ 0.511 (cde)	4.447 $\pm$ 0.809 (a)	2.845 $\pm$ 0.314 (ab)	1.808 $\pm$ 0.263 (bcd)
Okra	0.000 $\pm$ 0.000 (f)	0.006 $\pm$ 0.006 (f)	0.012 $\pm$ 0.012 (f)	0.227 $\pm$ 0.104 (ef)	2.216 $\pm$ 0.222 (c)
Bell pepper	0.000 $\pm$ 0.000 (f)	0.000 $\pm$ 0.000 (f)	0.016 $\pm$ 0.012 (f)	0.175 $\pm$ 0.052 (f)	1.216 $\pm$ 0.117 (de)
Tomato	0.000 $\pm$ 0.000 (f)	0.000 $\pm$ 0.000 (f)	0.000 $\pm$ 0.000 (f)	0.005 $\pm$ 0.003 (f)	0.058 $\pm$ 0.017 (f)
Green bean	0.002 $\pm$ 0.002 (f)	0.008 $\pm$ 0.008 (f)	0.037 $\pm$ 0.022 (f)	0.186 $\pm$ 0.042 (f)	0.246 $\pm$ 0.033 (f)
Eggplant	0.000 $\pm$ 0.000 (f)	0.018 $\pm$ 0.013 (f)	0.071 $\pm$ 0.028 (f)	0.076 $\pm$ 0.023 (f)	0.087 $\pm$ 0.013 (f)

<sup>1</sup> Means with the same letter in parenthesis are not significantly different ( $P=0.05$ ). <sup>2</sup>

Green tassel and silking refer to sweet corn only.

**Table 4. Mean proportion ( $\pm$  SEM) of the total stink bug days<sup>1</sup> attributed to the nymph and adult stages of brown marmorated stink bug during the growing season of six vegetable crops. Clarksville, MD. 2013.**

Vegetable crop	Proportion of the total stinkbug days <sup>2</sup>		
	Small nymphs (2 <sup>nd</sup> and 3 <sup>rd</sup> instars)	Large nymphs (4th and 5th instars)	Adults
Sweet corn	0.045 $\pm$ 0.013 (b)	0.022 $\pm$ 0.009 (b)	0.928 $\pm$ 0.021 (a)
Okra	0.055 $\pm$ 0.011 (ab)	0.160 $\pm$ 0.038 (ab)	0.783 $\pm$ 0.046 (ab)
Bell pepper	0.097 $\pm$ 0.015 (ab)	0.130 $\pm$ 0.028 (ab)	0.773 $\pm$ 0.035 (ab)
Tomato	0.042 $\pm$ 0.020 (b)	0.193 $\pm$ 0.064 (a)	0.768 $\pm$ 0.077(ab)
Green bean	0.118 $\pm$ 0.041 (ab)	0.232 $\pm$ 0.036 (a)	0.648 $\pm$ 0.058 (b)
Eggplant	0.158 $\pm$ 0.033 (a)	0.195 $\pm$ 0.039 (a)	0.645 $\pm$ 0.066 (b)

<sup>1</sup>Stink bug days for each life stage were derived by averaging the density per m<sup>2</sup> recorded over two sequential sampling dates and multiplying by the number of days between the sampling dates. Values of stink bug days were then accumulated over the entire cropping season. Proportions were calculated by dividing the accumulated stink bug days of each life stage by the total amount of stink bug days for each vegetable crop. <sup>2</sup> Means within a column with the same letter in parenthesis are not significantly different ( $P=0.05$ )

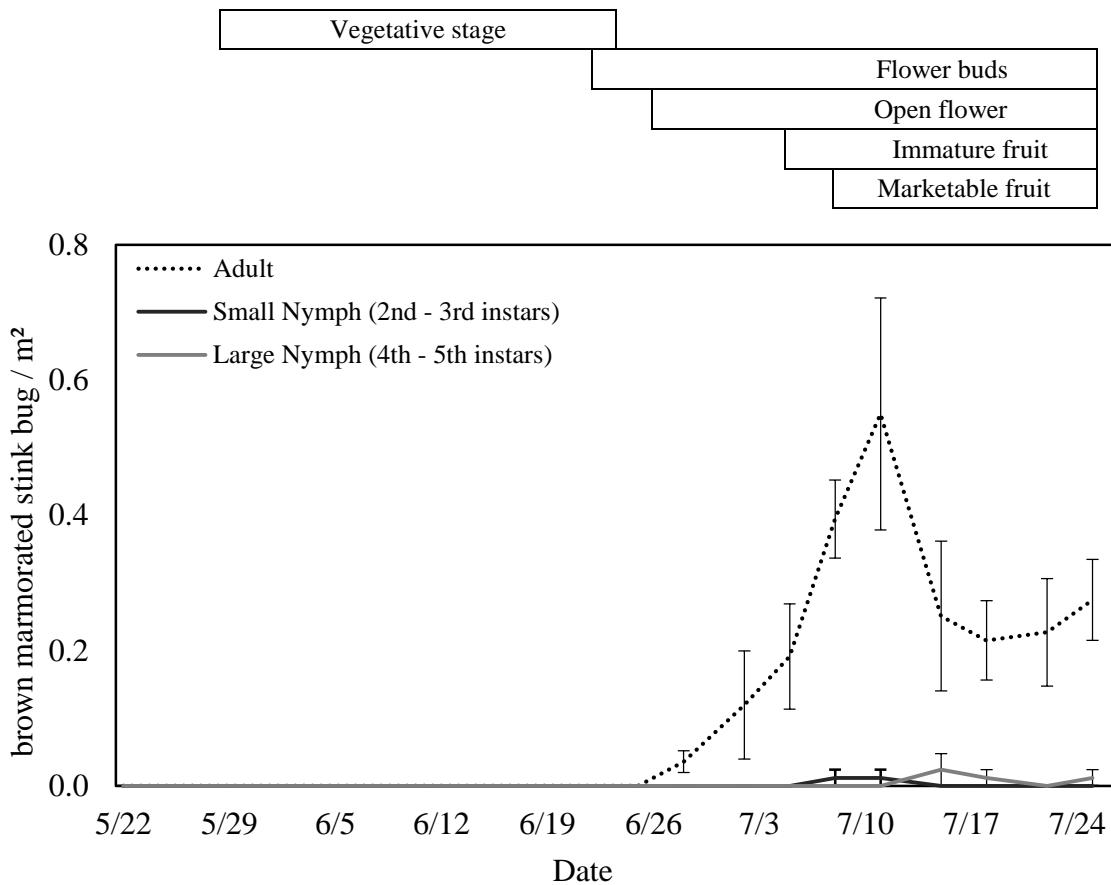


Figure 1. Mean density ( $\pm$  SEM) of brown marmorated stink bug life stages in an early season planting of green bean. Plants were direct-seeded on May 22. Horizontal bars above the graph depict the period of each development stage. Clarksville, MD. 2013.

