

**Risk Assessment and Improving Brown Marmorated Stink Bug (Stål), *Halyomorpha halys*,
Sampling in Virginia Soybean Systems**

Benjamin Lee Aigner

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D. Ames Herbert, Chair

Carlyle C. Brewster, Chair

Thomas P. Kuhar

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ABSTRACT

Brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), has become an important pest of soybean in the Mid-Atlantic US. To assess the influence of tree borders on BMSB infestations in soybean, twelve fields were sampled weekly using five 3-min visual counts of BMSB on tree of heaven (TOH) (*Ailanthus altissima*) and other host trees along a wooded border, on the adjacent soybean edge, 15 m and 30 m into the soybean field. At all locations, BMSB densities increased on TOH wooded borders in July, then, gradually moved into adjacent soybean borders later in the summer. BMSB did not move far from the field edge, with approximately half as many bugs being present at 15 m into the field and very few being detected 30 m into the field. These results validate the use of border sprays for BMSB control in soybean.

Additional studies conducted in 2013 and 2014 compared a visual plant inspection method with a standard sweep net strategy for sampling BMSB. Overall, the two methods were highly correlated with a correlation coefficient of $R=0.83$. Visual inspection appears to be an effective method for assessing BMSB populations in soybean.

One of the major factors affecting the distribution and establishment of invasive species is climate. The CLIMEX modeling software uses climatic and biological factors of species to predict the geographic risk for pest outbreaks. A climate simulation model was run with CLIMEX to determine the potential distribution of BMSB in Virginia based on temperature. To develop a more accurate model, factors like resource availability and source population would need to be considered.

General Audience Abstract

Brown marmorated stink bug (BMSB) is a major pest of many crops, including soybean. Before moving into soybean the bug has been observed on other preferred hosts (primarily tree of heaven) in neighboring forest edges. To determine the relationship of these hosts to BMSB and its movement into soybean fields we sampled these forest edges, the neighboring field edge, 15 m into the field, and 30 m into the field. Results showed that BMSB populations were high on the forest edges early in the season and increased rapidly on neighboring field edge when soybeans begin developing pods and seeds. Bugs rarely moved past the 15 m sample zone. These results validate the use of border sprays for BMSB control in soybean.

Sampling for BMSB with a sweep net has proven to be a challenge as the insect has a strong “startle response” where it will drop from the plant after slight disturbance. To assess the relationship between a standard sweep net sampling strategy and a new 2-min visual inspection method we sampled soybean fields in Virginia, Maryland and Delaware in 2013 and 2014. Data suggested that the 2-min visual inspection method can be used as an alternative to sweep net sampling.

Climate plays a critical role in the geographic distribution of species. We ran a climate simulation model with CLIMEX (modeling software) to determine the potential distribution of BMSB in Virginia based on temperature. To develop a more accurate model, additional factors like resource availability and distance from source population would need to be considered.

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Chapter One

Literature review: Biology, ecology, and risk assessment of brown marmorated stink bug in soybean systems

Nomenclature. The brown marmorated stink bug (BMSB) is in the family Pentatomidae within the insect order Hemiptera and was initially described as *Pentatoma halys* by Stål in 1855. It has formerly been referred to as *Poecilometis mistus* by Uhler in 1860, *Dalpada brevis* and *Dalpada remota* by Walker in 1867, and was synonymized by Josifov and Kerzhner as *Halyomorpha halys* in 1978 (Hoebeke and Carter 2003). Rider et al. (2002) reported that many in Japan still refer to it as *H. mista* and has been often confused with *H. picus*, a species native to India.

Origin, Invasion, and Distribution. BMSB is a species native to eastern China, Korea, and Japan. The first confirmed sighting of BMSB in the U.S. occurred in the fall of 1996 in Adams Island (Allentown), Pennsylvania (Hoebeke and Carter 2003). Based on genetic analysis, Xu et al. (2014) showed that its introduction to the U.S. likely stemmed from a single source in Beijing, China. It has been speculated that the bug was brought into Allentown via bulk container from either the port of Philadelphia or Elizabeth, NJ (Hamilton 2009). Since its introduction, it has spread rapidly to more than 40 states in the continental U.S., including Virginia, in which its first detection was in 2004 (Day et al. 2011). BMSB is currently considered a severe agricultural and nuisance pest in the Mid-Atlantic States, and a moderate agricultural and nuisance pest in the Midwest, Northeast, and Pacific Northwest. Many remaining states have reported detections as a nuisance but not yet as an economically significant agricultural pest (Rice et al. 2014).

Occurrences of BMSB have also been reported in Canada, Italy, Hungary, Switzerland, Greece and have been intercepted at ports in Australia and New Zealand, although, there are no accounts of established populations (Wermelinger et al. 2008, Harris 2010, Garipey et al. 2014, Milonas and Partsinevelos 2014, Vetek et al. 2014, Cesari et al. 2015, Haye et al. 2015). Climate models produced by Zhu et al. (2012) suggest that there is the potential for the establishment of populations of BMSB in the Southeast and Pacific Northwest that may eventually be of economic concern.

Life Cycle and Phenology. BMSB is a paurometabolous insect that develops to adulthood over five instars. Adults (Fig. 1.1) are brown, shield-shaped, 12-17 mm in length and 7-10 mm in width (Hoebeke and Carter 2003). They have black and white bands on their legs and antennae with a black and white “marbled” pattern on the outer edges of the abdomen (Hoebeke and Carter 2003). Beginning in early fall and into late fall, sexually immature adults seek overwintering sites (Watanabe et al. 1994) in manmade structures and well protected areas in the natural landscape where they will remain until either temperatures exceed 10°C or nutritional reserves have been depleted (Qin 1990, Hamilton 2008, Funayama 2012, Inkley 2012, Lee et al. 2014). In spring, adults will emerge from overwintering sites to feed, mate, and lay eggs on early season hosts, such as, tree of heaven (*Ailanthus altissima*), catalpa (*Catalpa speciosa*), paulownia (*Paulownia tomentosa*), and other fruiting trees (Bakken et al. 2015). Once females have completed ovarian development, they oviposit on the undersides of leaves in clusters of roughly 28 eggs (Fig. 1.2), which, are whitish-green in color making them well camouflaged (Nielsen and Hamilton 2009). First instars (Fig. 1.3) are black and red-orange in color and aggregate on the egg mass after hatching to acquire essential gut symbionts that are essential for development and competence as adults (Taylor et al. 2014). Once the first molt occurs, 3-6 days

after hatch, second instars begin to feed on foliage and fruiting structures of the host plant. Third, fourth and fifth instars (Fig. 1.4) generally molt 12-13, 19-20, and 26-27 days after hatching, respectively (Nielsen et al. 2008b, Rice et al. 2014).

In its native range, BMSB is documented to typically have 1-2 generations annually; however, 4-6 generations have been reported in southern China near Canton (Hoffman 1931). In the US, BMSB is likely univoltine in the upper Mid-Atlantic region (Nielsen and Hamilton 2009) and bivoltine in the ridge and valley regions of Virginia, West Virginia and North Carolina (Leskey et al. 2012c, Bakken et al. 2015). Based on average temperatures, up to five generations may be possible in tropical climates in the U.S. (Lee et al. 2013).

Nielsen et al. (2008b) researched the developmental rates of BMSB from egg to adulthood at constant temperatures ranging from 15-35°C. Incubation of eggs was shortest at 30°C (3 days) and the shortest total development time from egg to imaginal ecdysis occurred at 30°C.

Combined results of these studies showed that BMSB development from egg to imaginal ecdysis was significantly impacted by temperature and could occur in as little as 33 days (30°C) and as many as 122 days (17°C).

Additionally, temperature has been found to have a significant impact on the survival of BMSB with respect to both high and low lethal temperatures. Regarding lethal cold temperatures, it is reported that the degree of cold tolerance of BMSB is dependent upon sex, season, and acclimation location and was found to be within the range of -13°C to -18°C in bugs tested from Virginia and Minnesota, respectively (Cira et al. 2016). Aigner and Kuhar (2016) investigated lethal high temperature extremes by exposing BMSB to temperatures of 35, 38, 40, 42, 45, and 50°C in an incubator for times of 15 minutes, 1 hour, and 4 hours. At times of at least 1 hour, complete mortality occurred at temperatures of >45°C, and >90% mortality occurring at 42°C at

4 hours exposure. These studies are a vital contribution to understanding the inhibiting factors in population expansion and habitable range of the insect.

Pest Significance. Prior to the introduction of the BMSB, the two most dominant stink bug species in Virginia crop systems were the brown stink bug, *Euschistus servus* (Say), and the green stink bug, *Chinavia hilaris* (Say) (Underhill 1934, McPherson and McPherson 2000, Day and Kuhar 2003, Kamminga et al. 2009). Bakken et al. (2015) suggest that these remain the prominent species in the coastal plain regions of Virginia and North Carolina; however, studies conducted throughout the late 2000's have documented that BMSB has become the most dominant species occurring in raspberries, tree fruit, unmanaged hardwood trees, vegetables, soybeans, and grapes in the ridge and valley and southern piedmont regions of the state (Basnet et al. 2014, Basnet et al. 2015, Acebes-Doria et al. 2016, Bergmann et al. 2016). In addition to being a severe agricultural pest in a number of crops (e.g., fruiting vegetables, tree fruits, corn, and soybean), BMSB is an annual nuisance pest as it will invade man-made structures in search of overwintering sites in the fall (Inkley 2012, Kuhar et al. 2012f, Leskey et al. 2012b, Rice et al. 2014). The noxious odor emitted by BMSB, likely as a deterrent to predators, is composed primarily of (*E*)-2-decenal and tridecane and is a concern for some homeowners and business owners (Baldwin et al. 2014).

Adult and nymphal BMSB both feed on seeds, stems, leaves, fruits and/or pods of a variety of hosts by injecting plant digestive enzymes and extracting digested materials with their piercing-sucking mouthparts (stylets) (McPherson and McPherson 2000). BMSB was not recognized as a pest in Virginia and Pennsylvania until 2010, at which time pest pressure was greatest in this region and caused about \$37 million in losses to the apple industry (Leskey et al. 2012a). That same year some peach growers in Maryland reported to have lost ~100% of their yield and

similar losses can occur in sweet corn and soybean (Leskey and Hamilton 2010, Kuhar et al. 2012f, Cissel et al. 2015a). Stink bug damage to tomatoes and peppers commonly exceeds 30% in Virginia without insecticidal treatment (Kuhar 2012 a-e, 2013 a-c).

BMSB in Soybeans. Soybean production in Virginia has steadily grown over the years with more than 250,000 hectares harvested in 2015 at a value of ~\$193 million making it the most valuable field crop grown in Virginia (USDA/NASS 2015). BMSB was first detected in US soybean fields in Pennsylvania and New Jersey in 2006 and has been found to cause significant economic losses due to yield and quality reductions (Leskey et al. 2012b, Owens et al. 2013, Koch and Rich 2015). It advanced into soybean fields in Maryland, Delaware and Virginia in 2010 and by 2012 infestations had become widespread throughout these states (Cissel et al. 2015b). BMSB is now the predominant stink bug species found in soybeans in some Mid-Atlantic states (Nielsen and Hamilton 2009, Venugopal et al. 2014, Bakken et al. 2015) and is the area where populations and the risk to soybean crop damage is currently the greatest (Cissel et al. 2015b).

Soybean growth stages are classified as either vegetative or reproductive (McPherson and McPherson 2000). Vegetative stages are described from the time that the plant emerges from the soil until the flowering of the plant (Fehr et al. 1971, Fehr and Caviness 1977). The cotyledon stage is labeled VC in the classification index; V1 is the stage where only unifoliate nodes are present; V2 contains the first fully developed trifoliate leaf at the node above the unifoliate nodes; other vegetative stages (e.g., V3, V4, V5, etc.) contain three or more fully developed trifoliate leaves at nodes above the unifoliate node (McPherson and McPherson 2000). The reproductive stages are described as R1—R8 in the classification index. Flowering stages are classified as R1 (beginning bloom) and R2 (full bloom) and can occur simultaneously. Stages

R3—R4 are beginning and full pod development stages and R5—R6 are beginning and full seed development stages, respectively. Senescence occurs at stage R7 and full maturity is reached at R8 (Fehr et al. 1971, Fehr and Caviness 1977). Native species of stink bug are attracted to the crop in significant numbers beginning at the R3 stage of development (Thomas et al. 1974).

Feeding injury to soybean by BMSB is similar to that caused by native stink bug species, which, insert their stylets into the pods and seeds of the plant and extract digested plant materials (McPherson and McPherson 2000). Insertion of the stylet causes initial damage to the cell upon penetration, then, salivary enzymes spread to break down cell walls and proteins (Depieri and Panizzi 2011, Owens et al. 2013). Feeding during pod development results in aborted pods and underdeveloped flat pods. In later stages of seed development, this generally causes shriveled, deformed, and/or aborted seeds (Corrêa-Ferreira and Azevedo 2002). Internal damage to the seed has a white chalky appearance and can be identified externally by a dark sunken spot (Miner 1966).

Additionally, stink bug damage in soybean can cause “stay-green” syndrome, a delay in maturation where the plant attempts to compensate for loss of seed (Daugherty et al. 1964, Russin et al. 1987, Rice et al. 2014). This condition typically occurs on the edges of soybean fields where BMSB infestations originate in adjacent forested borders containing alternate tree hosts, especially the invasive tree of heaven (Venugopal 2014, Bakken et al. 2015). This can cause complete yield loss within a field due to the difficulties associated with harvesting soybeans that have not fully matured (Hill et al. 2006, Owens et al. 2013, Cissel et al. 2015b). Chapter two of this thesis presents research findings on the patterns of infestation of BMSB on soybean field edges and fields from adjacent wooded borders.

BMSB Sampling in Soybeans. Traditional sampling and monitoring techniques for native stink bug species, particularly *N. viridula*, in soybeans are described by Todd and Herzog (1980) and include fumigation cages, ground cloth sampling, black light trapping, vacuum sampling, and sweep net sampling. Nielsen et al. (2011) showed that monitoring with large pyramid traps and aggregation pheromones can help detect invading populations sooner than other methods, but is more expensive. Because of its ease of use, the sweep net technique has become the universal standard for sampling for stink bugs, however, BMSB has brought new challenges in accurately depicting population densities in soybean fields. A ‘startle response’ by the insect causes it to drop to the ground after very little disturbance making it difficult to properly detect actual densities (Cissel et al. 2015b). Thresholds for BMSB in soybean growing states have been set at similar levels due to the similarities in damage that it causes compared to native species (~5 bugs per 15 sweeps). Chapter 3 presents research findings on a new visual sampling method for BMSB and a comparison with the current standard sweep net method.

