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Short communication

Comparison of Two Sampling Methods for Assessing *Halyomorpha halys* (Hemiptera: Pentatomidae) Numbers in Soybean Fields

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Abstract

Sampling soybean fields for the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), can be challenging. Both adults and nymphs have a "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, in Virginia, Delaware, and Maryland, we evaluated a visual plant inspection method that consisted of counting the number of brown marmorated stink bug nymphs and adults seen on soybean plants in a 2-min inspection period while walking carefully between two rows. After a 30-min interval, which allowed the stink bugs to reposition in the canopy, the area was resampled using 15 sweeps with a 38-cm-diameter sweep net. In total, 76 soybean fields and 2,042 paired comparisons were used to determine a strong linear relationship between sampling methods ($y = 0.984x + 0.4359$, $R^2 = 0.6934$, where $y =$ brown marmorated stink bugs/2-min visual count and $x =$ brown marmorated stink bugs/15 sweeps). An average visual count of 5.4 brown marmorated stink bugs in 2 min 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 brown marmorated stink bug populations in soybeans.

Key words: *Halyomorpha halys*, soybean, stink bug, sampling

Since its introduction to the United States in the late 1990s, the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), has become a well-known pest of many agricultural crops, including soybeans (*Glycine max*) (Nielsen et al. 2011, Owens et al. 2013, Rice et al. 2014). In some mid-Atlantic states, brown marmorated stink bug is now the predominant stink bug species that is found in soybeans (Nielsen et al. 2011).

Brown marmorated stink bug adults and nymphs, with the exception of first instars, which feed on egg chorion (Taylor et al. 2014), attack soybean pods and seeds (Owens et al. 2013). Brown marmorated stink bugs feed by inserting their piercing-sucking mouth parts, injecting plant digestive enzymes, and extracting the plant fluids (Peiffer and Felton 2014). Stink bug 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 de Azevedo 2002, Owens et al. 2013). In the field, brown marmorated stink bugs 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 pod (Cissel et al. 2015). Feeding by brown marmorated stink bugs causes delayed plant development, often referred to as "stay green" syndrome (Hill et al. 2006). As a result, portions of the field with heavy brown marmorated stink bug infestations, often on edges, remain green while the remainder of the field dries down, reaching the R8 (full maturity) stage. This can significantly affect harvesting and yield (Owens et al. 2013).

Integrated pest management programs rely upon accurate pest sampling to determine the potential for economic crop loss (Pedigo and Rice 2013). In soybeans, sweep net sampling has been a standard method for assessing stink bug and other pest populations in the United States since the 1970s (Kogan and Pitre 1980). Economic thresholds for stink bugs in soybean have been developed for different soybean plant densities and for different regions of the United States. Owens et al. (2013) concluded that damage done by brown marmorated stink bugs should be considered equivalent to that of native stink bug species. A threshold of approximately 5 stink bugs per 15 sweeps is recommended in many states (North Carolina