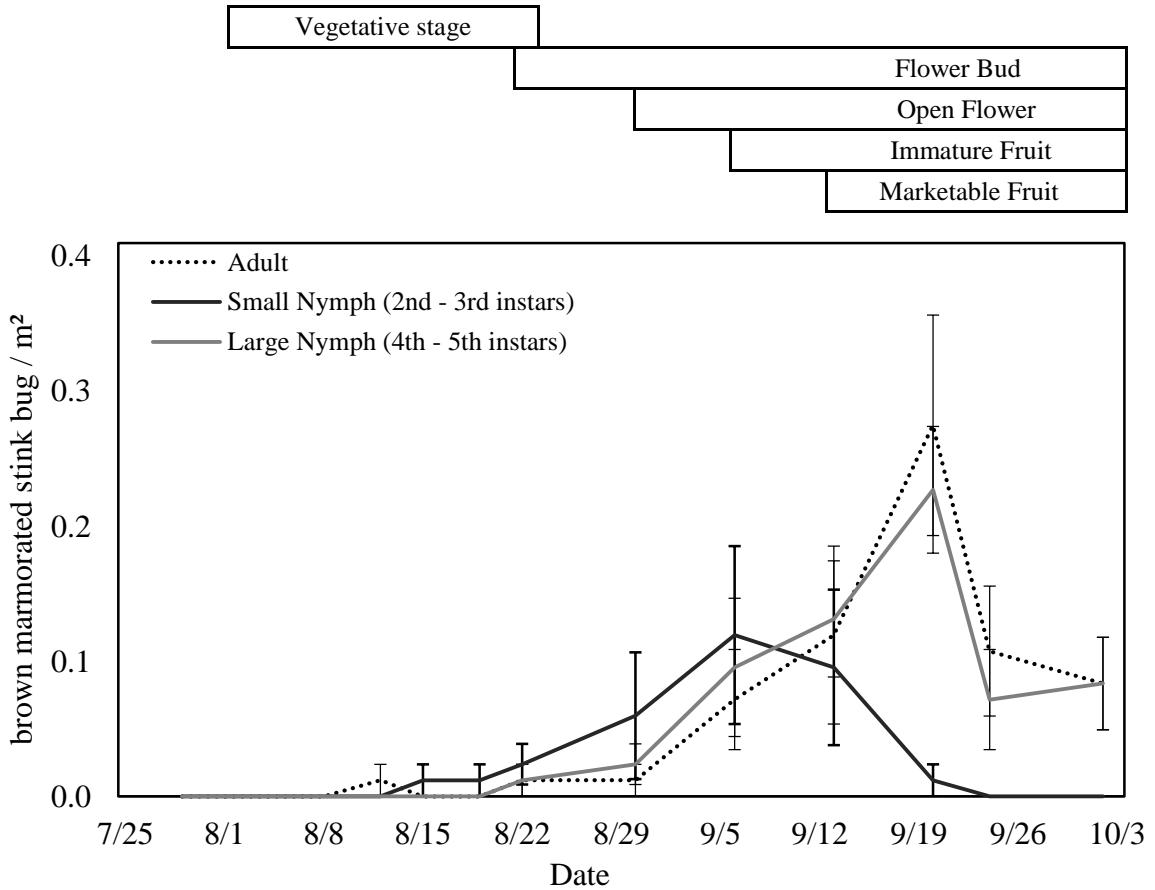


Figure 2. Mean density ( $\pm$  SEM) of brown marmorated stink bug life stages in a late season planting of green bean. Plants were direct-seeded on July 24. Horizontal bars above the graph depict the period of each development stage. Clarksville, MD. 2013.