BMSB Management. BMSB has proven more difficult to manage than other species of native stink bugs due to its wide host range, lack of specific natural enemies, reproduction in large numbers, unique overwintering capabilities, and increased survival due to global warming (Lee et al. 2013). In several cropping systems such as fruiting vegetables, tree fruit, and soybeans, multiple applications of insecticides have been used to treat for BMSB. However, many of the broad spectrum insecticides registered for stink bugs can cause secondary pest issues and damage to important pollinator species. As BMSB is a border driven pest, border treatment applications have been found to be effective in soybean systems (Herbert et al. 2015) and could be implemented in crop systems with similar infestation patterns (e.g., corn, apple, peach). In comparison to treating entire fields, treating just 12-m into the field prevented further

advancement and reduced insecticide use by 85-95% (Leskey et al. 2012d and e, Leskey et al. 2014).

CLIMEX Modeling. Climate is one of the key factors that limits the spread of habitation of species in the natural world. With the CLIMEX modeling software (Hearne Scientific Software Pty. Ltd., Melbourne, Australia) it is possible to map the potential geographic distribution and likelihood of establishment of BMSB based on historical climate data and limiting biological factors. Zhu et al. 2012 used ecological niche modeling to make a direct climate comparison between the native range of BMSB and areas of invasion. This study suggests that much of the Mid-Atlantic and Midwest regions of the US are highly suitable for the establishment of the pest as well as areas in the Pacific Northwest. Chapter four presents research findings on the potential geographic distribution of BMSB in Virginia using the CLIMEX model.

References Cited

- Aigner, J.D. and T.P. Kuhar. 2016.** Lethal high temperature extremes of the brown marmorated stink bug (Hemiptera: Pentatomidae) and efficacy of commercial heat treatments for control in export shipping cargo. *J. Agr. Urban Entomol.* 32: (1) 1—6.
- Acebes-Doria, A. L., T. C. Leskey, and C. J. Bergh. 2016.** Host plant effects on *Halyomorpha halys*(Hemiptera: Pentatomidae) nymphal development and survivorship. *Eviron. Entomol.* In Press.
- Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert Jr., D., T. P. Kuhar, and J. Walgenbach. 2015.** Occurrence

of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. *Environ. Entomol.* 44: 1011-1021.

Baldwin, R.L., A. Zhang, S.W. Fultz, S. Abubeker, C. Harris, E.E. Connor, and D.L Van Hekken. 2014. Hot topic: brown marmorated stink bug odor compounds do not transfer into milk by feeding bug-contaminated corn silage to lactating dairy cattle. *J. Dairy Sci.* 97:1877–1884

Basnet, S., L. M. Maxey, C. A. Laub, T. P. Kuhar, and D. G. Pfeiffer. 2014. Stink bugs (Hemiptera: Pentatomidae) in primocane-bearing raspberries in Southwestern Virginia. *J. Entomol. Sci.* 49: 304–312.

Basnet, S., T. P. Kuhar, C. A. Laub, and D. G. Pfeiffer. 2015. Seasonality and Distribution Pattern of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Virginia Vineyards. *J. Econ. Entomol.* 108(4): 1902 – 1909.

Bergmann, E. J . P. D. Venugopal, H . M. Martinson, M. J. Raupp, P. M. Shrewsbury. 2016. Host Plant Use by the Invasive *Halyomorpha halys* (Stål) on Woody Ornamental Trees and Shrubs. *PLoS ONE* 11 (2): e0149975. doi:10.1371/journal.pone.0149975

Biddinger, D., J. Tooker, A. Surcica, and G. Krawzyk. 2012. Survey of native biocontrol agents of the brown marmorated stink bug in Pennsylvania fruit orchards and adjacent habitat, pp. 47-54, Pennsylvania Fruit News.

Cesari, M., L. Maistrello, F. Ganzerli, P. Dioli, L. Rebecchi, and R. Guidetti. 2015. A pest alien invasion in progress: potential pathways of origin of the brown marmorated stink bug *Halyomorpha halys* populations in Italy. *J. Pest Sci.* 88: 1-7.

- Cira, T. M., R.C. Venette, J. Aigner, T. Kuhar, D.E. Mullins, S.E. Gabbert, and W.D. Hutchison. 2016.** Cold Tolerance of *Halyomorpha halys* (Hemiptera: Pentatomidae) Across Geographic and Temporal Scales. *Environ. Entomol.* 1 – 8. doi: 10.1093/ee/nvv220.
- Cissel, W. J., C. E. Mason, J. Whalen, J. Hough-Goldstein, and C. R. R. Hooks. 2015a.** Effects of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) Feeding Injury on Sweet Corn Yield and Quality. *J. Econ. Entomol.* 108(3): 1 – 7.
- Cissel, B., J. Whalen, G. Dively, C. Hooks, T. Patton, P. D. Venugopal, B. Aigner, J. Hogue, A. Herbert, T. Kuhar, S. Malone, E. Seymore. 2015b.** Brown marmorated stink bug biology and management in Mid-Atlantic soybeans. Virginia Cooperative Extension. ENTO – 168NP.
- Daugherty, D. M., M. H. Neustadt, C. W. Gehrke, L. E. Cavanah, L. F. Williams, and D. E. Green. 1964.** An evaluation of damage to soybeans by brown and green stink bugs. *J. Econ. Entomol.* **57**: 719-722.
- Day, E. R. and T. P. Kuhar. 2003.** Stink Bugs Fact Sheet, Revised 2003. Virginia Coop. Ext. Publ. No. 444-621.
- Day, E. R., T. McCoy, D. Miller, T. P. Kuhar, and D. G. Pfeiffer. 2011.** Brown marmorated stink bug. 2902-1100. (http://www.pubs.ext.vt.edu/2902/2902-1100/2902-1100_pdf.pdf)
- Funayama, K. 2012.** Nutritional states of post-overwintering adult brown-marmorated stink bugs, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae). *Jpn. J. Appl. Entomol. Zool.* 56: 12 - 15.
- Gariepy, T., T. Haye, H. Fraser, and J. Zhang. 2014.** Occurrence, genetic diversity, and potential pathways of entry of *Halyomorpha halys* in newly invaded areas of Canada and

- Switzerland. J. Pest Sci. 87: 17-28.
- Hamilton, G. C. 2009.** Brown marmorated stink bug. Am. Entomol. 55: 19.
- Harris, A. C. 2010.** *Halyomorpha halys* (Hemiptera: Pentatomidae) and *Protaetia brevitarsis* (Coleoptera: Scarabaeidae: Cetoniinae) intercepted in Dunedin. The Weta 40: 42-44.
- Haye, T., T. Garipey, K. Hoelmer, J. P. Rossi, J. C. Streito, X. Tassus, and N. Desneux. 2015.** Range expansion of the invasive brown marmorated stinkbug, *Halyomorpha halys*: an increasing threat to field, fruit and vegetable crops worldwide. J. Pest Sci. 88: 665-673.
- Herbert D., W. Cissel, J. Whalen, G. Dively, C. Hooks, T. Patton, D. Venugopal, T. Kuhar, B. Aigner, S. Malone, J. Hogue, E. Seymore. 2015.** Brown Marmorated Stink Bug Biology and Management in Mid-Atlantic Soybeans. Virginia Cooperative Extension Pub. No. ENT-168NP.
- Hoffmann W.E. 1931.** A pentatomid pest of growing beans in South China. Peking Nat. Hist. Bull. 5: 25–26.
- Hoebeke, E. R., and M. E. Carter. 2003.** *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol Soc. Washington 105: 225-237.
- Inkley, D. B. 2012.** Characteristics of home invasion by the brown marmoratedstink bug (Hemiptera: Pentatomidae). J. Entomol. Sci. 47: 125–130.
- Josifov, M., and I. Kerzhner. 1978.** Heteroptera aus Korea. II. Teil (Aradidae, Berytidae, Lygaeidae, Pyrrhocoridae, Rhopalidae, Alydidae, Coreidae, Urostylidae, Acanthosomatidae, Scutelleridae, Pentatomidae, Cydnidae, Plataspidae). Fragm. Faun. 23: 137-196.

- Kamminga, K.L. 2008.** Species survey, monitoring and management of economically important stink bug species in eastern Virginia. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA.
- Koch, R. L., and W. A. Rich. 2015.** Stink bug (Hemiptera: Heteroptera: Pentatomidae) feeding and phenology on early-maturing soybean in Minnesota. *J. Econ. Entomol.* 108: 2335-2343.
- Kuhar, T., H. Doughty, K. Kamminga, and L. Lilliston. 2012a.** Evaluation of insecticides using bean dip bioassay for control of brown marmorated stink bug, 2011. *Arthropod Manag. Tests* 37: L1.
- Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012b.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 1. *Arthropod Mgt. Tests* 38: E37. online publ. DOI: 10.4182/amt.2012.E37.
- Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012c.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 2. *Arthropod Mgt. Tests* 38: E38. online publ. DOI: 10.4182/amt.2012.E38.
- Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012d.** Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia-2011-Experiment 3. *Arthropod Mgt. Tests* 38: E39. online publ. DOI: 10.4182/amt.2012.E39.

Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012e.

Evaluation of insecticides for the control of brown marmorated stink bug in tomatoes in Virginia, 2011. *Arthropod Mgt. Tests* 37: E72. online publ. DOI: 10.4182/amt.2012.E72.

Kuhar, T. P., K. L. Kamminga, J. Whalen, G. P. Dively, G. Brust, C. R. R. Hooks, G.

Hamilton, and D. A. Herbert. 2012f. The pest potential of brown marmorated stink bug on vegetable crops. Online. *Plant Health Prog.* doi:10.1094/PHP-2012-0523-01-BR

Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2013a.

Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 1. *Arthropod Mgt. Tests* 38: E39. online publ. DOI: 10.4182/amt.2013.E39.

Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2013b.

Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 2. *Arthropod Mgt. Tests* 38: E40. online publ. DOI: 10.4182/amt.2013.E40.

Kuhar T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2013c.

Evaluation of insecticides for the control of brown marmorated stink bugs in bell peppers in Virginia, 2012-Experiment 3. *Arthropod Mgt. Tests* 38: E41. online publ. DOI: 10.4182/amt.2013.E41.

Kuhar, T.P., J.A. Morehead, and T. Dimeglio. 2015. Diagnosing stink bug injury to

vegetables. Virginia Coop. Ext. Pub. No. ENTO-173NP.

<https://pubs.ext.vt.edu/ENTO/ENTO-173/ENTO-173.html>

- Lee, D.-H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. 2013.** Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environ. Entomol.* 42: 627-641.
- Lee, D.-H., J. P. Cullum, J. L. Anderson, J. L. Daugherty, L. M. Beckett, and T. C. Leskey. 2014.** Characterization of overwintering sites of the invasive brown marmorated stink bug in natural landscapes using human surveyors and detector canines. *PLoS One* 9: e91575.
- Leskey, T. C., and Hamilton G.C. 2010.** Brown marmorated stink bug working group meeting. http://projects.ipmcenters.org/Northeastern/FundedProjects/ReportFiles/Pship2010/Pship2010-Leskey-ProgressReport-237195-Meeting-2010_11_17.pdf. Assessed April 30, 2012.
- Leskey, T. C., D.-H. Lee, B. D. Short, and S. E. Wright. 2012a.** Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): analysis of insecticide lethality. *J. Econ. Entomol.* 105: 1726-1735.
- Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. A. Hoelmer, D.-H. Lee, and S. E. Wright. 2012b.** Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. *Outlooks Pest Manag.* 23: 218-226.
- Leskey, T. C., D.-H. Lee, B. D. Short & S. E. Wright. 2012c.** Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): Analysis of insecticide lethality. *J. Econ. Entomol.* 105: 1726–1735.

- Leskey, T. C., S. E. Wright, B. D. Short, and A. Khrimian. 2012d.** Development of behaviorally based monitoring tools for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) in commercial tree fruit orchards. *J. Entomol. Sci.* 47: 76-85.
- Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. 2012e.** Impact of the invasive brown marmorated stink bug, *Halyomorpha halys*(Stål), in Mid-Atlantic tree fruit orchards in the United States: Case studies of commercial management. *Psyche: A J. Entomol.* 2012: 1-14.
- Leskey, T. C., B. D. Short, and D. H. Lee. 2014.** Efficacy of insecticide residues on adult *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards. *Pest. Manag. Sci.* 70: 1097-1104.
- McPherson, J. E., and R. M. McPherson. 2000.** Stink Bugs of Economic Importance in America North of Mexico. CRC Press, Boca Raton, FL. 253 pp.
- Milonas, P., and G. Partsinevelos. 2014.** First report of brown marmorated stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in Greece. *EPPO Bulletin* 44: 183-186.
- Nielsen, A. L., P. W. Shearer, and G. C. Hamilton. 2008a.** Toxicity of insecticides to *Halyomorpha halys* (Hemiptera: Pentatomidae) using glass-vial bioassays. *J. Econ. Entomol.* 101: 1439-1442.
- Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008b.** Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* 37: 348-355.

- Nielsen, A. L., and G. C. Hamilton. 2009.** Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. *J. Econ. Entomol.* 102: 1133-1140.
- Nielsen, A.L., G.C. Hamilton and P.W. Shearer. 2011.** Seasonal phenology and monitoring of the non-native *Halyomorpha halys* in soybean. *Environ. Entomol.* 40(2):231–238.
- Owens, D., D. A. Herbert, G. Dively, D. Reising, and T. Kuhar. 2013.** Does feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) reduce soybean seed quality and yield? *J. Econ. Entomol.* 106: 1317-1323.
- Qin, W. 1990.** Occurrence rule and control techniques of *Halyomorpha picus*. *Plant Prot.* 16: 22–23.
- Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Garipey, G. Hamilton, T. Haye, D. A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. P. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G. Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, J. F. Tooker. 2014.** Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *J. Integr. Pest Manag.* 5: A1-A13.
- Rider, D., L. Zheng, and I. Kerzhner. 2002.** Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera). II. Pentatominae. *Zoosyst. Rossica* 11: 135-153.
- Russin, J. S., M. B. Layton, D. B. Orr, D. J. Boethel. 1987.** Within-plant distribution of, and partial compensation for, stink bug (Heteroptera: Pentatomidae) damage to soybean seeds. *J. Econ. Entomol.* 80: 215-220.

