

Edge Effects Influence the Abundance of the Invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) in Woody Plant Nurseries

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ABSTRACT The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), has caused severe economic losses in the United States and is also a major nuisance pest invading homes. In diverse woody plant nurseries, favored host plants may be attacked at different times of the season and in different locations in the field. Knowledge of factors influencing *H. halys* abundance and simple methods to predict where *H. halys* are found and cause damage are needed to develop effective management strategies. In this study, we examined *H. halys* abundance on plants in tree nurseries as a function of distance from field edges (edge and core samples) and documented the abundance in tree nurseries adjoining different habitat types (corn, soybean, residential areas, and production sod). We conducted timed counts for *H. halys* on 2,016 individual trees belonging to 146 unique woody plant cultivars at two commercial tree nurseries in Maryland. Across three years of sampling, we found that *H. halys* nymphs and adults were more abundant at field edges (0–5 m from edges) than in the core of fields (15–20 m from edges). Proximity of soybean fields was associated with high nymph and adult abundance. Results indicate that monitoring efforts and intervention tactics for this invasive pest could be restricted to field edges, especially those close to soybean fields. We show clearly that spatial factors, especially distance from edge, strongly influence *H. halys* abundance in nurseries. This information may greatly simplify the development of any future management strategies.

KEY WORDS adjacent habitat, edge effect, invasive species, brown marmorated stink bug, tree nursery

The brown marmorated stink bug [*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)], is a highly invasive, polyphagous herbivore, feeding on at least 169 known species of plants in its introduced range (Hoebeke and Carter 2003, Bergmann et al. 2014, Rice et al. 2014). First detected in Allentown, PA, in the mid-1990s, *H. halys* has become a widespread pest in the mid-Atlantic and has established populations in at least 41 states and the District of Columbia (Leskey et al. 2012a, Hoebeke and Carter 2003, CAB International [CABI] 2014, Rice et al. 2014). *H. halys* is a pest of many field and specialty crops, including soybean, field and sweet corn, orchard crops, vegetables, and woody and herbaceous ornamentals (Nielsen and Hamilton 2009, Kuhar et al. 2012, Leskey et al. 2012b, Lee et al. 2013, Venugopal et al. 2014). *H. halys* has been reported from woody plant nurseries in the mid-Atlantic states since 2010, where it has attained high densities on a wide variety of trees and shrubs (Martinson et al. 2013, Bergmann et al. 2014, E.J.B. unpublished data). Over the past 5 yr, *H. halys* has damaged crops and caused serious economic losses in various cropping

especially in the mid-Atlantic United States (Leskey et al. 2012a, CABI 2014, Rice et al. 2014).

Large populations of *H. halys* are a concern to managers of ornamental plants in nurseries and landscapes because of potential for direct damage, indirect damage, and nuisance problems. On ornamental plants, *H. halys* may cause direct feeding damage by distorting and discoloring fruits and causing discolored foliage and wet spots on trunks of trees at feeding sites (Leskey et al. 2012a, Martinson et al. 2013). Indirect damage may result if *H. halys* transmits plant diseases, as has been reported in Asia (Hoebeke and Carter 2003, Leskey et al. 2012a, Lee et al. 2013). Finally, *H. halys* overwinters in urban landscapes and structures, leading to significant problems as a nuisance pest (Watanabe et al. 1994, Hoebeke and Carter 2003, Inkley 2012). The nuisance potential would certainly increase in landscapes where *H. halys* populations build up on favored cultivars of ornamentals.

Like many stink bugs, *H. halys* preferentially feed on reproductive structures of plants, though they can be found feeding on leaves and stems as well (Hoebeke and Carter 2003, Rice et al. 2014). Upon emergence from overwintering, these pests colonize preferred tree hosts and agricultural fields at the beginning of the growing season, and they move among habitats as different crops reach preferred phenological stages

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(Leskey et al. 2012a, Lee et al. 2013). Such movement among habitats will be constrained by dispersal distance and the relative suitability of plants on each side of a habitat border (Kennedy and Margolies 1985, Ekbom et al. 2000, Kennedy and Storer 2000). For ornamental plant nurseries, the abundance and potential damage caused by *H. halys* may be contingent on the type and proximity of adjacent habitat and its potential as a source of *H. halys*.