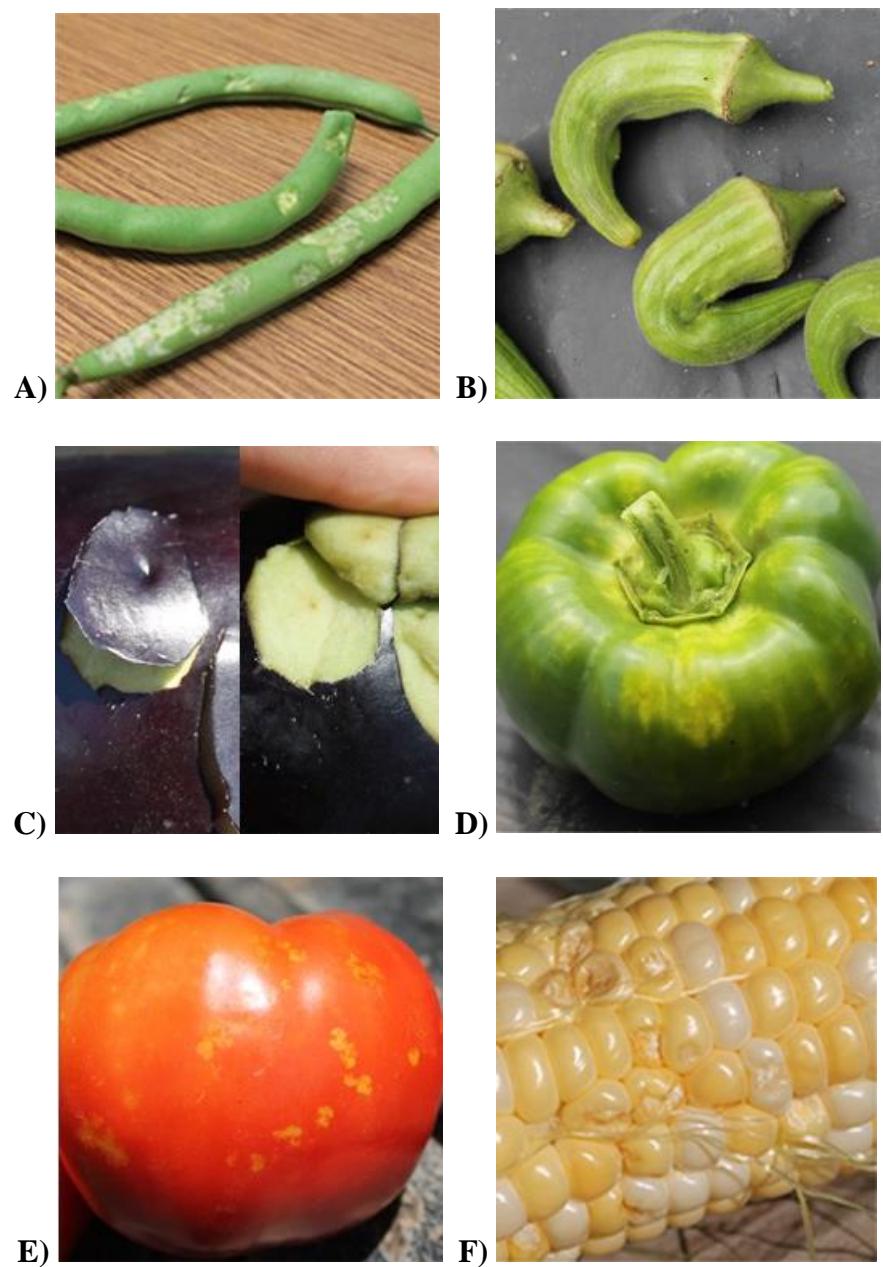


Figure 3. Feeding injury caused by the brown marmorated stink bug on: A) green beans (*Phaseolus vulgaris*), B) okra (*Abelmoschus esculentus*), C) eggplant (*Solanum melongena*), D) bell pepper (*Capsicum annuum*), E) tomato (*Solanum lycopersicum*), and F) sweet corn (*Zea mays* convar. *saccharata* var. *rugosa*).

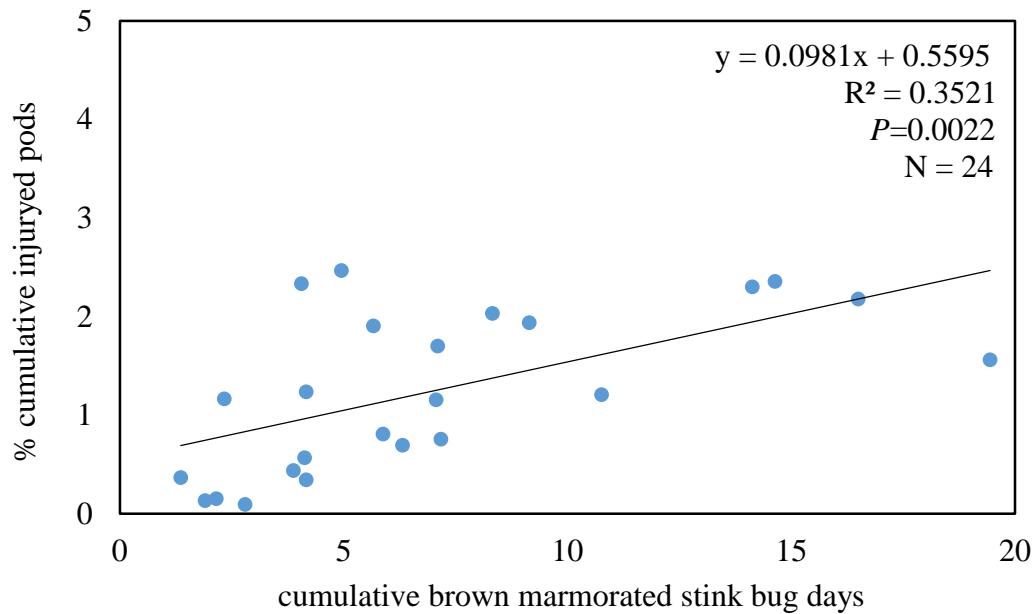


Figure 4. Percent of cumulative injured green bean pods due to brown marmorated stink bug feeding as a function of cumulative total stink bug days over the entire crop cycle. Clarksville, MD, 2013. The cumulative percentage of injured pods is equal to the accumulated number of harvested pods divided by the accumulated number of injured pods at each harvest date. Stink bug days were calculated by averaging the numbers stink bug/m<sup>2</sup> from the current and last sampling events, and then multiplying that value by the number of days between the sampling events.

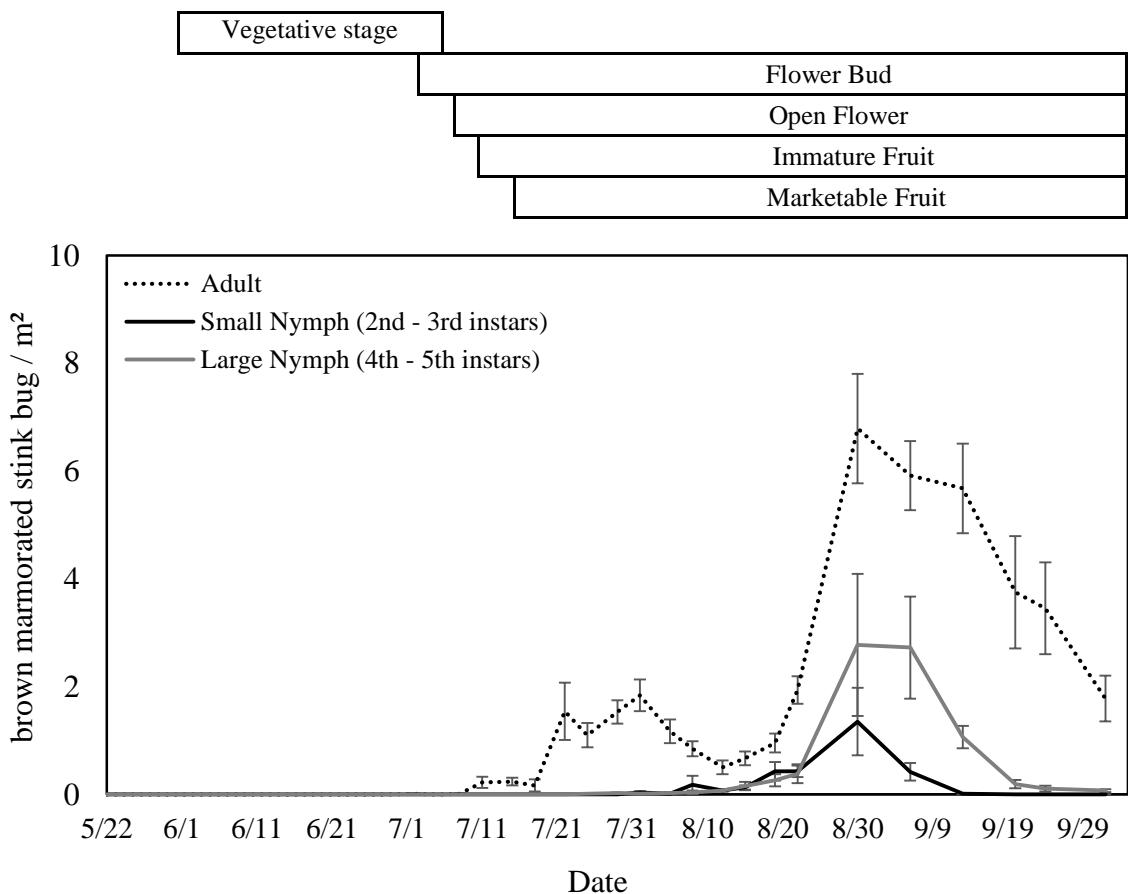


Figure 5. Mean density ( $\pm$  SEM) of brown marmorated stink bug life stages in okra.