- Taylor, C. M., P. L. Coffey, B. D. DeLay, and G. P. Dively. 2014.** The importance of gut symbionts in the development of the brown marmorated stink bug, *Halyomorpha halys* (Stål). PLoS One 9: e90312.
- United States Department of Agriculture [USDA]: National Agricultural Statistics Service (NASS). 2015.** <https://quickstats.nass.usda.gov/results/DD5654E7-31C4-34DE-AE81-A11F5DB0F326>. Accessed on: August 2016.
- Vetek, G., V. Papp, A. Haltrich, and D. Redei. 2014.** First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa 3780: 194-200.
- Underhill, G.W. 1934.** The green stink bug. Virginia Agric. Exp. Stn. Bull. 294: 1-26.
- Watanabe, M., R. Arakawa, Y. Shinagawa, and T. Okazawa. 1994.** Anti-invading methods against the brown marmorated stink bug, *Halyomorpha mista*, in houses. Jpn. J. Sanit. Zool. 45: 311–317.
- Wermelinger, B., D. Wyniger and B. Forster. 2008.** First records of an invasive bug in Europe: *Halyomorpha halys* Stål (Heteroptera: Pentatomidae), a new pest on woody ornamentals and fruit trees? Bull. Soc. Entomol. Suisse 81:1-9.
- Xu, J., D. M. Fonseca, G. C. Hamilton, K. A. Hoelmer, and A. L. Nielsen. 2014.** Tracing the origin of US brown marmorated stink bugs, *Halyomorpha halys*. Biol. Invasions 16: 153-166.
- Zhu, G., W. Bu, Y. Gao, and G. Liu. 2012.** Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). PLoS One 7: e31246.

Fig 1.1



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

Fig. 1.2



Brown marmorated stink bug egg mass (Photo Credit: Yurika Alexander).

Fig. 1.3



First instar brown marmorated stink bug nymphs (Photo Credit: Wil Hershberger).

Fig 1.4



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

Chapter Two

Relationship of brown marmorated stink bug, *Halyomorpha halys*, infestations in tree borders to subsequent patterns of movement into soybean fields

(B.L. Aigner, T.P. Kuhar, D.A. Herbert, C.C. Brewster, J.W. Hogue, and J.D. Aigner. 2016. Manuscript in Preparation for Submission to: Journal of Economic Entomology)

ABSTRACT

The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), is an important pest of soybean in the Mid-Atlantic U.S. In order to assess the influence of non-managed wooded borders on *H. halys* infestation patterns in soybean, twelve soybean fields in Orange and Madison Counties, Virginia were sampled each week from July to October in 2013 or 2014 for *H. halys*. At each location, five 2-minute visual counts of *H. halys* life stages were made on Tree of Heaven (*Ailanthus altissima*) and other favorable host trees along a wooded border, on the adjacent soybean edge, 15 m into the soybean field, and 30 m into the field. Seasonal data showed a clear trend at all locations of *H. halys* densities building up on *Ailanthus altissima*-dominated wooded borders in July, then, gradually moving into adjacent soybean field edges later in the summer. *Halyomorpha halys* did not move far from the invading field edge, with approximately half as many bugs being present at 15 m into the field and very few being detected 30 m into the field. These results have implications for continued management using field border sprays, particularly on edges adjacent to woods.

Key words *Halyomorpha halys*, *Ailanthus altissima*, soybeans, stink bug

Introduction

Since its invasion into the U.S. in the late 1990s, the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), has established itself as a conspicuous household nuisance and important agricultural pest throughout the Mid-Atlantic U.S. (Rice et al. 2014). Soybean is among the many crops attacked by this polyphagous pentatomid (Nielsen et al. 2011). Feeding during pod development stages results in aborted pods and underdeveloped flat pods. In later stages of seed development, this generally causes shriveled, deformed, and/or aborted seeds (Owens et al. 2013). Stink bug feeding in soybean also can cause “stay-green” or “green stem” syndrome where the plant delays maturation in an effort to compensate for seed that may have been aborted due to feeding (Hill et al. 2006, Rice et al. 2014). Complete yield loss can result from mechanical issues that likely ensue from attempting to harvest soybeans that have not fully senesced (Cissel et al. 2015). A better understanding of the landscape and ecological factors influencing *H. halys* populations should aid in the development of effective management strategies (Wallner et al. 2014). Venugopal et al. (2014) analyzed the effect of adjacent habitat on *H. halys* infestations in soybeans in Maryland and reported that fields adjacent to wooded, crop and building habitats harbored higher densities of stink bugs than those adjacent to open habitats. Stay green syndrome also appeared to be largely associated with field borders. Additional studies have shown that non-managed forested areas, in particular, are thought to be important season-long reservoirs for dispersal of *H. halys* to agricultural crops (Bakken et al. 2015). In Virginia, we have observed particularly high densities of *H. halys* on the invasive tree of heaven, *Ailanthus altissima*, which has become the most dominant tree species along the edges of forests and roadways in Virginia (McAvoy et al. 2012). Moreover, based on annual surveys of soybeans across Virginia from 2011 to 2012, the soybean fields having highest *H. halys* pest pressure were typically bordered by woods containing *A. altissima*. Thus, in order to

better understand the relationship between *H. halys* and *A. altissima*-dominated wooded borders, the seasonal abundance of *H. halys* life stages was sampled on this tree species adjacent to soybean fields, and at different distances into the soybean field. A better understanding of *H. halys* infestation patterns in fields should aid in the development of effective and efficient pest management strategies for soybeans and other crops.

Materials and Methods

Five soybean fields in Orange County and three fields in Madison County were sampled once a week from early July to October in 2013 (Table 2.1). In 2014, four fields in Madison County were sampled in the same months. All fields that were chosen had at least one border of woods with *A. altissima* and other favorable host trees such as mulberry (*Morus nigra*) and Princess Tree (*Paulownia tomentosa*) (Bakken et al. 2015). At each field, five randomly spaced 2-minute visual samples were taken in four separate sample zones. These zones are known in this experiment as the wooded border (containing *A. altissima* and alternate host trees), the adjacent soybean field edge, 15-m into the soybean field, and 30-m into the soybean field. All *H. halys* adults, nymphs, and eggs were recorded.

Statistical Analysis. Analysis of variance procedure was used to analyze the effect of sample zone on stink bug population densities of nymphs and adults each year. Additionally, repeated measures analysis of variance procedure was used to analyze the effect of sample zone on density of *H. halys* nymphs and adults over sample dates each year. Fisher's Protected LSD was used to separate means of all analyses.

Results and Discussion

Populations of *H. halys* were found at all twelve field locations. Generally, there was a consistent seasonal population dynamics trend at all sample locations over the two year sampling periods. However, densities in 2014 were much lower, thus, yielded slightly different results in sample zone dynamics. In 2013, the highest occurrences of nymphs and adults were in the field edges and wooded borders, respectively (Fig. 2.1A and C). Also, adults traveled relatively farther into fields as they are more mobile than nymphs. Sample zone had a significant effect on the densities of both nymphs ($F=47.316$; $df=3$, 28.6; $p\leq 0.0001$) and adults ($F=9.766$; $df=3$, 27.2; $p\leq 0.0002$) in 2013. Sample zone dynamics were similar in 2014, however, highest occurrences of nymphs and adults occurred in the wooded borders rather than the field edges (Fig. 2.2A and C). Neither nymphs nor adults traveled much past the field edge. Additionally, sample zone had a significant effect on the densities of both nymphs ($F=9.751$; $df=3$, 12.8; $p\leq 0.0013$) and adults ($F=15.574$; $df=3$, 11.9; $p\leq 0.0002$) in 2014.

In July, *H. halys* nymph densities increased on *A. altissima* and other host trees in the wooded borders in 2013 (Fig. 2.1B and D) and 2014 (Fig. 2.2B and D). By August in many of the fields, *H. halys* nymphs and adults began appearing on the adjacent soybean borders with densities peaking on the crop during the growth stages R5 and R6 in late August which are the beginning and full seed development stages (Fehr and Caviness 1977). Venugopal et al. (2015a) also found the highest *H. halys* densities on R5 to R6 stage soybeans. The effects of sample zone with respect to date on populations of *H. halys* nymphs ($F=10.768$; $df=30$, 228.5; $p\leq 0.0001$) and adults ($F=2.094$; $df=30$, 227.4; $p\leq 0.0013$) in 2013 were significant. Again, in 2014, the effects of sample zone with respect to date on populations of both nymphs ($F=3.701$; $df=30$, 98.7; $p\leq 0.0001$) and adults ($F=2.508$; $df=30$, 97.1; $p\leq 0.0004$) were significant.

There was a significant effect of sampling zone on density of *H. halys* with respect to date in both years with most bugs not moving far from the invading field edge; approximately half as many bugs being present at 15-m into the field and very few being detected in the field at 30-m in 2013 (Fig. 2.1B and D) and 2014 (Fig. 2.2B and D). These results are very similar to those of Venugopal et al. (2015a) for soybeans and corn sampled in Maryland, and follow a general pattern for *H. halys* being a border inhabiting agricultural pest in tree crops as well (Blaauw et al. 2014, Joseph et al. 2014, Venugopal et al. 2015b).

Our study confirms that *H. halys* populations increase numbers on *A. altissima* and other mixed hardwood tree hosts during the early summer (Bakken et al. 2015) when soybean is in vegetative stages of growth. As the crop matures into pod and seed development stages *H. halys* densities begin to gradually increase at the field edge and have a high tendency to remain on field edges throughout the soybean growing season. As pod and seed development begins to occur, *H. halys* move into the edge in order to feed until the plant is no longer a desirable food source. Treating only field edges has been introduced and exploited as an IPM strategy that was very quickly adopted by growers and agricultural consultants. This study contributed to a multistate project on the biology and management of *H. halys* in Mid-Atlantic soybeans, the results and implications of which have been published as a field management bulletin (see Cissel et al. 2015).

References Cited

- Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert Jr., D., T. P. Kuhar, and J. Walgenbach. 2015.** Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. *Environ. Entomol.* 44: 1011-1021.
- Blaauw, B. R., D. Polk, and A. L. Nielsen. 2014.** IPM-CPR for peaches: Incorporating behaviorally-based methods to manage *Halyomorpha halys* and key pests in peach. *Pest Management Science* 71: 1513-1522.
- Cissel, B., J. Whalen, G. Dively, C. Hooks, T. Patton, P. D. Venugopal, B. Aigner, J. Hogue, A. Herbert, T. Kuhar, S. Malone, and E. Seymore. 2015.** Brown marmorated stink bug biology and management in Mid-Atlantic soybeans. Virginia Cooperative Extension. ENTO – 168NP.
- Fehr, W.R. and C.E. Caviness. 1977.** Stages of soybean development. Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa.
- Hill, C.B, G.L. Hartman, R. Esgar, and H.A. Hobbs. 2006.** Field evaluation of Green Stem Disorder in soybean cultivars. *Crop Sci.* 46:879–885.
- Joseph, S. V., J. W. Stallings, T. C. Leskey, G. Krawczyk, D. Polk, B. Butler, and J. C. Bergh. 2014.** Spatial distribution of brown marmorated stink bug (Hemiptera: Pentatomidae) injury at harvest in Mid-Atlantic apple orchards. *Journal of Economic Entomology* 107: 1839-1848.

- McAvoy, T., A. L. Snyder, N. Johnson, S. M. Salom, and L. T. Kok. 2012.** Road survey of the invasive Tree-of-Heaven (*Ailanthus altissima*) in Virginia. *Invasive Plant Sci. Manage.* (Oct-Dec 2012): 506-512.
- Nielsen, A. L., and G. C. Hamilton. 2009.** Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. *J. Econ. Entomol.* 102: 1133-1140.
- Nielsen, A. L., G. C. Hamilton, and P. W. Shearer. 2011.** Seasonal phenology and monitoring of the non-native *Halyomorpha halys* in soybean. *Environ. Entomol.* 40(2):231–238.
- Owens, D., D. A. Herbert, , G. Dively, D. Reising, and T. Kuhar. 2013.** Does feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) reduce soybean seed quality and yield? *J. Econ. Entomol.* 106: 1317-1323.
- Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Garipey, G. Hamilton, T. Haye, D. A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. P. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G. Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, J. F. Tooker. 2014.** Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *J. Integr. Pest Manag.* 5: A1-A13.
- Venugopal, P. D., P. L. Coffey, G. P. Dively, and W. O. Lamp. 2014.** Adjacent habitat influence on stink bug (Hemiptera: Pentatomidae) densities and the associated damage at field corn and soybean edges. *PLoS ONE* 9 (10):e109917.

Venugopal, P. D., G. P. Dively, and W. O. Lamp. 2015. Spatiotemporal dynamics of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) in and between adjacent corn and soybean fields. *J. Econ. Entomol.* 108(5):2231-2241. doi: 10.1093/jee/tov188.

Venugopal, P. D., H. M. Martinson, E. J. Bergmann, P. M. Shrewsbury, and M. J. Raupp. 2015. Edge effects influence the abundance of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) in woody plant nurseries. *Environ. Entomol.* 44(3):474-479. doi: 10.1093/ee/nvv061.

Wallner, A. M., G. C. Hamilton, A. L. Nielsen, N. Hahn, E. J. Green, and C. R. Rodriguez-Saona. 2014. Landscape factors facilitating the invasive dynamics and distribution of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), after arrival in the United States. *PLoS ONE* 9(5): e95691. doi:10.1371/journal.pone.0095691

Table 2.1. Field sampling locations and date of first sample at each site.

First Sample Date (Soybean Growth Stage)	Location	GPS Coordinates	
7/9/2013 (R1)	Orange 1	38.2355	-78.1097
7/9/2013 (V6)	Orange 2	38.2125	-78.2419
7/9/2013 (V6)	Orange 3	38.1727	-78.248
7/9/2013 (V6)	Orange 4	38.1722	-78.2444
7/9/2013 (V7)	Orange 5	38.1755	-78.243
7/25/2013 (R2)	Madison 1	38.2883	-78.1411
7/25/2013 (R2)	Madison 2	38.3005	-78.1411
7/25/2013 (R2)	Madison 3	38.3297	-78.13
7/18/2014 (R2)	Madison 1	38.2998	-78.1450
7/18/2014 (R2)	Madison 2	38.3030	-78.1489
7/18/2014 (R2)	Madison 3	38.3069	-78.1357
7/18/2014 (R2)	Madison 4	38.3356	-78.1274

Figure Captions

Fig. 2.1A. Mean \pm SEM number of *H. halys* nymphs observed in different zones around or in soybeans in central Virginia in 2013. Bars of different zones characterized by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Fig.2.1B. Mean \pm SEM number of *H. halys* nymphs observed in different zones around or in soybeans over time in central Virginia in 2013.