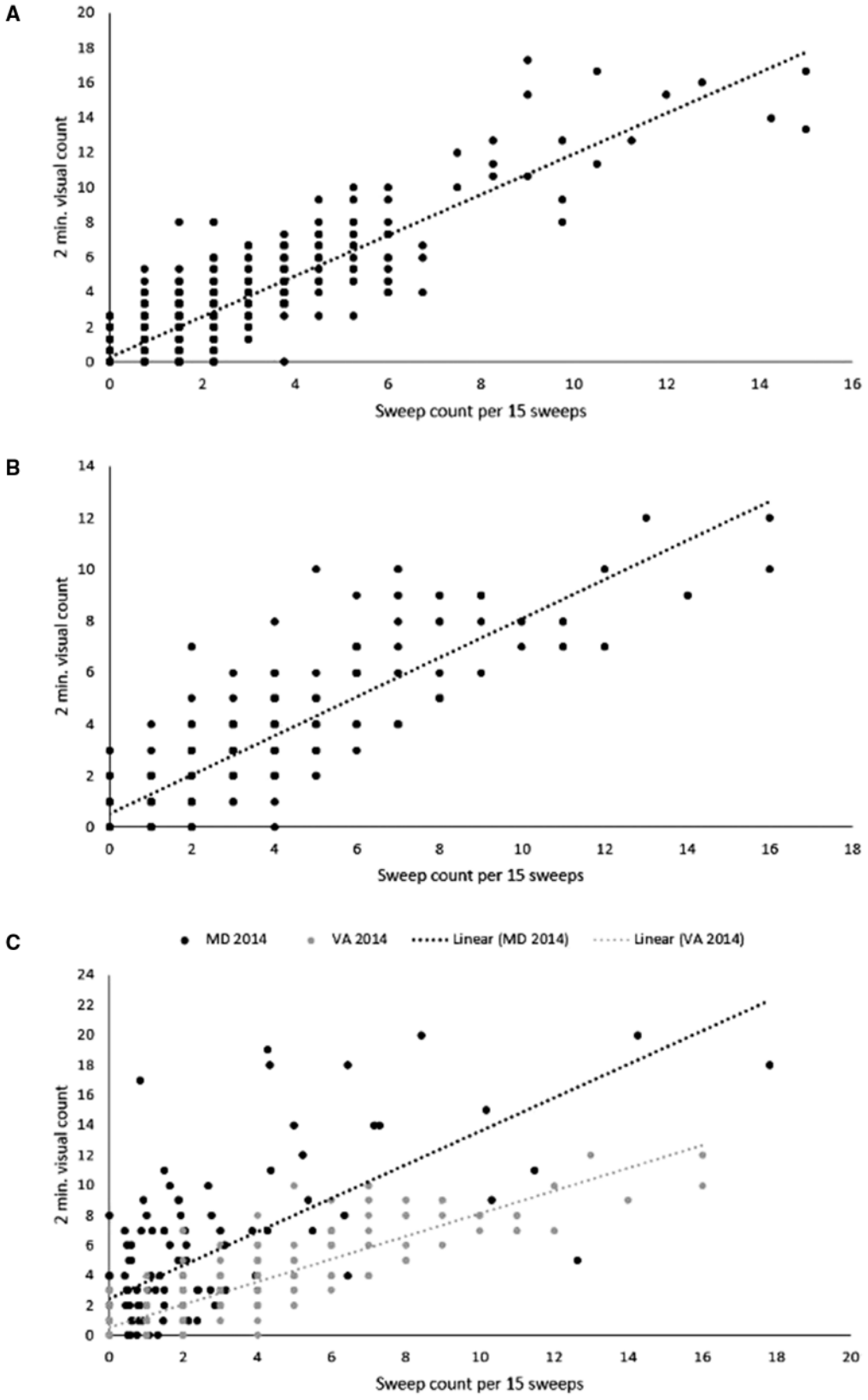

Fig. 1. Relationships between numbers of brown marmorated stink bug adults and nymphs observed in a 2-min visual plant assessment compared with the numbers caught in a 15-sweep net sample. (A) 1,431 paired sample comparisons in Virginia soybean fields in 2013; (B)135 paired sample comparisons in Maryland soybean fields in 2013; (C) 127 and 327 paired sample comparisons in 2014 in Maryland and Virginia, respectively; and (D) 2,042 paired comparisons for combined 2013 and 2014 data from Virginia, Maryland, and Delaware.