Plants were direct-seeded on May 22. Horizontal bars above the graph depict the period of each development stage. Clarksville, MD. 2013.

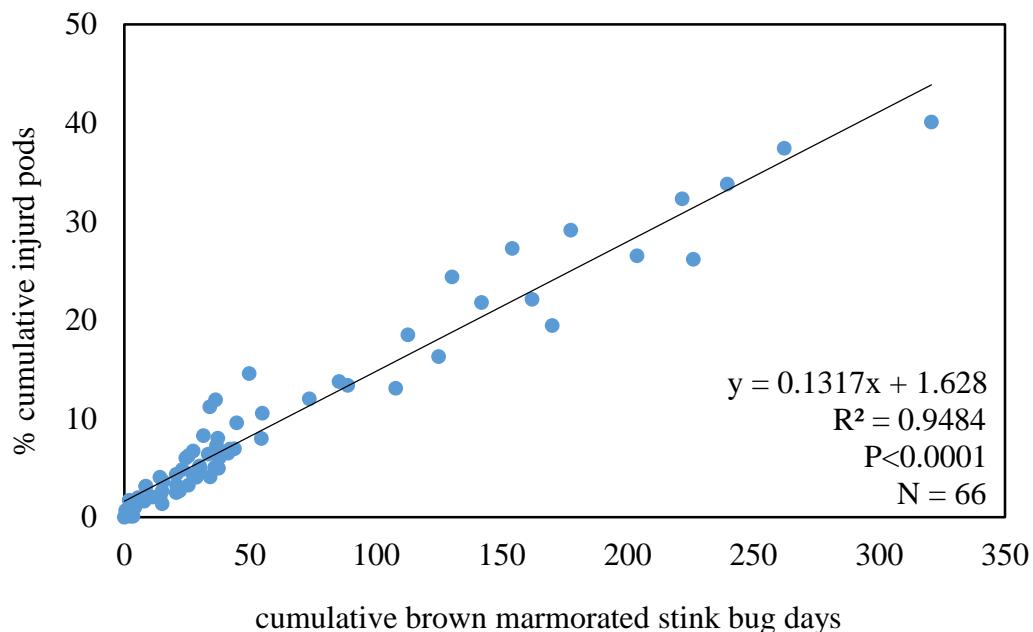


Figure 6. Percent of cumulative injured okra pods due to brown marmorated stink bug feeding as a function of cumulative total stink bug days over the entire crop cycle. Clarksville, MD, 2013. The cumulative percentage of injured pods is equal to the accumulated number of harvested pods divided by the accumulated number of injured pods at each harvest date. Stink bug days were calculated by averaging the numbers stink bug/m<sup>2</sup> from the current and last sampling events, and then multiplying that value by the number of days between the sampling events.

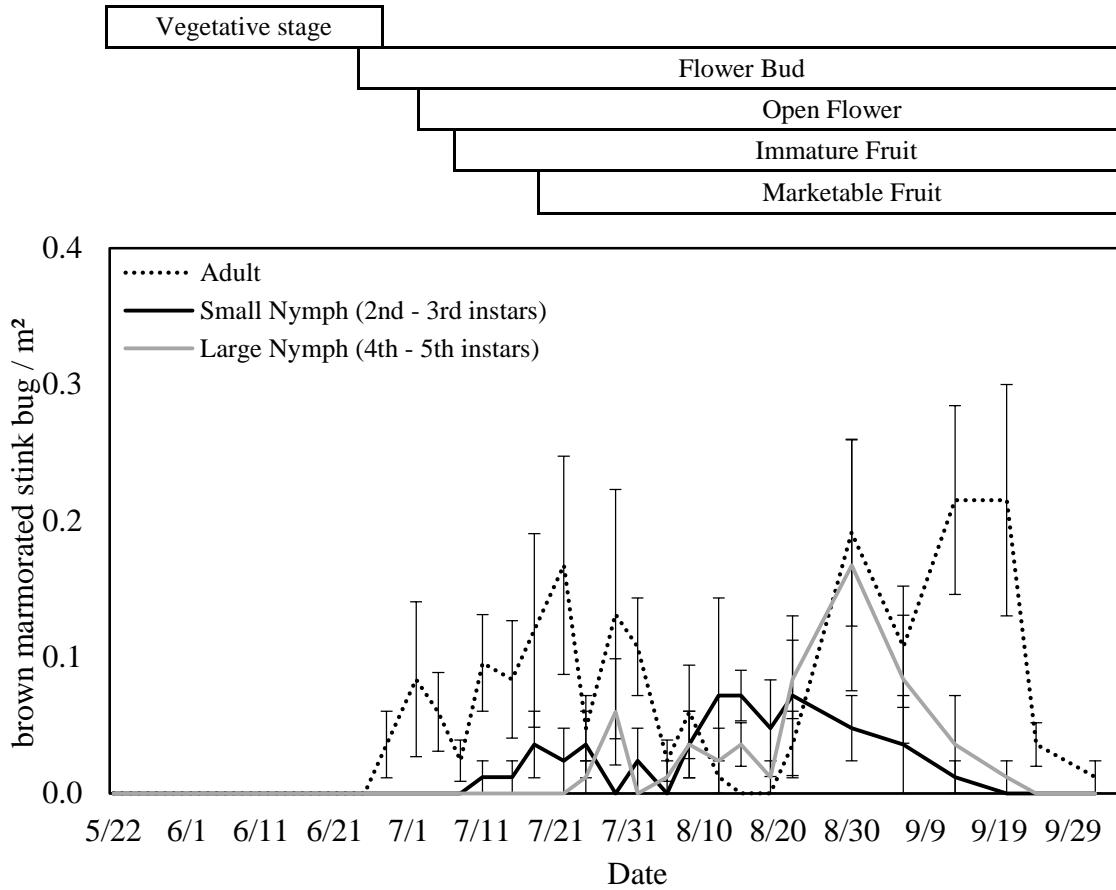


Figure 7. Mean density ( $\pm$  SEM) of brown marmorated stink bug life stages in eggplant.