Fig.2.1C. Mean \pm SEM number of *H. halys* adults observed in different zones around or in soybeans in central Virginia in 2013. Bars of different zones characterized by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Fig.2.1D. Mean \pm SEM number of *H. halys* adults observed in different zones around or in soybeans over time in central Virginia in 2013.

Fig. 2.2A. Mean \pm SEM number of *H. halys* nymphs observed in different zones around or in soybeans in central Virginia in 2014. Bars of different zones characterized by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Fig. 2.2B. Mean \pm SEM number of *H. halys* nymphs observed in different zones around or in soybeans over time in central Virginia in 2014.

Fig. 2.2C. Mean \pm SEM number of *H. halys* adults observed in different zones around or in soybeans in central Virginia in 2014. Bars of different zones characterized by the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

Fig. 2.2D. Mean \pm SEM number of *H. halys* adults observed in different zones around or in soybeans over time in central Virginia in 2014.

Fig. 2.1

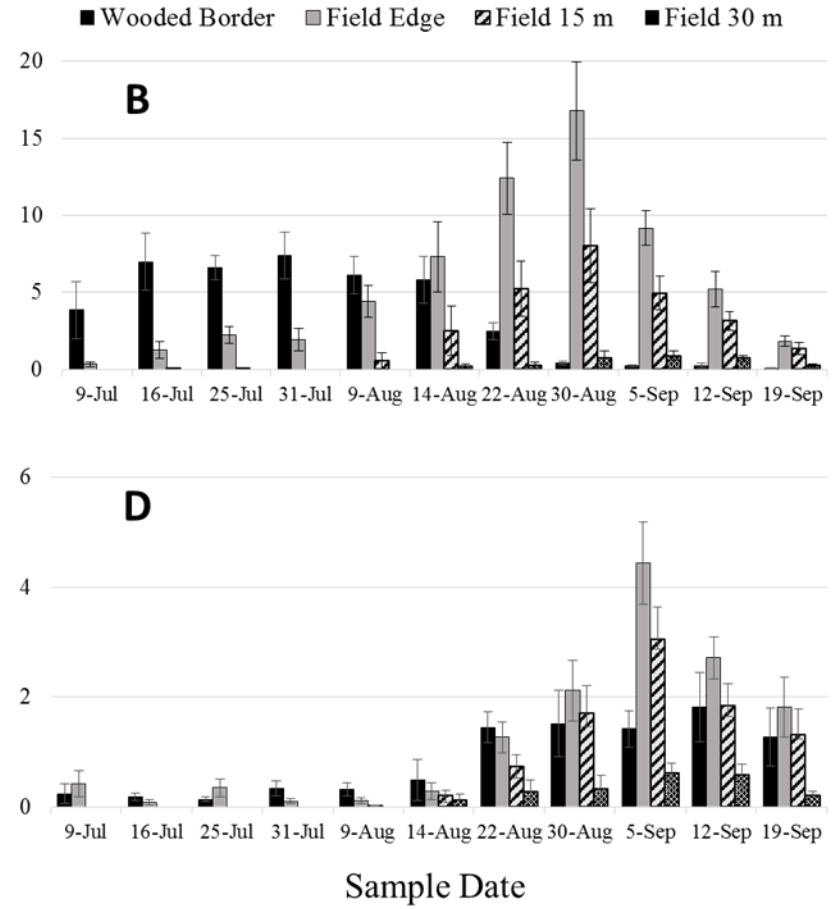
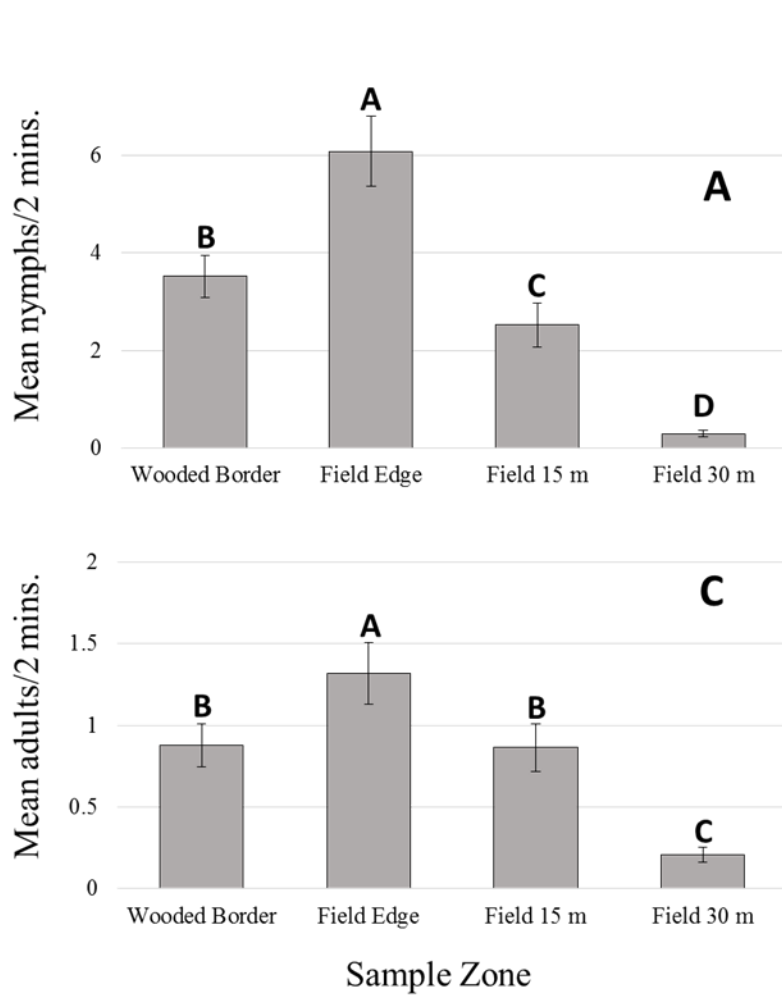
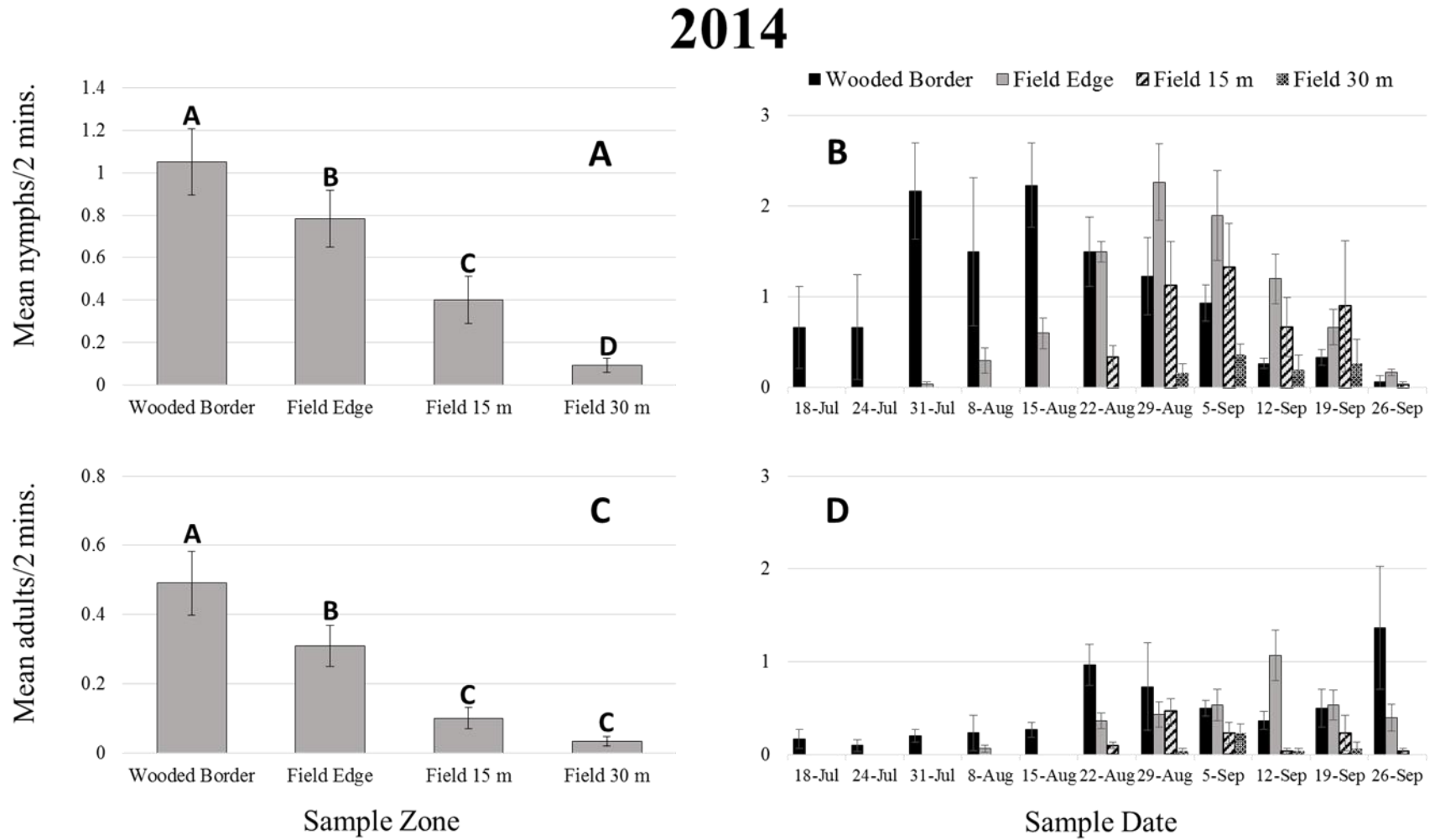


Fig. 2.2



Chapter Three

A new visual sampling method for assessing *Halyomorpha halys* (Hemiptera: Pentatomidae) numbers in soybean fields

(B.L. Aigner, D.A. Herbert, G.P. Dively, D. Venugopal, J. Whalen, B. Cissel, T.P. Kuhar, C.C. Brewster, J.W. Hogue, and E. Seymore. 2016. Manuscript accepted for publication to: Journal of Economic Entomology. September 2016)

ABSTRACT

Sampling soybean fields for brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), has proven to be a challenge. Both adults and nymphs have a strong “startle response” and drop to the ground with even the slightest disturbance. This behavior could reduce the effectiveness of the traditional sweep net and ground cloth sampling methods. In 2013 and 2014, we evaluated a visual plant inspection method in Virginia, Delaware and Maryland by comparing BSMB visual counts to the numbers caught using a sweep net. The visual inspection method consisted of counting the number of BMSB nymphs and adults seen on the plants in a 2-minute inspection period while walking carefully down and between two rows. The ends of each sample area were marked with flags. After a 30-minute interval, which allowed the stink bugs to reposition in the canopy, each flagged area was sampled using 15 sweeps with a 38-cm diameter sweep net. Comparison of visual and sweep net counts by state and year showed a reasonably close relationship between the numbers of BSMB observed by the two sampling methods. The best result was obtained when sampling data from all states and years were combined for a total of 76 soybean fields with 2,042 paired comparisons ($y = 0.984x + 0.4359$, $R^2 = 0.6934$ where $y = 2$ min. visual count and $x = \text{sweep count}/15$ sweeps). An average visual count of 5.4 BMSB in 2 minutes was estimated as being equivalent

to the current economic threshold of 5 stink bugs per 15 sweeps. Visual inspection appears to be an effective method for assessing BMSB populations in soybean.

Introduction

Since its introduction to the United States in the late 1990s, the brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), has become a well-known pest of many agricultural crops (e.g., apples, peaches, sweet corn, peppers, tomatoes, field corn and soybeans) in the Mid-Atlantic region (Kuhar et al. 2012, Leskey et al. 2012, Rice et al. 2014). Soybeans (*Glycine max*), in particular, are a preferred host for BMSB, which is capable of causing economic losses due to yield and quality reductions (Nielsen and Hamilton 2009, Owens et al. 2013a). BMSB was first detected in United States soybean fields in Pennsylvania and New Jersey in the mid-2000's (Nielsen and Hamilton 2009), and was observed in soybean fields in Maryland, Delaware, and Virginia by 2010. By 2012, infestations of the insect were found to be wide-spread in most of the major soybean growing areas throughout these states. Furthermore, in some Mid-Atlantic states, BMSB is now the predominant stink bug species found in soybean (Nielsen and Hamilton 2009, Bakken et al. 2015). As of 2014, minor infestations of BMSB also have been reported in soybeans in other states such as Tennessee and Kentucky.

Brown marmorated stink bug adults and nymphs, with the exception of first instars, which are non-feeding, attack soybean pods and seeds (Owens et al. 2013a). Similar to native and long-established stink bug species [e.g., *Chinavia hilaris* (Say), *Nezara viridula* (Linnaeus), and *Euschistus servus* (Say)], BMSB feeds on plant parts by inserting their piercing-sucking mouth parts, injecting plant digestive enzymes, and extracting the plant fluids (McPherson and McPherson 2000, Peiffer and Felton 2014). Feeding injury to soybean seed in the early stages of

pod development, R3 (beginning pod) to R4 (full pod) (Fehr and Caviness 1977), can result in aborted pods or underdeveloped flat pods. Feeding injury to larger developing seeds, R5 (beginning seed) and R6 (full seed), results in shriveled, deformed, or even aborted seeds (Corrêa-Ferreira and Azevedo 2002). In the field, BMSB damaged pods are prematurely yellow with brown speckles on the pod wall as a result of insect feeding. Seed damage is visible upon opening and inspection of the damaged pod (Cissel et al. 2015). Feeding by BMSB has also been shown to cause delayed plant development, often referred to as “stay green” syndrome (Rice et al. 2014). In response to stink bug feeding, soybeans will prolong development in an effort to produce more seed to compensate for what may have been lost. As a result, at the end of the season, portions of the field with heavy BMSB infestations remain green while the remainder of the field dries down, reaching the R8 (full maturity) stage (Rice et al. 2014). Stay green syndrome can result in highly significant yield loss because of the difficulties associated with harvesting soybeans that have not fully matured at the same time as the remainder of the field (Hill et al. 2006, Owens et al. 2013a).