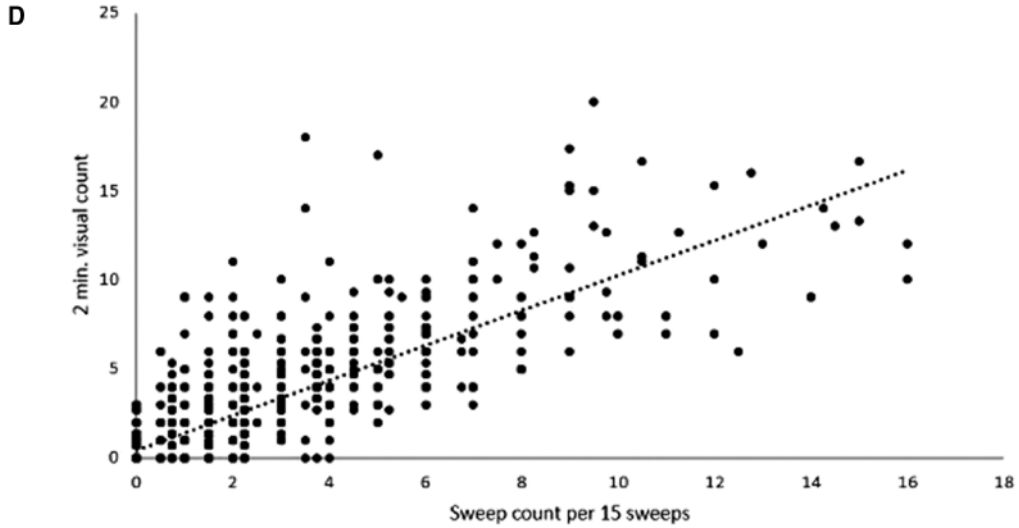

Fig. 1. Continued

Table 1. Regression statistics for the relationship between numbers of brown marmorated stink bug nymphs and adults per 2-min visual plant inspection and per 15 sweeps in soybean fields in Virginia, Maryland, and Delaware

Year	State	Paired comparisons	Fields sampled	Linear regression equation[a]	R^2	Predicted visual count when $x = 5$[b]
2013	VA	1,431	20	$y = 1.1661x + 0.2788$	0.80	6.1
	MD	135	2	$y = 2.0641x + 1.0919$	0.71	11.4
2014	VA	327	19	$y = 0.7566x + 0.5438$	0.75	4.3
	MD	127	2	$y = 1.1183x + 2.4263$	0.49	8.0
	DE	168	35	$y = 0.7065x + 0.3605$	0.16	3.9
2013 2014	VA, MD, DE	2,042	76	$y = 0.984x + 0.4359$	0.69	5.4

[a] x and y represent the number of brown marmorated stink bug adults and nymphs per 15 sweeps and per 2-min 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 per 15 sweeps.

Cooperative Extension 2013, Herbert 2015, Studebaker 2016). Sampling soybeans for brown marmorated stink bugs using sweep netting, however, has proven to be a challenge. Unlike native stink bug species such as *Euschistus* spp. and *Chinavia hilaris* Say, brown marmorated stink bug adults and nymphs have a strong "startle response" and drop to the ground when disturbed (Cissel et al. 2015). We evaluated a visual plant inspection method as an alternative and compared it with numbers of brown marmorated stink bugs caught using the standard sweep net method in 76 soybean fields in the mid-Atlantic United States.

Materials and Methods

In 2013 and 2014, sampling of brown marmorated stink bug adults and nymphs was conducted in 76 arbitrarily selected soybean fields in Virginia, Maryland, and Delaware (Table1). Fields were revisited and sampled from July through October. In all fields, plants were on narrow row spacing (38–46-cm centers), except in Maryland, where fields were on a 91.5-cm center (wide row).

In each field, brown marmorated stink bug was 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 2 min, viewing a 1-m-wide area parallel to the field edge, and counting all brown marmorated stink bugs that were observed. The beginning and end points of each sampling area (~6 m in length) were flagged to ensure that the same area would be used for the subsequent sweep net sampling. After a 30-min interval, which allowed the stink bugs to reposition in the plant canopy, the flagged area was sampled with 15 sweeps using a 38-cm sweep net by the "Lazy-8" method described by Kogan and Pitre (1980b). All brown marmorated stink bug 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 brown marmorated stink bug adults and nymphs observed during the 2-min visual inspection and sweep net sampling. Separate analyses were carried out for the data collected in soybean fields in Virginia, Maryland, and Delaware in each year, and for the combined data collected from Virginia, Maryland, and Delaware in both years. Pairs of data where both visual inspection and sweep net counts were zeroes were excluded from the analysis because they provided no quantitative comparison. All statistical analyses were carried out using JMP Pro 11.0 (SAS 2013).