Six week old plants were transplanted on May 22. Horizontal bars above the graph

depict the period of each plant development stage. Clarksville, MD. 2013.

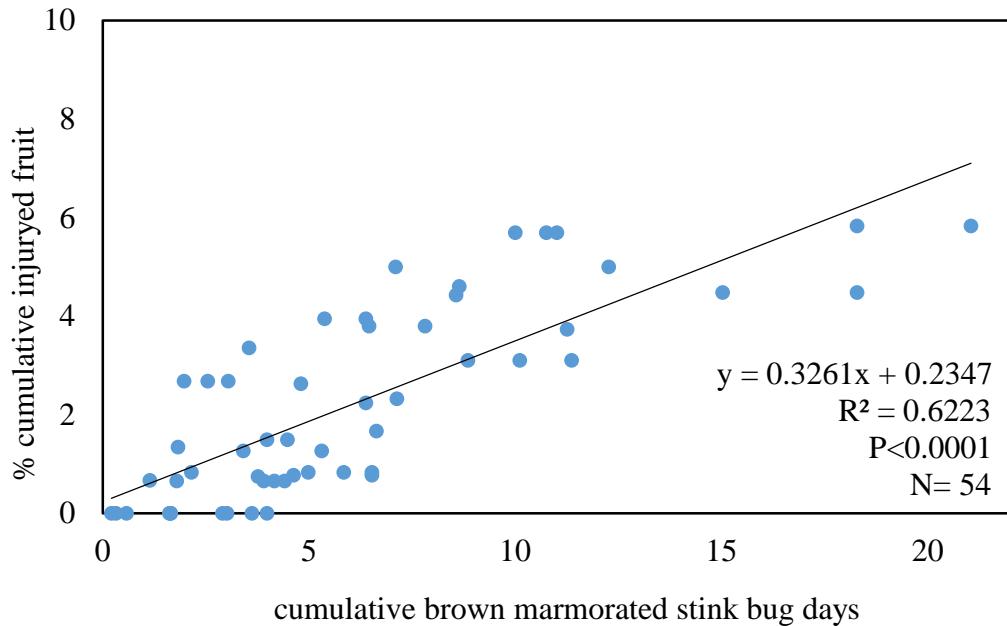


Figure 8. Percent of cumulative injured eggplant fruit due to brown marmorated stink bug feeding as a function of cumulative total stink bug days over the entire crop cycle.

Clarksville, MD, 2013. The cumulative percentage of injured fruit is equal to the accumulated number of harvested fruit divided by the accumulated number of injured fruit at each harvest date. Stink bug days were calculated by averaging the numbers stink bug/m<sup>2</sup> from the current and last sampling events, and then multiplying that value by the number of days between the sampling events.

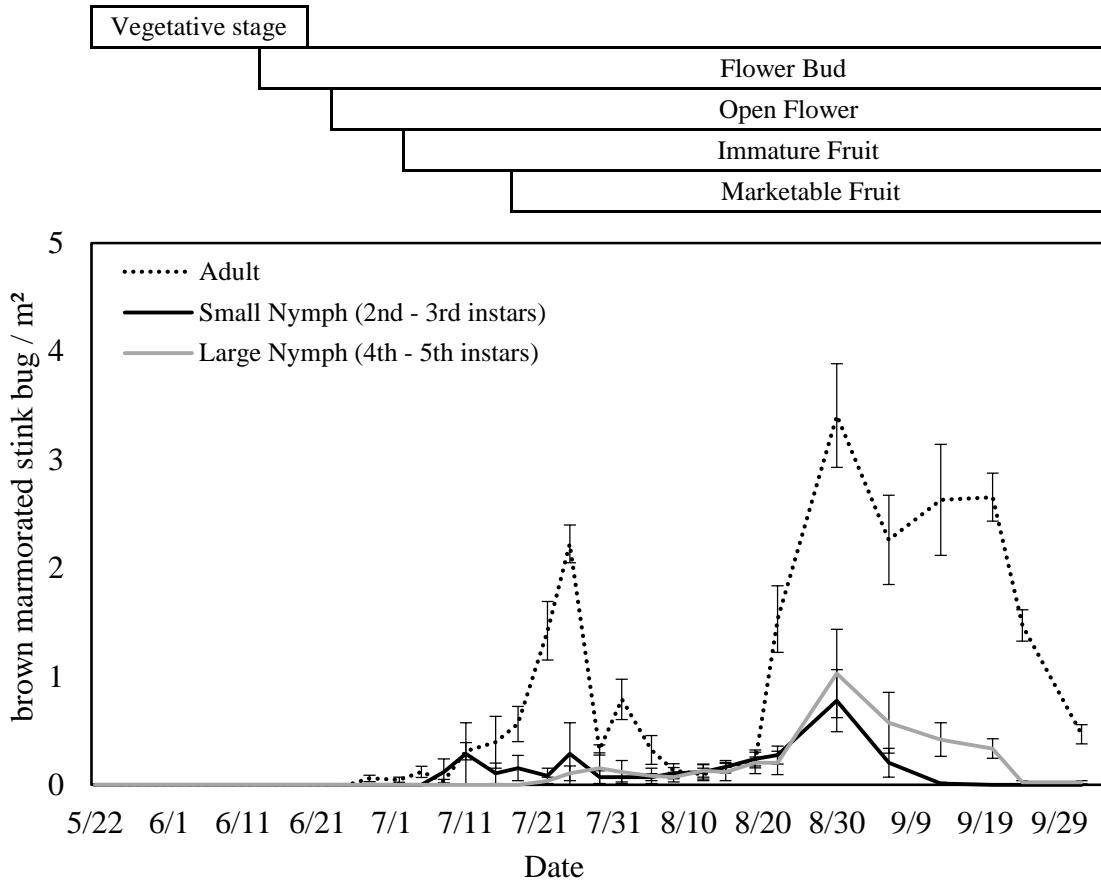


Figure 9. Mean density ( $\pm$  SEM) of brown marmorated stink bug life stages in bell pepper. Six week old plants were transplanted on May 22. Horizontal bars above the graph depict the period of each development stage. Clarksville, MD. 2013.