Assessment of a pest population is a key component in the development of an integrated pest management (IPM) program for dealing with the threat of potential economic loss in a crop system (Pedigo and Rice 2013a). Decision making with regard to management is based upon economic injury levels and economic thresholds developed by determining the lowest number of insects in a sample that will cause significant loss in quality and yield of a crop (Pedigo and Rice 2013a). Sampling is an important process for estimating pest abundance and an essential component for decision making in an IPM program. Although there are many sampling methods for determining pest abundance (e.g., sweep net, ground cloth, light trap, sticky card, etc.), the

method of choice should depend on the type of pest and characteristics of the crop system (Pedigo and Rice 2013b).

The sweep net sampling method has been a standard for assessing native stink bug populations in soybean in the United States since the 1970s (Todd and Herzog 1980). As such, economic thresholds have been based on the average number of BSMB caught per sweep given some number of random samples in a field. Sampling soybean fields for BMSB using sweep netting, however, has proven to be a challenge compared with sampling for native stink bug species. Both BMSB adults and nymphs have a strong ‘startle response’ and drop to the ground with even the slightest disturbance (personal observation). This makes using traditional sampling methods for BMSB—sweep nets and ground cloths—difficult and could lead to an underestimation of densities. To that end, we developed an alternative sampling method that involves careful visual inspection of plants in the field. We evaluated the visual plant inspection method by comparing numbers of BMSB observed with this method to the numbers caught using the standard sweep net method.

Materials and Methods

BMSB Sampling. In 2013 and 2014, sampling of BMSB adults and nymphs was conducted in 76 arbitrarily-selected soybean fields in Virginia, Maryland and Delaware (Table 3.1). Fields were revisited and sampled from July through October when BMSB populations diminished. In all fields, plants were on narrow row spacing (38 to 46-cm centers) except in Maryland where fields were on a 91.5-cm center (wide row).

In each field there were a number of paired comparisons of the number of BMSB sampled using the standard sweep net and a visual plant inspection method. Visual sampling

was conducted 4.5-m from the field edge as the sampler carefully walked between the planted rows for two minutes, viewing a 1-m wide area parallel to the field edge, and counting all BMSB that were observed. The beginning and end points of each sampling area were flagged to ensure that the same area would be used for the subsequent sweep net sampling. After a 30-minute interval, which allowed the stink bugs to reposition in the plant canopy, the flagged area was sampled with 15 sweeps using 38-cm sweep net (Bioquip, Rancho Dominguez, CA) by the 'Lazy-8' method described by Kogan and Pitre (1980). All BMSB adults and nymphs found in the sweep net after sampling were counted.

Statistical Analysis. Standard linear regression and correlation analyses (Zar 1984) were used to assess the relationship of the number of BMSB adults and nymphs observed during the 2-minute visual inspection and sweep net counts. Separate analyses were carried out for the sampling data collected in soybean fields in Virginia and Maryland in each year, for the combined data collected in Virginia and Maryland in 2014, and for the combined data collected from Virginia, Maryland, and Delaware in all years. In addition, statistical pairs where both visual inspection count and sweep net count were zeroes, were excluded because they provided no quantitative comparison. All statistical analyses were carried out using JMP Pro 11.0 (SAS 2013) at a significance level of $\alpha = 0.05$.

Results

In observed soybean fields in Virginia, Maryland and Delaware in 2013 and 2014 all regressions showed positive correlations and were fairly consistent. In Virginia in 2013 and 2014, the two sampling methods showed the highest correlations with $R = 0.89$ and $R = 0.86$, respectively (Figs. 3.1, 3.2). Samples compared from Maryland in 2014 also resulted in a high

correlation ($R = 0.84$) of insects counted between the two methods (Fig. 3.3). The results of analysis of the sampling data from Delaware for both years showed a much lower correlation ($R = 0.39$), which may be attributed to low population densities in the sampled areas. It has been documented that BMSB occur in lower numbers and do not become well established in the coastal plains of the Mid-Atlantic region (Bakken et al. 2015).

At a threshold of the 5 bugs per 15 sweeps, higher numbers of BMSB were observed in 2 minutes in wide row planted fields (8.0) compared with narrow row planted fields (4.3) (Table 3.1, Fig. 3.4). This difference could be due to several factors—for example, in wide row plantings, the sampler can ‘see’ stink bugs from the top of the canopy to the bottom (Owens et al. 2013b), whereas the sweep net sample is mainly confined to the upper canopy—which would mean more could be seen than swept. The best result was obtained when data from all states and years were combined (76 fields, 2,042 paired comparisons) and showed that an average visual count of 5.4 BMSB per 2 minutes was equivalent to the threshold of 5 BMSB per 15 sweeps (Table 3.1, Fig. 3.5).

Overall, the results show that there is a reasonably close relationship between the numbers of BMSB sampled by the visual inspection method and by sweep netting. Currently, many soybean growing states have the economic threshold for BMSB set at 5 bugs per 15 sweeps (e.g., AR^a, DE^b, LA^c, MD^d, MS^e, NC^f, TN^g, VA^h). Table 3.1 displays the visual count that corresponds to this sweep net threshold ($x = 5$). For example, for Virginia in 2013, 6.1 BMSB were observed in 2 minutes when at the 5 stink bug per 15 sweep threshold (Table 3.1, Fig. 3.1). In 2014, 4.3 and 3.9 were observed in 2 minutes at the 5 per 15 sweep threshold, in Virginia and Delaware, respectively (Table 3.1, Figure 3.2).

Not all results were this close. In Maryland fields in 2014, visual counts tended to be higher compared with sweep net counts (11.4 BMSB observed at the 5 per 15 sweep threshold; Table 3.1, Fig. 3.3); a greater number of BMSB were observed in the 2 minute visual inspection than were caught in 15 sweeps.

Discussion

Maryland fields differed from the other fields in the study in that some had very high numbers of BMSB, up to 40 individuals per 15 sweeps. It appears that in fields with very high BMSB populations, the sampler can see more BMSB in relation to what can be caught in 15 sweeps. However, fields with such high populations would likely be so obviously above threshold that sampling using any method would not be necessary to determine the need for treatment.

Our conclusion from this study is that the visual inspection method may be an effective and preferred alternative method for determining the action threshold for BMSB in soybean, especially in fields with narrow row spacing. Stepping a few rows into a field and doing a 2-minute visual count while carefully walking down between rows appears to be a simple and relatively accurate way of assessing the need for an insecticide field-edge treatment for BMSB in soybean. Visually observing 5 BMSB adults or nymphs in a 2-minute search appears to be very close to the 5 stink bugs per 15 sweep threshold currently being used in several states (Table 3.1). Having a simple assessment method could encourage more soybean field sampling by growers and crop advisors and lead to more conscientious treatment decisions regarding BMSB management.

Acknowledgements

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References Cited

- Bakken, A.J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert, D. Reisig, T. P. Kuhar, and J. F. Walgenbach. 2015.** Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. *Environ. Entomol.* 44(4): 1011–1021 (2015); DOI: 10.1093/ee/nvv092
- Cissel B., J. Whalen, G. Dively, C. Hooks, T. Patton, P. D. Venugopal, B. Aigner, J. Hogue, A. Herbert, T. Kuhar, S. Malone, E. Seymore. 2015.** Brown marmorated stink bug biology and management in Mid-Atlantic soybeans. Virginia Cooperative Extension. ENTO – 168NP.
- Corrêa-Ferreira B. S. and J. de Azevedo. 2002.** Soybean seed damage by different species of stink bugs. *Agric. Forest Entomol.* 4: 145–150.
- Fehr, W. R. and C. E. Caviness. 1977.** Stages of soybean development. Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa. Special Report 80.
- Hill C. B., G. L. Hartman, R. Esgar, and H. A. Hobbs. 2006.** Field evaluation of green stem disorder in soybean cultivars. *Crop Sci.* 46(2): 879-885.
- Kogan M. and H. N. Pitre. 1980.** General sampling methods for above-ground population of soybean arthropods, Pg. 30-60. *In* M. Kogan and D.C. Herzog (eds.), *Sampling Methods in Soybean Entomology*. Springer-Verlag, New York, New York, USA.
- Kuhar T. P., K. L. Kamminga, J. Whalen, G. P. Dively, G. Brust, C.R.R. Hooks, G. Hamilton, and D. A. Herbert. 2012.** The pest potential of brown marmorated stink bug on vegetable crops. *Plant Health Prog. Online*. Doi: 10.1094/ PHP–2012–0523–01–BR.

- Leskey T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. A. Hoelmer, D.-H. Lee, and S. E. Wright. 2012.** Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Pestic. Outlook. 218-226.
- McPherson J. E. and R. M. McPherson. 2000.** Stink bugs of economic importance in America North of Mexico. CRC, Boca Raton, FL.
- Nielsen A. L. and G. C. Hamilton. 2009.** Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in northeastern United States. Ann. Entomol. Soc. Am. 102: 608–616.
- Nielsen A. L., G. C. Hamilton, and D. Matadha. 2011.** Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae) in soybean. Environ. Entomol. 40(2): 231-238.
- Owens D. R., D. A. Herbert, Jr., G. P. Dively, D. D. Reising, and T. P. Kuhar. 2013a.** Does feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) reduce soybean quality and yield? J. Econ. Entomol. 106(3): 1317-1323.
- Owens, D., D. A. Herbert, T. Kuhar and D. Reising. 2013b.** Effects of temperature and relative humidity on the vertical distribution of stink bugs (Hemiptera: Pentatomidae) within soybean canopies and implications for field sampling. J. Entomol. Sci. 48 (2): 90-98.
- Pedigo L.P. and M. E. Rice. 2013a.** Economic Decision Levels for Pest Populations, Pg. 255-285. In L. P. Pedigo and M. E. Rice (eds.), Entomology and Pest Management 6th Edition. Prentice-Hall of India, New Delhi, India.

Pedigo L. P. and M. E. Rice. 2013b. Surveillance and Sampling, Pg. 213-254. *In* L. P. Pedigo and M. E. Rice (eds.), Entomology and Pest Management 6th Edition. Prentice-Hall of India, New Delhi, India.

Peiffer, M. and G. W. Felton. 2014. Insights into the saliva of the brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae). PLoS ONE. 9 (2): e88483 DOI: 10.1371/journal.pone.0088483

Rice K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H.

Fraser, T. Gariepy, G. Hamilton, T. Haye, A. Herbert, K. Hoelmer, C. R. Hooks, A.

Jones, G. Krawczyk, T. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G.

Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D.

Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, and J. F. Tooker. 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *J. Integr. Pest Manag.* 5: 1–13.

Todd J. W. and D. C. Herzog. 1980. Sampling phytophagous Pentatomidae on soybean, Pg. 438-478. *In* M. Kogan and D.C. Herzog (eds.), *Sampling Methods in Soybean Entomology*. Springer-Verlag, New York, New York, USA.

Zar J. H. 1984. Comparing Simple Linear Regression Equations, Pg. 292-305. *In* J. H. Zar [ed.], *Biostatistical Analysis*, 2nd Edition, Prentice Hall, Englewood Cliffs, New Jersey, USA.

Table 3.1. Regression statistics comparing the number of brown marmorated stink bug nymphs and adults observed in a 2-minute visual plant inspection with number caught per 15 sweeps, and the number observed in 2 minutes when number swept was equal to 5.

Year	State	Number of Paired Comparisons	Number of Fields Sampled	Linear Regression Equation ^a	R ²	BMSB visual threshold when $x = 5^b$
2013	VA ⁱ	1,431	20	$y = 1.1661x + 0.2788$	0.8016	6.1
2014	MD ⁱ	135	2	$y = 2.0641x + 1.0919$	0.7137	11.4
	MD	127	2	$y = 1.1183x + 2.4263$	0.486	8.0
	VA	327	19	$y = 0.7566x + 0.5438$	0.7466	4.3
	DE ⁱ	168	35	$y = 0.7065x + 0.3605$	0.1559	3.9
2013, 2014	DE, MD, VA	2,042	76	$y = 0.984x + 0.4359$	0.6934	5.4

^a x and y represent the number of BMSB adults and nymphs per 15 sweeps and per 2-minute visual inspection, respectively; all regression lines were significant ($P < 0.05$).

^b Current economic threshold in many soybean growing states with sweep net sampling = 5 stink bugs/15 sweeps.

Figure Captions

Fig. 3.1. Number of BMSB adults and nymphs observed in a 2-minute visual plant assessment compared with the number caught in a 15-sweep net sample – 1,431 paired sample comparisons in Virginia soybean fields in 2013.

Fig. 3.2. Number of BMSB adults and nymphs observed in a 2-minute visual plant assessment compared with the number caught in a 15-sweep net sample – 327 paired sample comparisons in Virginia soybean fields in 2014.

Fig. 3.3. Number of BMSB adults and nymphs observed in a 2-minute visual plant assessment compared with the number caught in a 15-sweep net sample – 135 paired sample comparisons in Maryland soybean fields in 2014.

Fig. 3.4. Number of BMSB adults and nymphs observed in a 2-minute visual plant assessment compared with the number caught in a 15-sweep net sample – 127 paired sample comparisons in Maryland and 327 paired sample comparisons in Virginia soybean fields in 2014.

Fig. 3.5. Number of BMSB adults and nymphs observed in a 2-minute visual plant assessment compared with the number caught in a 15-sweep net sample – 2,042 paired comparisons in 2013 and 2014 in Virginia, Delaware, and Maryland.