Results and Discussion

A strong positive linear relationship in brown marmorated stink bug numbers for the 2-min visual and sweep net sampling was obtained for all comparisons ($r > 0.70$), except for data from Delaware ($r =$

0.39), where much lower densities of brown marmorated stink bugs were observed. Regression analysis of the 2-min visual brown marmorated stink bug count on numbers per 15 sweeps for Virginia and Maryland showed that for each year (2013 and 2014), the slopes of the fitted line for Maryland were greater (Table 1; Fig. 1B and C) than those for Virginia (Fig. 1A and C). The difference may be attributed to Maryland soybeans having very high densities of brown marmorated stink bugs on certain sample dates (>100 bugs per 2-min count) compared with a maximum of 10 bugs per 2-min count at any of the Virginia fields. In addition, Maryland soybean fields were also planted on wide-row (91.5 cm) spacing, which made it much easier to see stink bugs within the plant canopy when walking compared with the narrow-row (<46 cm) spacing in the Virginia and Delaware fields. Nonetheless, when data from all states and years were combined (76 fields, 2,042 paired comparisons), a highly significant linear relationship ($y = 0.984x + 0.4359$, $R^2 = 0.69$, $P < 0.05$; Table 1; Fig. 1D) was observed between the number of brown marmorated stink bugs per 2-min visual count (y) and the numbers per 15 sweeps (x).

Currently, many soybean-growing states have an economic threshold for stink bugs of 5 bugs per 15 sweeps (North Carolina Cooperative Extension 2013, Herbert 2015, Studebaker 2016). Using the linear relationship obtained for the combined data, an average visual count of 5.4 brown marmorated stink bugs per 2 min corresponds to the threshold of 5 brown marmorated stink bugs per 15 sweeps. Thus, there is roughly a 1:1 ratio in sample density between the two methods, making threshold translations simple.

In conclusion, the visual inspection method is an effective alternative method for determining the action threshold for brown marmorated stink bugs in soybeans, especially in fields with narrow row spacing. Numbers of brown marmorated stink bugs observed during a 2-min sample are roughly equivalent to the numbers caught from 15 sweeps of a net. Because the visual method does not require any equipment and is simple, it could encourage more soybean field sampling by growers and crop advisors and lead to more conscientious treatment decisions regarding stink bug management.

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Footnote: a. VA Counties sampled: Albemarle, Amelia, Appomattox, Bedford, Buckingham, Campbell, Cumberland, Fluvanna, Goochland, Green, Louisa, Madison, Nottoway, Orange, Powhatan, Prince Edward, and Franklin. MD Counties sampled: Howard and Washington. DE Counties sampled: New Castle, Kent, and Sussex.

References Cited

Cissel, B., J. Whalen, G. Dively, C. Hooks, T. Patton, P. D. Venugopal, B. Aigner, J. Hogue, A. Herbert, T. Kuhar, et al. 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. For. 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.

Herbert, D. A. 2015. Pest management guide: field crops 2015. Virginia Coop. Ext. Pub. No. 456-016. (https://pubs.ext.vt.edu/456/456-016/Section04-Insects-1.pdf)

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.

Kogan, M., and H. N. Pitre. 1980. General sampling methods for aboveground population of soybean arthropods, pp. 30–60. *In* M. Kogan and D. C. Herzog (eds.), Sampling methods in soybean entomology. Springer-Verlag, New York, NY.

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: 231–238.

North Carolina Cooperative Extension 2013. Thresholds: Soybeans. Online publication. (http://soybeans.ces.ncsu.edu/thresholds/, accessed 1 October 2016).

Owens, D. R., D. A. Herbert, Jr., G. P. Dively, D. D. Reisig, and T. P. Kuhar. 2013. Does feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) reduce soybean quality and yield? J. Econ. Entomol. 106: 1317–1323.

Pedigo, L. P., and M. E. Rice. 2013. Economic decision levels for pest populations, pp. 255–285. *In* L. P. Pedigo and M. E. Rice (eds.), Entomology and pest management, 6th Edn. 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: 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, et al. 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). J. Integr. Pest Manage. 5: 1–13.

Studebaker, G. 2016. 2016 Insecticide Recommendations for Arkansas. University of Arkansas Division of Agriculture Research and Extension Pub. No. MP144. Online publication. (https://www.uaex.edu/publications/pdf/mp144/mp144.pdf, accessed 1 October 2016).

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.

Zar, J. H. 1984. Comparing simple linear regression equations, pp. 292–305. *In* Biostatistical Analysis, 2nd Edn, Prentice Hall, Englewood Cliffs, NJ.