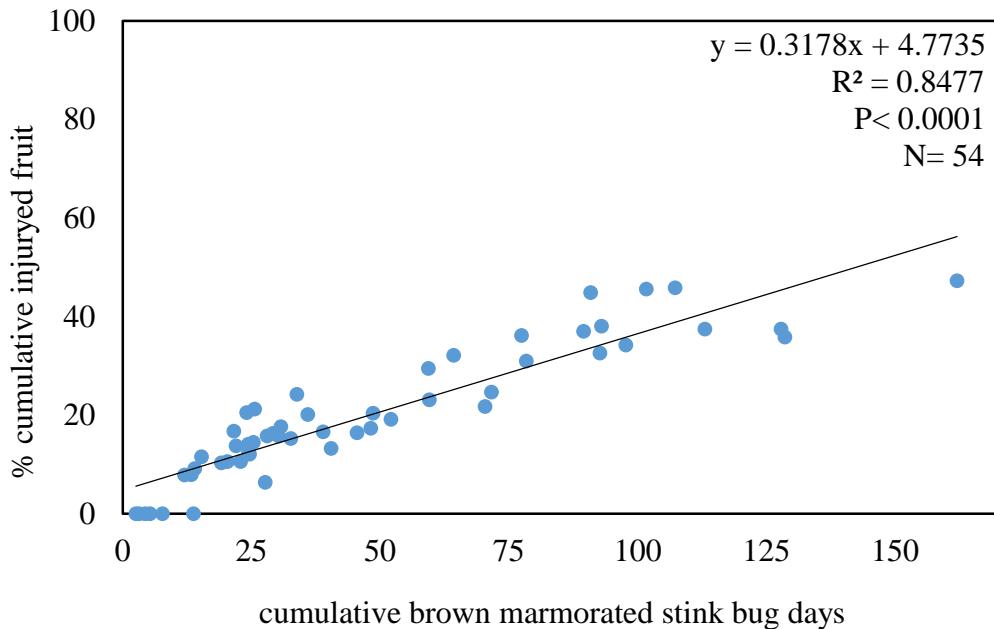


Figure 10. Percent of cumulative injured bell pepper fruit due to brown marmorated stink bug feeding as a function of cumulative total stink bug days over the entire crop cycle. Clarksville, MD, 2013. The cumulative percentage of injured fruit is equal to the accumulated number of harvested fruit divided by the accumulated number of injured fruit at each harvest date. Stink bug days were calculated by averaging the numbers stink bug/m<sup>2</sup> from the current and last sampling events, and then multiplying that value by the number of days between the sampling events.

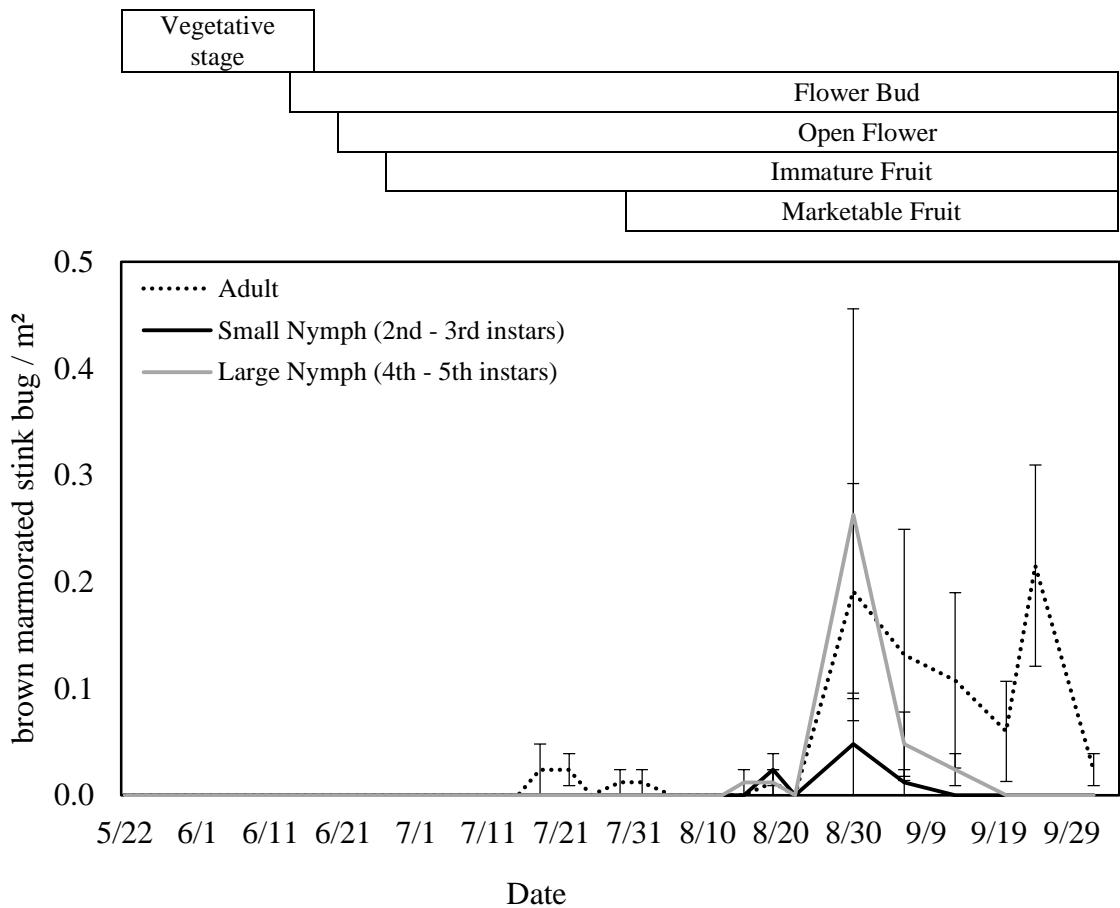


Figure 11. Mean density ( $\pm$  SEM) of brown marmorated stink bug life stages in tomato.

Six week old plants were transplanted on May 22. Horizontal bars above the graph depict the period of each development stage. Clarksville, MD. 2013.