Figure 3.1

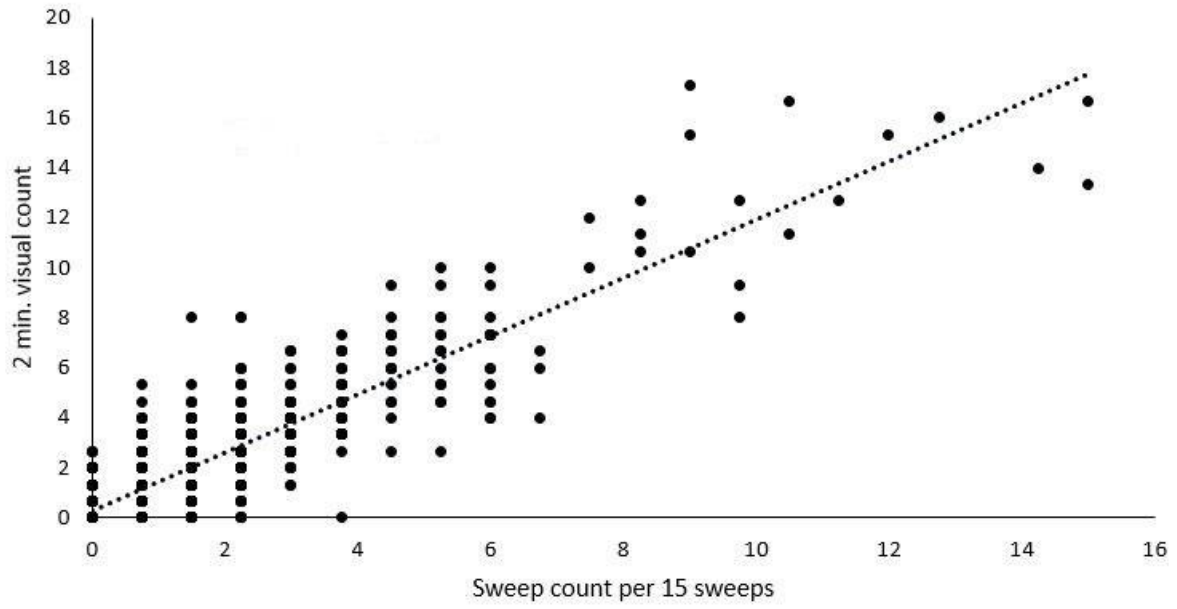


Figure 3.2

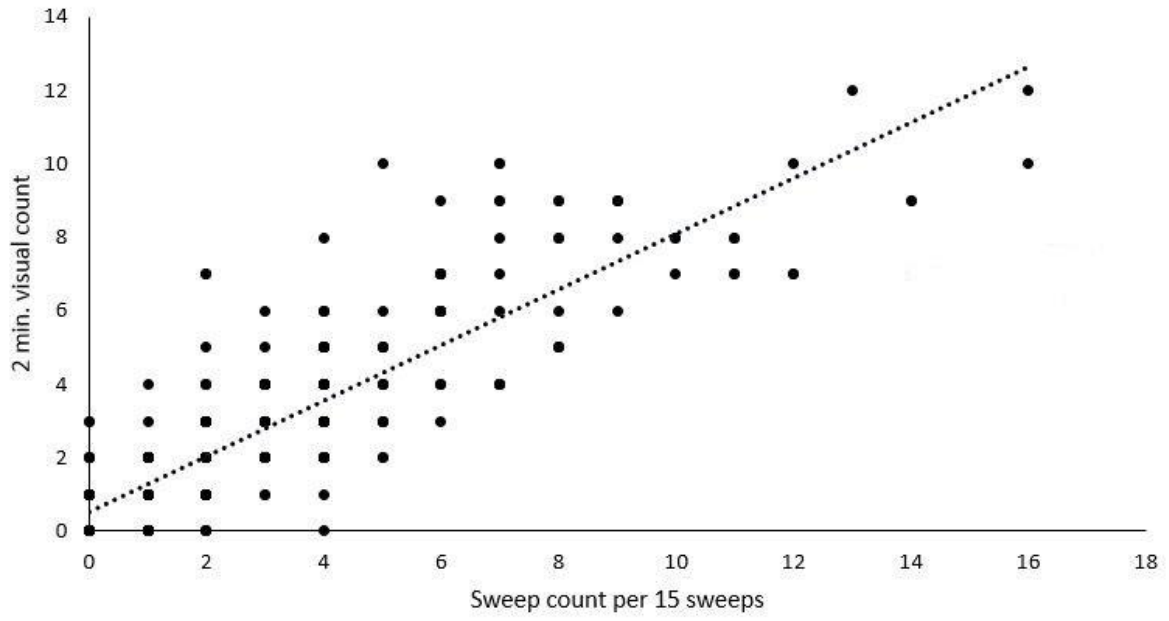


Figure 3.3

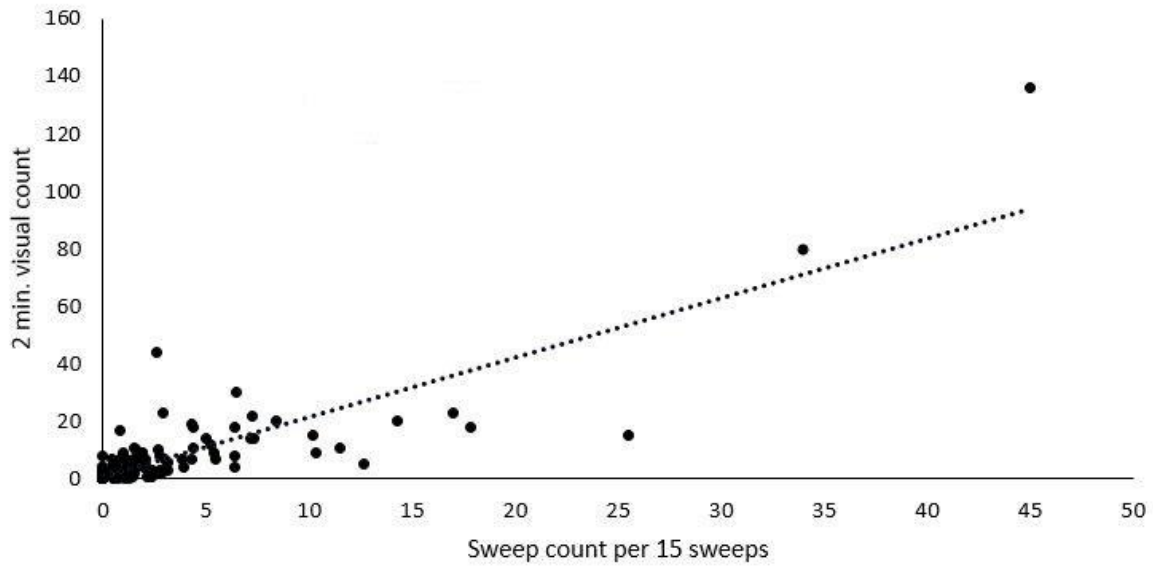


Figure 3.4

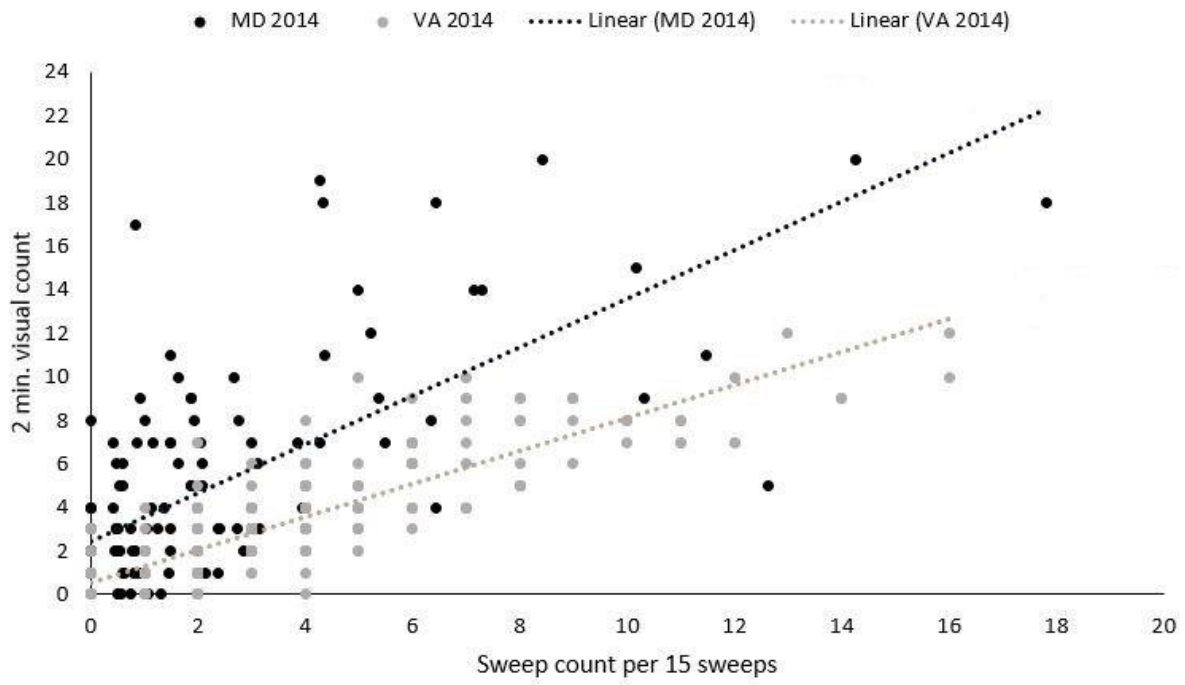
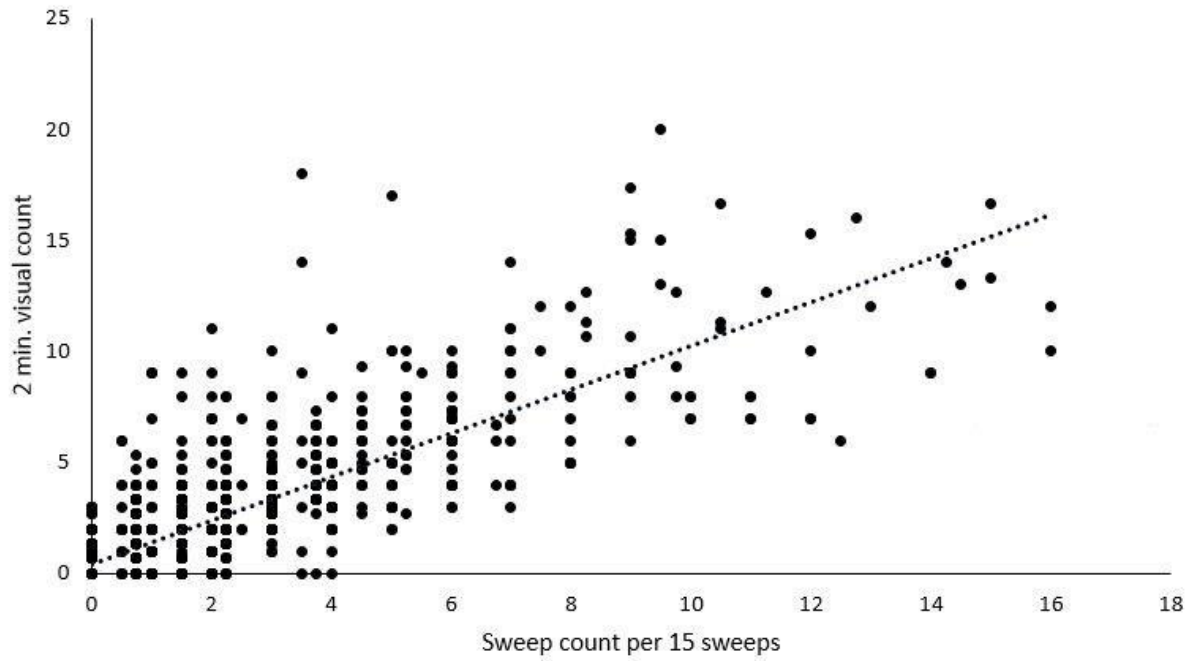


Figure 3.5



Footnotes

- a. **Studebaker, G. 2015.** Insecticide Recommendations for Arkansas.
<https://www.uaex.edu/publications/pdf/mp144/mp144.pdf>

- b. **Whalen, J. 2015.** Stink Bug on the Rise in Soybeans.
<http://extension.udel.edu/kentagextension/tag/stinkbug/>

- c. **Schowalter, T.D. 2015.** 2015 Louisiana Insect Pest Management Guide.
<http://www.lsuagcenter.com/NR/rdonlyres/3A39E4CE-5E4D-4CC0-9744-2F3AEC362003/100995/9Soybean2015.pdf>

- d. **Brust, G. 2015.** Common Stink Bugs of the Mid-Atlantic, Hemiptera: Pentatomidae.
https://extension.umd.edu/sites/default/files/docs/articles/CommonStinkbugsOfMidAtlantic_0.pdf

- e. **Catchot, A. 2015.** 2015 Insect Control Guide for Agronomic Crops.
<http://msucares.com/pubs/publications/p2471.pdf>

- f. **North Carolina Cooperative Extension. 2013.** Thresholds.
<http://soybeans.ces.ncsu.edu/thresholds/>

- g.* **University of Tennessee Cooperative Extension. 2015.** 2015 Soybean Insect Control Recommendations. http://www.utcrops.com/cotton/cotton_insects/pubs/PB1768-Soybean.pdf
- h.* **Herbert D.A. 2015.** Pest Management Guide: field crops 2015. <https://pubs.ext.vt.edu/456/456-016/Section04-Insects-1.pdf>
- i.* **VA Counties:** Albemarle, Amelia, Appomattox, Bedford, Buckingham, Campbell, Cumberland, Fluvanna, Goochland, Green, Louisa, Madison, Nottoway, Orange, Powhatan, Prince Edward, and Franklin. **MD Counties:** Howard and Washington. **DE Counties:** New Castle, Kent, and Sussex.

Chapter Four

Using CLIMEX to assess potential risk for brown marmorated stink bug in Virginia

(B.L. Aigner, C.C. Brewster, D.A. Herbert, and T.P. Kuhar)

Introduction

Climate is a primary limiting factor in the distribution and establishment of species around the globe. The accidental introduction of brown marmorated stink bug (BMSB), *Halyomorpha halys*, to the U.S. in the late 1990's has resulted in severe economic losses in a number of agricultural systems in some regions of the country (Kuhar et al. 2012, Leskey et al. 2012a, Rice et al. 2014). Due to the economic threat of BMSB it is important to understand the potential geographic range of distribution and establishment. Having the knowledge of potential risk can help growers anticipate the need for management in predicted high risk areas. Typically, 1-2 generations occur annually in its native range, however, 4-6 generations have been reported near Canton in southern China (Hoffman 1931). The pest is likely univoltine in the upper Mid-Atlantic region (Nielsen and Hamilton 2009) and bivoltine in the mountainous regions of Virginia, West Virginia and North Carolina (Leskey et al. 2012b, Bakken et al. 2015). Up to five generations may be possible in tropical climates in parts of the southeast based on average temperatures (Lee et al. 2013).