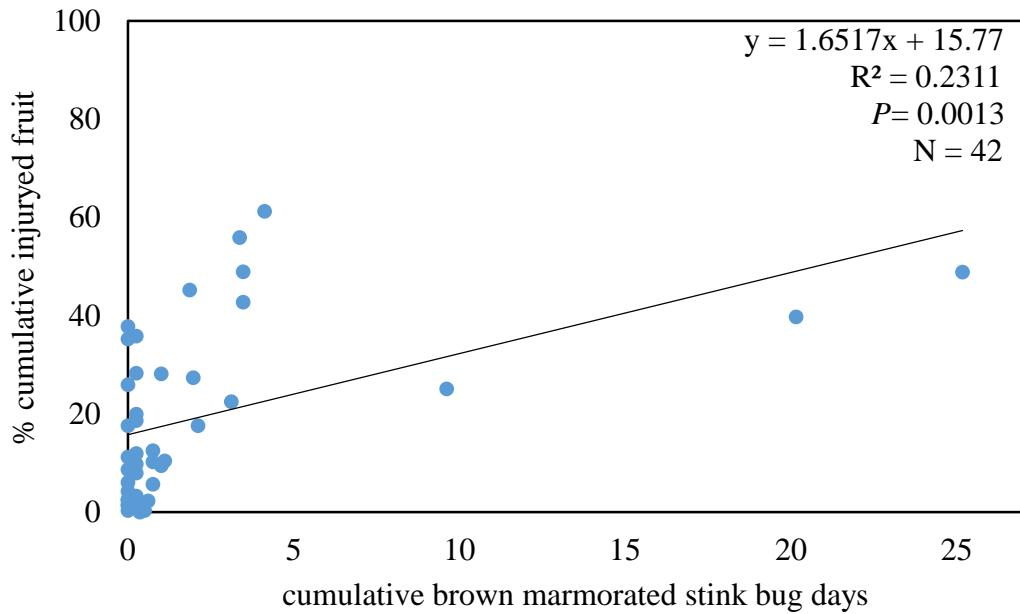


Figure 12. Percent of cumulative injured tomato fruit due to stink bug feeding as a function of cumulative total stink bug days over the entire crop cycle. Clarksville, MD, 2013. The cumulative percentage of injured fruit is equal to the accumulated number of harvested fruit divided by the accumulated number of injured fruit at each harvest date. Stink bug days were calculated by averaging the numbers stink bug/m<sup>2</sup> from the current and last sampling events, and then multiplying that value by the number of days between the sampling events.

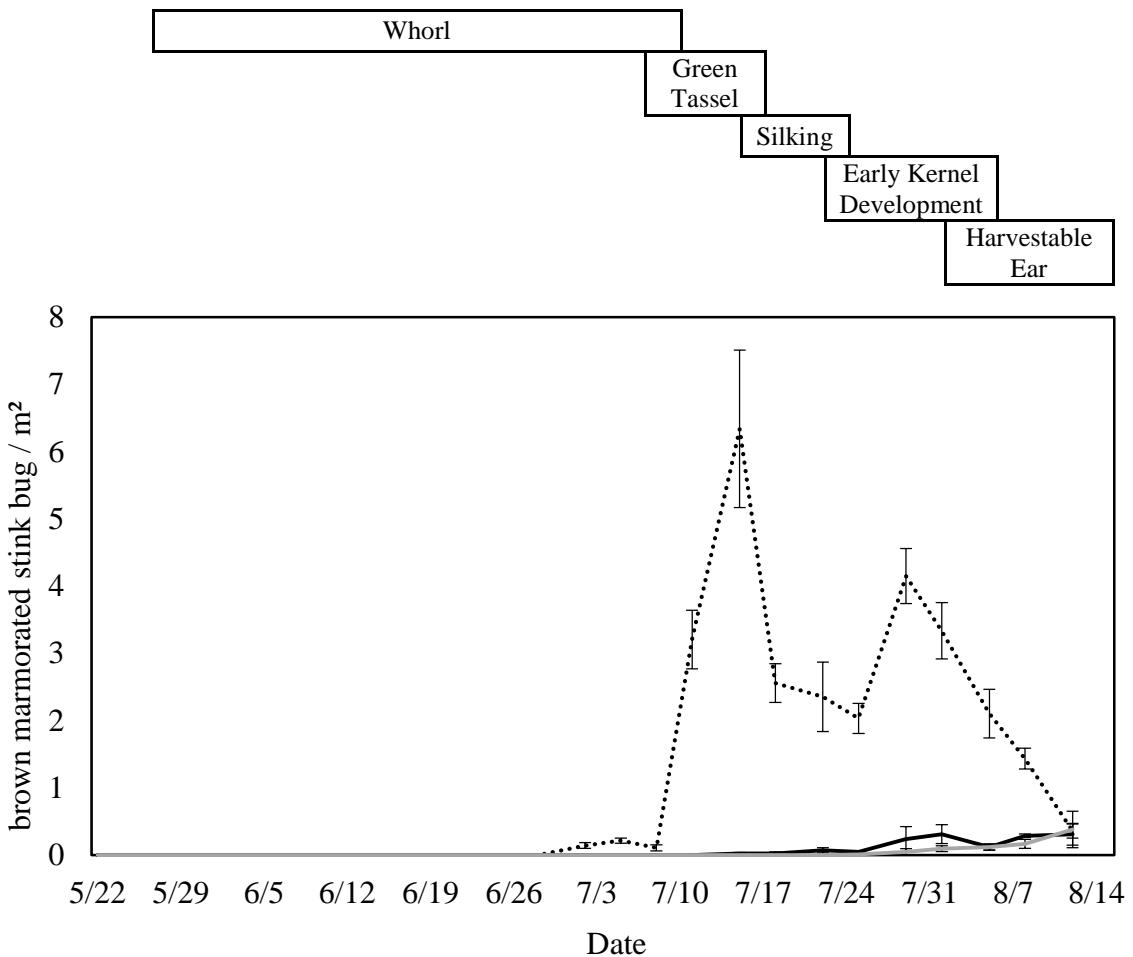


Figure 13. Mean density ( $\pm$  SEM) of brown marmorated stink bug life stages in sweet corn. Plants were direct-seeded on May 22. Horizontal bars above the graph depict the period of each development stage. Clarksville, MD. 2013.

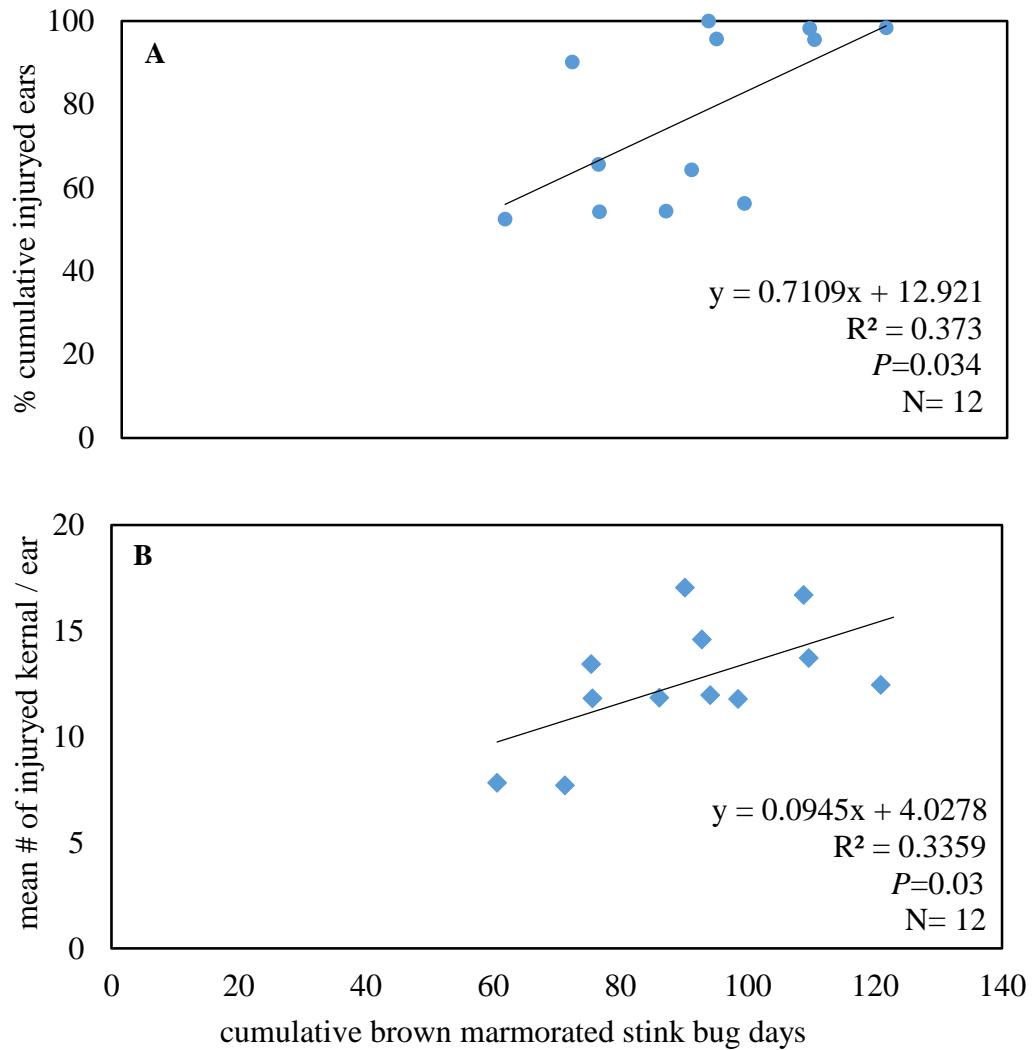


Figure 14. A.) Percent of cumulative injured sweet corn ears due to brown marmorated stink bug feeding, B.) mean injured kernels per ears of sweet corn as a. function of cumulative total stink bug days over the entire crop cycle. Clarksville, MD, 2013. The cumulative percentage of injured ears is equal to the accumulated amount of harvested ears divided by the accumulated amount of injured ears. Stink bug days were calculated by averaging the stink bug/m<sup>2</sup> value from the current and last sampling events, and then multiplying that value by the number of days between the sampling events.

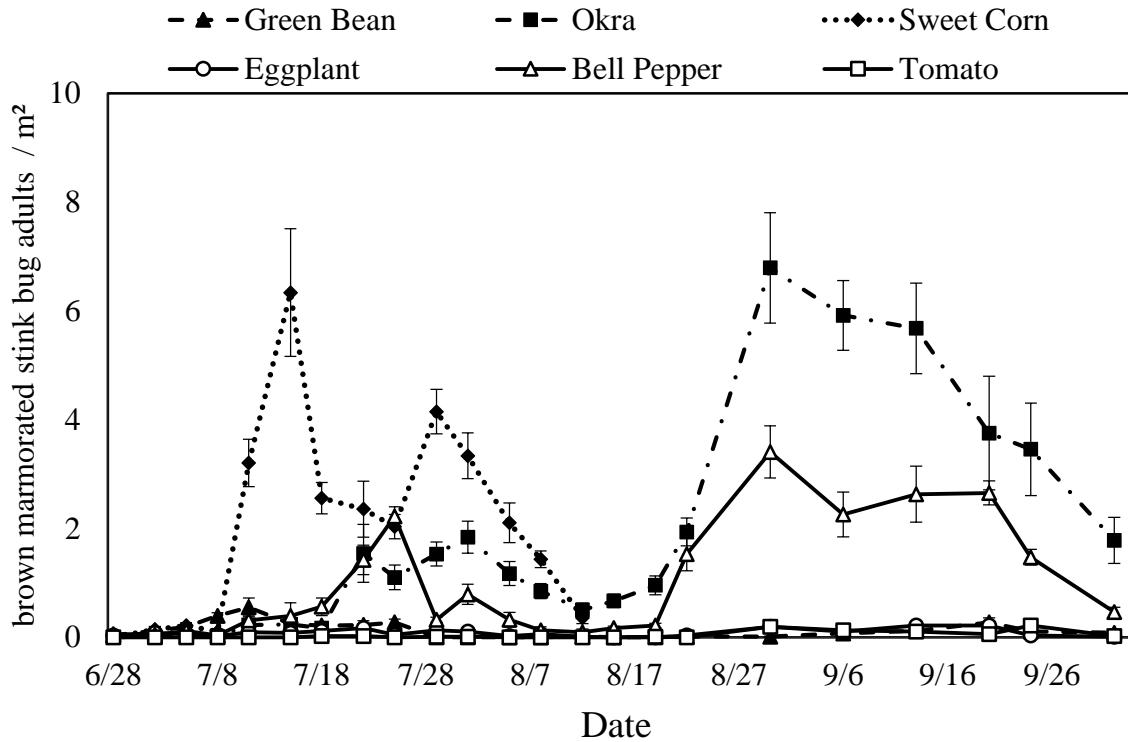


Figure 15. Mean density ( $\pm$  SEM) of brown marmorated stink bug adults over the growing season of six vegetable crops. Clarksville, MD. 2013. Green bean plants were pulled up and replanted on July 25. Sweet corn plots were harvested and cut down on August 12.

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