Nielsen et al. (2008) found that minimum and maximum temperature thresholds for BMSB development are 17°C and 33°C, respectively. The degree of cold tolerance of BMSB is dependent upon sex, season, and acclimation location and was found to be within the range of

-13°C to -18°C in bugs tested from Virginia and Minnesota, respectively (Cira et al. 2016). Moreover, significant lethal effects to BMSB with regard to high temperature extremes occur at around 42°C with prolonged exposure (Aigner and Kuhar 2016).

The CLIMEX modeling software (Hearne Scientific Software Pty. Ltd., Melbourne, Australia) can be used to provide an estimation of the geographic range distribution of invasive or established species by directly comparing the climatic conditions of the native host range with any number of locations of concern (Poutsma et al. 2008, Taylor and Kumar 2012, Park et al. 2014, Shabani and Kumar 2014). Biological parameters of BMSB were used in the CLIMEX model to simulate the potential distribution of the pest throughout Virginia. The model was validated by making a visual comparison of the CLIMEX simulated distribution with a previously developed global niche model by Zhu et al. (2012).

Materials and Methods

CLIMEX is one of several niche modeling software that can be used to simulate and predict the potential geographic distribution of species in relation to climate, primarily temperature, moisture, and light (Sutherst et al. 2004). With CLIMEX, the overall growth potential of the species is expressed by the Annual Growth Index (GI_A), which ranges between 0–100. The interaction of GI_A with stress indices such as extreme wetness, coldness, and heat results in an index of climatic suitability known as the Ecoclimatic Index (EI), which is an overall measure of favorableness of each geographic location in the area of interest for species survival. The EI also varies from 0–100 with a geographic location having an EI = 0 considered

climatically unsuitable for the species; EI = 1–3, 4–10, 11–25, and >25 represent marginal, moderate, favorable, and highly favorable geographic locations for species survival, respectively.

Fitting CLIMEX Parameters. CLIMEX uses a Species Parameter File (SPF) to store all of the model parameters for the species of interest. Parameters in the SPF for modeling *H. halys* distribution are shown in Table 4.1. CLIMEX provides several SPF templates (e.g., Wet Tropical, Mediterranean, Temperate, Semi-Arid, and Desert), one of which can be used as a starting point in developing a specific SPF for the species of interest. The Temperate template was selected initially and parameters in this SPF were modified accordingly based on data obtained from current literature on the insect so that the predicted worldwide distribution of *H. halys* matched the distribution predicted by Zhu et al. (2012).

CLIMEX Climate Data. CLIMEX provides a climate data set, which was derived from a global spline grid of data with a resolution of 0.5° from the Climate Research Unit at Norwich, U.K. Whereas, these data are sufficient for investigating the worldwide distribution of a species, they lack the resolution for more fine scale investigations of species distribution, such as the distribution of *H. halys* in Virginia. CLIMEX, only contains information on climate from 3 weather stations in Virginia; however, the software gives users the ability to import higher resolution climate data for specific locations. Climate data for Virginia from 165 weather stations were obtained from the National Center for Environmental Information (www.ncdc.noaa.gov) and modified to fit the formatting requirements of CLIMEX (Fig. 4.1).

CLIMEX Simulation of *H. halys* Distribution. Simulations of *H. halys* distribution were initially carried out and parameters in the SPF were adjusted accordingly until a reasonable visual correspondence was obtained between the projected distribution and the distribution

reported in Zhu et al. (2012). Once a correspondence between the two distributions was obtained simulations were carried out specifically for Virginia by using the full set of weather station data.

Results and Discussion

Reasonable correspondence for the worldwide distribution of *H. halys* and the distribution in the contiguous U.S. were obtained between the CLIMEX Ecoclimatic Index (EI) and the distributions predicted by the Zhu et al. (2012) model. These models predict high risk growth potential of *H. halys* within its native range in Asia and in the Southeastern states, the coastal Pacific Northwest, and a portion of the Mid-Atlantic (Figs. 4.2 and 4.3). In addition, the simulations showed regions of moderate risk growth potential in states in the Midwest, Southern Great Plains, Texas, and the Pacific Northwest (Fig 4.2). Low risk growth potentials are predicted to occur in other areas (Fig. 4.2).

In Virginia, CLIMEX predicted that the coastal plains region of the state would provide an environment for the highest growth potential of *H. halys* with one additional patch in the southwestern piedmont region (Fig. 4.4). The remaining southern piedmont counties are at moderate risk followed by the Valley and Ridge and Appalachian Plateau regions. The distribution of *H. halys* predicted by CLIMEX appears to contrast with observations of *H. halys* population levels and potential for population growth reported by others (e.g., Venugopal et al. 2016). One reason for this is that CLIMEX predictions are based solely on climate, and as noted by the developers of CLIMEX, there are factors other than climate, such as the availability of host plants, that limit species distribution (Sutherst et al. 2004).

References Cited

- Aigner, J.D. and T.P. Kuhar. 2016.** Lethal high temperature extremes of the brown marmorated stink bug (Hemiptera: Pentatomidae) and efficacy of commercial heat treatments for control in export shipping cargo. *J.Agr. Urban Entomol.* 32: (1) 1—6.
- Aljaryian, R., L. Kumar, and S. Taylor. 2016.** Modelling the current and potential future distributions of the sunn pest *Eurygaster integriceps* (Hemiptera: Scutelleridae) using CLIMEX. *Pest Manag. Sci.*; 72: 1989–2000.
- Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert Jr., D., T. P. Kuhar, and J. Walgenbach. 2015.** Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. *Environ. Entomol.* 44: 1011-1021.
- Cira, T. M., R.C. Venette, J. Aigner, T. Kuhar, D.E. Mullins, S.E. Gabbert, and W.D. Hutchison. 2016.** Cold Tolerance of *Halyomorpha halys* (Hemiptera: Pentatomidae) Across Geographic and Temporal Scales. *Environ. Entomol.* 1 – 8. Doi: 10.1093/ee/nvv220.
- Hoffmann W.E. 1931.** A pentatomid pest of growing beans in South China. *Peking Nat. Hist. Bull.* 5: 25–26.
- Haye, T., A. Abdallah, T. Gariepy, and D. Wyniger. 2014.** Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, *Halyomorpha halys* in Europe. *J. Pest Sci.* 87:407-418

- Kuhar, T. P., K. L. Kamminga, J. Whalen, G. P. Dively, G. Brust, C. R. R. Hooks, G. Hamilton, and D. A. Herbert. 2012.** The pest potential of brown marmorated stink bug on vegetable crops. Online. Plant Health Prog. doi:10.1094/PHP-2012-0523-01-BR.
- Kumar, S., L. G. Neven, H. Zhu, and R. Zhang. 2015.** Assessing the global risk of establishment of *Cydia pomonella* (Lepidoptera: Tortricidae) using CLIMEX and MaxEnt niche models. J. Econ. Entomol. 1-12.
- Kumar, S., W.L. Lee, and L.G. Neven. 2016.** Mapping global potential risk of establishment of *Rhagoletis pomonella* (Diptera: Tephritidae) using MaxEnt and CLIMEX niche models. J. Econ. Entomol. 1-11
- Lee, D.-H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. 2013.** Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environ. Entomol. 42: 627-641.
- Legaspi, B.C., Jr., and J.C. Legaspi. 2010.** Field-Level Validation of a CLIMEX Model for *Cactoblastis cactorum* (Lepidoptera: Pyralidae) Using Estimated Larval Growth Rates. Environ. Entomol. 39(2):368-377.
- Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. A. Hoelmer, D.-H. Lee, and S. E. Wright. 2012a.** Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23: 218-226.

- Leskey, T. C., D.-H. Lee, B. D. Short & S. E. Wright. 2012b.** Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): Analysis of insecticide lethality. *J. Econ. Entomol.* 105: 1726–1735.
- Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008.** Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* 37: 348-355.
- Nielsen, A., and G.C. Hamilton. 2009.** Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. *Annals of the Entomological Society of America* 102:608-616.
- Nielsen, A. L., and G. C. Hamilton. 2009.** Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. *J. Econ. Entomol.* 102: 1133-1140.
- Nielsen, A.L., S. Chen, and S.J. Fleischer. 2016.** Coupling developmental physiology, photoperiod, and temperature to model phenology and dynamics of invasive heteropteran, *Halyomorpha halys*. *Frontiers in Physiology*, 7: 1-12.
- Park, J-J., H-h. Mo, G-S. Lee, S-E. Lee, J-H. Lee, and K. Cho. 2014.** Predicting the potential geographic distribution of *Thrips palmi* in Korea, using the CLIMEX model. *Entomol. Res. (Korea)* 44: 47-57. Doi: 10.1111/1748-5967.12049
- Poutsma, J., A. J. M. Loomans, B. Aukema, and T. Heijerman. 2008.** Predicting the potential geographical distribution of the harlequin ladybird, *Harmonia axyridis*, using the CLIMEX model. *BioControl* 53: 103-125. Doi: 10.1007/s10526-007-9140-y.
- Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Garipey, G. Hamilton, T. Haye, D. A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. P. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G.**

- Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, and J. F. Tooker. 2014.** Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *J. Integr. Pest Manag.* 5: A1-A13.
- Shabani, F., and L. Kumar. 2014.** Sensitivity analysis of CLIMEX parameters in modeling potential distribution of *Phoenix dactylifera* L. *PLoS ONE* 9(4): e94867. Doi: 10.1371/journal.pone.0094867.
- Sutherst, R. W., G. F. Maywald, W. Bottomley, and A. Bourne. 2004.** CLIMEX v2: user's guide. CSIRO, Queensland, Australia
- Taylor, S. and L. Kumar. 2012.** Potential distribution of an invasive species under climate change scenarios using CLIMEX and soil drainage: A case study of *Lantana camara* L. in Queensland, Australia. *J. Environ. Manage.* 114: 414-422.
- Venugopal, P.D., G.P. Dively, A. Herbert, S. Malone, J. Whalen, and W. Lamp. 2016.** Contrasting role of temperature in structuring regional patterns of invasive and native pestilential stink bugs. *PLoS ONE* 11(2):e0150649.
- Yonow, T., V. Hattingh, and M. de Villiers. 2013.** CLIMEX modelling of the potential global distribution of the citrus black spot disease caused by *Guignardia citricarpa* and the risk posed to Europe. *Crop Protection* 44:18-28.
- Zhu, G., Bu, W., Gao, Y., Liu G. 2012.** Potential geographic distribution of brown marmorated stink bug invasion (*Halyomorpha halys*). *PLoS ONE*. 7(2): e31246. Doi: 10.1371/journal.pone.0031246.

Table 4.1. CLIMEX parameter file for *Halyomorpha halys*

Parameter	Description	Value
Temperature Index (TI)		
DV0	Limiting low temperature	5
DV1	Lower optimal temperature	15
DV2	Upper optimal temperature	26
DV3	Limiting high temperature	35
Moisture Index (MI)		
SM0	Limiting low moisture	0
SM1	Lower optimal moisture	0.3
SM2	Upper optimal moisture	5
SM3	Limiting high moisture	8
Diapause Index (DI)		
DPD0	Diapause induction daylength	10
DPT0	Diapause induction temperature	-3
DPT1	Diapause termination temperature	15
DPD	Diapause development days	-150
DPSW	Summer/Winter diapause indicator	0
Cold Stress (CS)		
TTCS	Cold stress temperature threshold	-13.9
THCS	Cold stress temperature rate	0
DTCS	Cold stress degree-day threshold	15
DHCS	Cold stress degree-day rate	-0.0001
TTCSA	Cold stress temperature threshold (average)	-10
THCSA	Cold stress temperature rate (average)	-0.04
Heat Stress		
TTHS	Heat stress temperature threshold	35
THHS	Heat stress temperature rate	0.005
DTHS	Heat stress degree-day threshold	0
DHHS	Heat stress degree-day rate	0
Dry Stress (DS)		
SMDS	Dry stress threshold	0.2
HDS	Dry stress rate	-0.005
Wet Stress (WS)		
	Wet stress threshold	2.5
	Wet stress rate	0.002
PDD	Degree-days per generation	588

Figure Captions

Fig. 4.1. Map of the location of the 165 weather stations in Virginia from which climate data were obtained for prediction of Brown Marmorated stink bug, *Halyomorpha halys* distribution.

Fig. 4.2. Comparison of the worldwide distribution of Brown Marmorated stink bug, *Halyomorpha halys* predicted by Zhu et al. (2012) with the distribution predicted by the CLIMEX model.

Fig. 4.3. Comparison of the distribution of Brown Marmorated stink bug, *Halyomorpha halys* in the contiguous U.S. predicted by Zhu et al. (2012) with the distribution predicted by the CLIMEX model.

Fig. 4.4. Distribution of Brown Marmorated stink bug, *Halyomorpha halys* predicted by the CLIMEX model for Virginia. Areas of high growth potential are dark green and those of low growth potential are light green.

Fig. 4.1

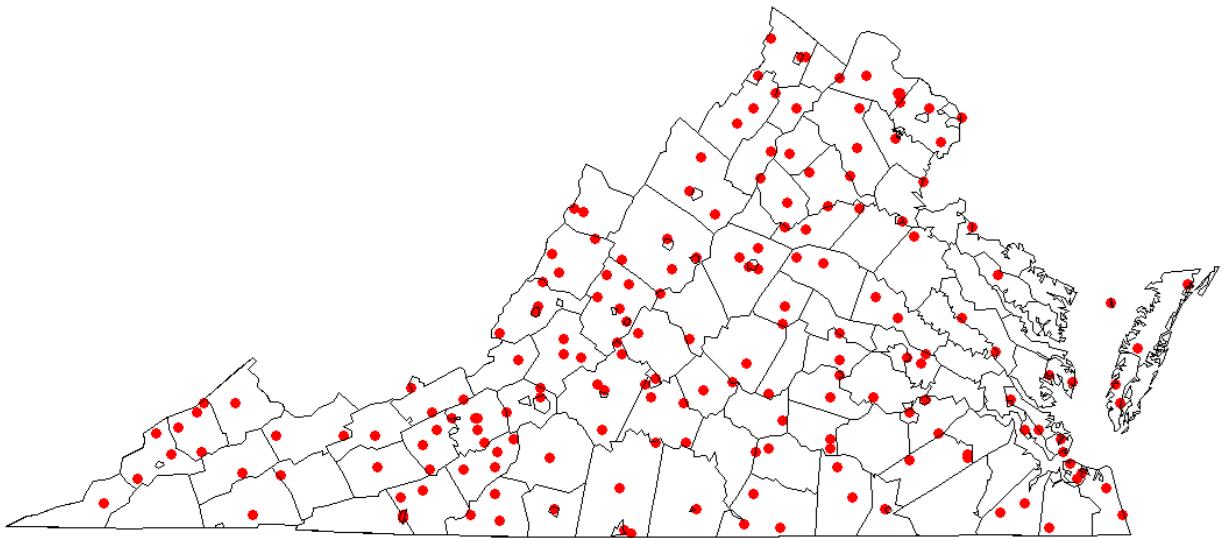
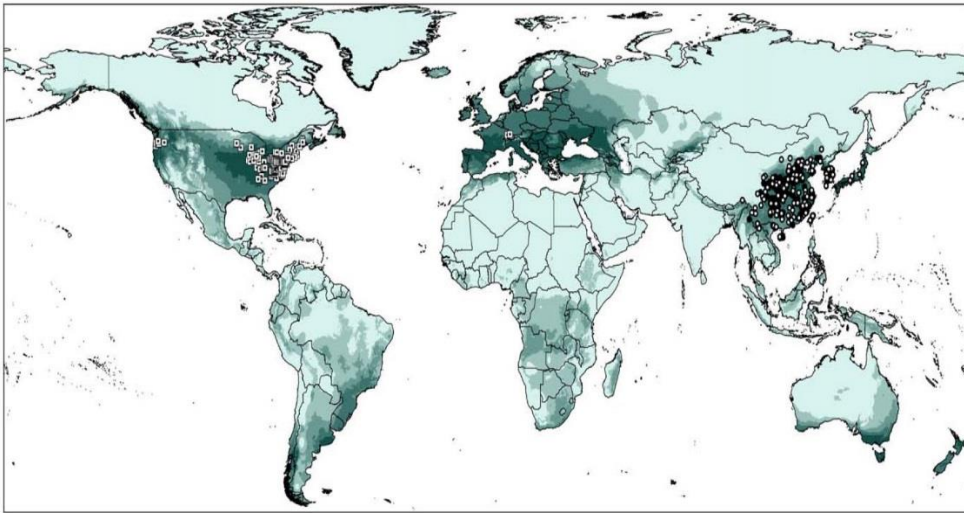
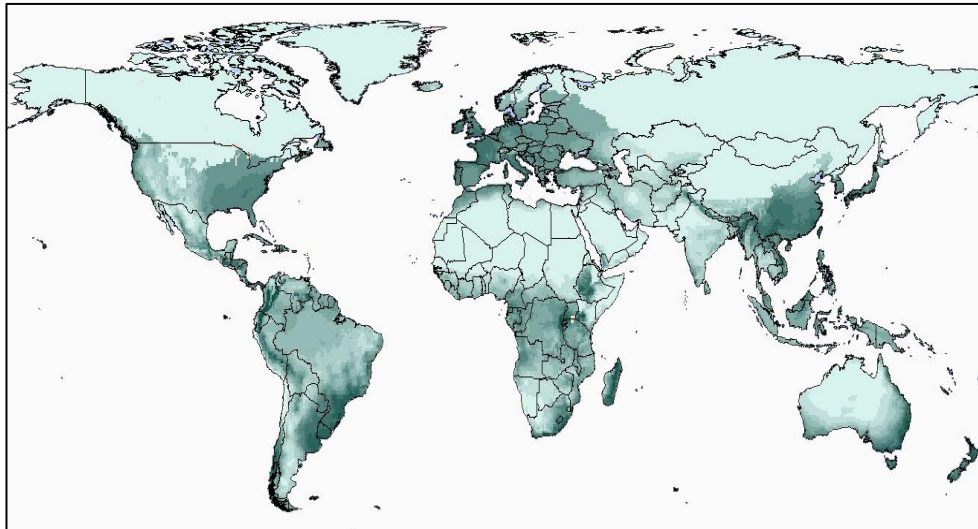
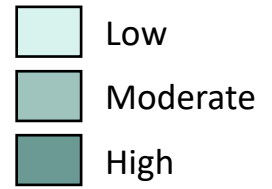


Fig. 4.2



Zhu et al. (2012)

Growth Potential



CLIMEX Ecoclimate Index (EI)

Fig. 4.3

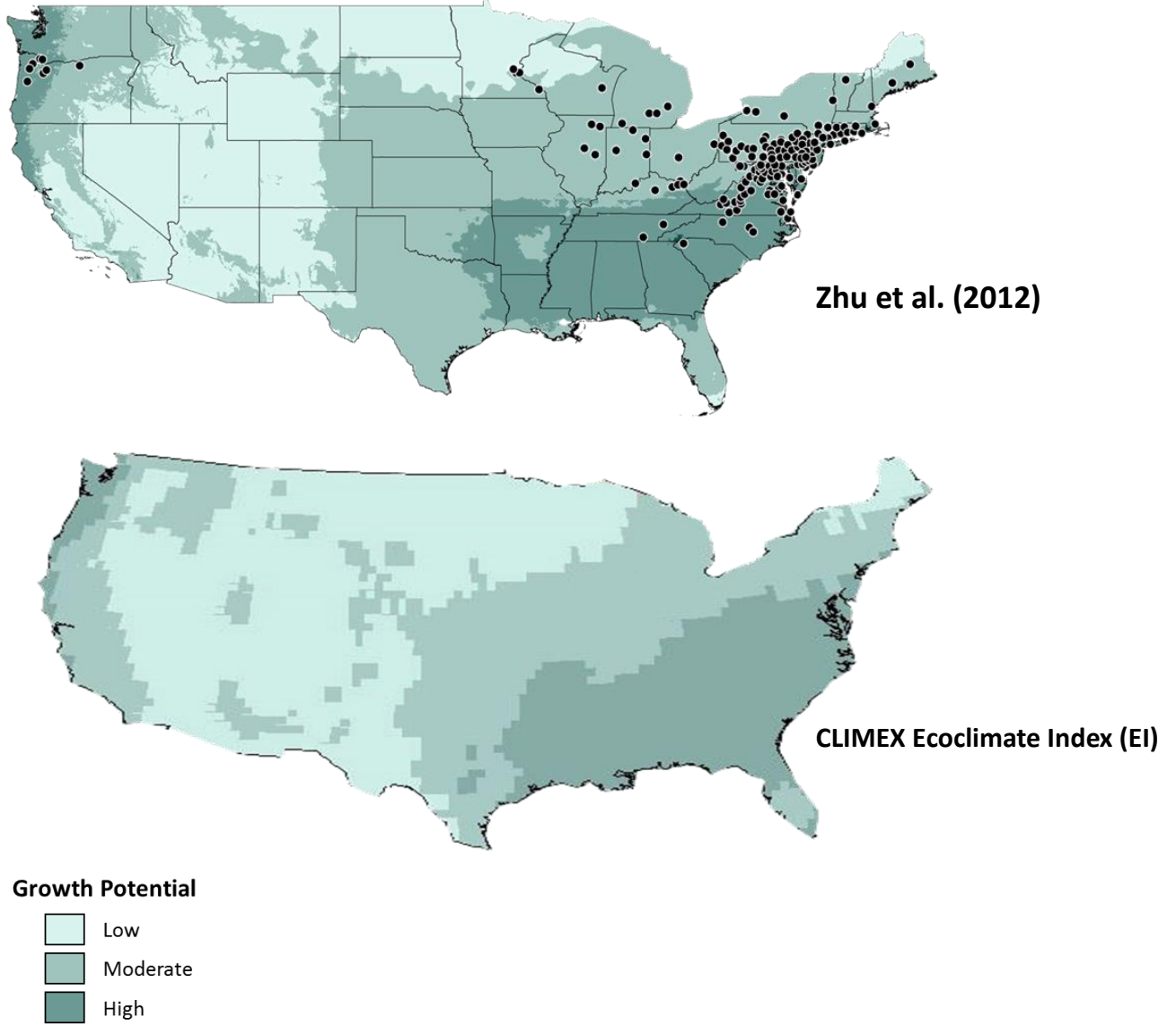
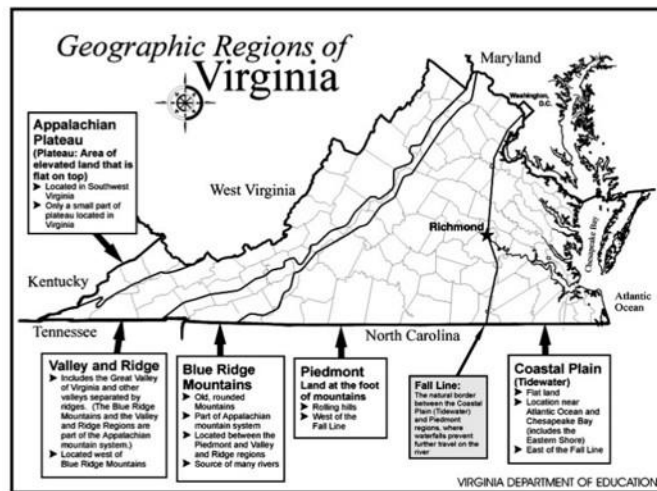
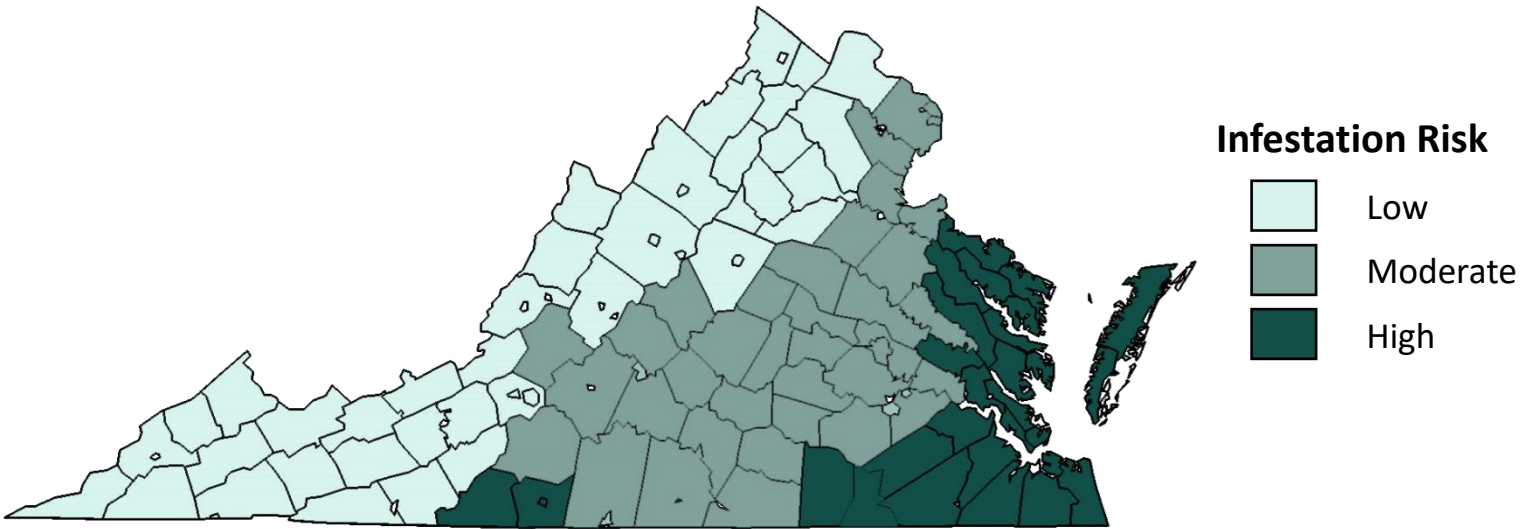


Fig. 4.4



Chapter Five

Summary

Brown marmorated stink bug (BMSB) has been observed on tree borders adjacent to soybean fields early in the season prior to its movement into soybean. To assess the influence of tree borders on BMSB infestations in soybean, twelve soybean fields in Orange and Madison Counties were sampled each week throughout the season. At each location, five 3-min visual counts of BMSB were made on tree of heaven (TOH) and other favorable host trees along a wooded border, on the adjacent soybean edge, 15 m into the soybean field, and 30 m into the field. Seasonal data showed a clear trend at all locations of BMSB populations increasing numbers on TOH wooded borders in July, then, gradually moving into adjacent soybean borders later in the summer. BMSB did not move far from the field edge, with approximately half as many bugs being present at 15 m into the field and very few being detected 30 m into the field. These results have implications for continued management in soybean using field border sprays, particularly on field edges adjacent to woods.

Both BMSB adults and nymphs have a strong ‘startle response’ and drop to the ground after very little disturbance, thus, making sampling difficult. This behavior could reduce the effectiveness of the traditional sweep net and ground cloth sampling methods. In 2013 and 2014, studies evaluated a visual plant inspection method in Virginia, Delaware and Maryland by comparing BSMB visual counts to the numbers caught using a sweep net. The visual inspection method consisted of counting the number of BMSB nymphs and adults seen on the plants in a 2-minute long inspection period while walking carefully down and between two rows. The ends of each sample area were marked with flags. After a 30-minute interval, which allowed the stink bugs to reposition in the canopy, each flagged area was sampled using 15 sweeps with a 38-cm

diameter sweep net. Regression analyses compared visual and sweep net counts by state/year and combined. Using these regressions and the current economic threshold for BSMB in Virginia of 5 stink bugs per 15 sweeps, indicated that overall, there is a close relationship between the numbers sampled by the two methods. The best result was obtained when data from all states and years were combined (76 fields, 2,042 paired comparisons). An average count of 5.4 BMSB observed in 2 minutes was equivalent to the threshold level of 5 BMSB per 15 sweeps ($Y = 0.984X + 0.4359$, $R^2=0.6934$). The 2-min visual inspection appears to be an effective method for assessing BMSB populations in soybean.

Knowing the potential distribution range of a pest can help growers anticipate the need for management before an economic impact occurs. The CLIMEX modeling software uses climatic and biological factors of species to predict the geographic risk for pest outbreaks. To determine the potential distribution of BMSB in Virginia a climate simulation model was run with CLIMEX. First, CLIMEX parameters were adjusted to correspond with a previously predicted host range distribution map. Once these maps closely resembled one another the focus area was reduced to include only Virginia. By increasing the number of weather stations in Virginia we were able to expand the weather database used by CLIMEX. Results suggest high growth potential in the coastal plains region of Virginia with one patch in the southwest piedmont region. The remaining piedmont region modeled a moderate growth potential while the ridge and valley region suggests low potential for growth. This does not compare well with previous work on the distribution range of BMSB. To develop a more accurate model, factors like resource availability and source population would need to be considered.