The distribution of stink bugs in a field can be strongly influenced by a distinct edge effect whereby abundance is highest at the immediate edge of a given habitat type and tapers off quickly from the edge. This edge-mediated abundance is reported in various cropping systems for many stink bug species in the United States, including *H. halys* (Toscano and Stern 1976; Tillman et al. 2009; Pease and Zalom 2010; Reay-Jones 2010; Reeves et al. 2010; Tillman 2010, 2011; CABI 2014; Venugopal et al. 2014).

As *H. halys* move both within and among crops throughout the season (Leskey et al. 2012a, CABI 2014, Rice et al. 2014), assessments of the infestation patterns of *H. halys* populations invading tree nurseries are needed. Whereas much effort over the past 5 yr has emphasized *H. halys* dynamics within tree fruit, vegetables, and field crops (Leskey et al. 2012b, Kuhar et al. 2012, Pfeiffer et al. 2012, Brennan et al. 2013, Basnet et al. 2014, Venugopal et al. 2014), knowledge of colonization of woody plant nurseries remains unknown. Nurseries present unique challenges to understanding and managing this highly polyphagous pest, as production fields in nurseries typically encompass vastly higher plant diversity than other agricultural crops. Thus, efforts aimed at predicting where *H. halys* may be present in nurseries based on edge effects and adjacent habitat may greatly simplify the development of management strategies in these diverse systems.

The primary objective of this study was to determine whether *H. halys* abundance in woody plant nurseries differed based on the distance from the edge of the nursery to adjacent habitats. We hypothesized greater abundance at the edge of the nursery than further into the planting blocks (hereafter, the core). As the abundance of *H. halys* in nurseries may depend on the type of adjacent habitat (Venugopal et al. 2014; Blaauw et al. 2014), we also provide preliminary comparisons of *H. halys* abundance on trees in woody plant nursery fields adjacent to production sod, soybean, corn, and low-density residential land.

Materials and Methods

Study Design and Data Collection. This study was conducted over 3 yr in two commercial woody plant nurseries located in Frederick and Montgomery Counties, MD. For each nursery, timed visual surveys of *H. halys* life stages were recorded over a discrete observation period each year on trees at the edges and in the core of production fields. Production fields were located adjacent to four types of habitat: low-density residential, production sod, field corn, and soybeans.

Table 1. Number of nursery trees sampled at different distances from field edges (edge [0–5 m] or core [15–20 m]) and adjacent habitats from 2011–2013

Year	Site	Edge	Core	Adjacent habitat	Cultivars
2011	Raemelon	96	99	Sod	37
2011	Raemelon	277	260	Soy	77
2012	Raemelon	147	147	Corn	42
2012	Ruppert location 1	87	87	Corn	11
2012	Ruppert location 2	333	333	Residential	38
2013	Raemelon	204	204	Soy	52
2013	Ruppert location 1	108	75	Soy	13
2013	Ruppert location 2	336	333	Residential	39
Totals ^a :		1,588	1,538		146 unique

Sites included Raemelon Farm in Adamstown, MD, and two locations at Ruppert Nurseries in Laytonsville, MD.

^a Total number of trees visited includes some trees visited in multiple years.

In 2011, surveys were conducted at Raemelon Farm in Adamstown in western MD (39.299813 latitude; –77.478700 longitude). In 2012 and 2013, surveys were conducted at both Raemelon Farm and two sites at Ruppert Nurseries in Laytonsville in central MD (39.212633; 77.142759; also see Table 1 for sampling details). Production fields at Ruppert Nurseries consisted of 20 rows of 25–35 ornamental trees and shrubs. Fields at Raemelon were larger, consisting of 80–150 rows. At both nurseries, rows were predominantly spaced 3 m apart, depending on the size of the plant, which ranged in height from 1 to 4 m. A stratified sampling scheme was followed in which six trees in each row were surveyed, three at the edge and three in the core, and each individual tree were the unit of measurement or replicates. “Edge” trees were located within 5 m of the field edge (tree positions 1–3), and “core” trees were located beyond 15 m from the edge (tree positions 9–11; Fig. 1). Table 1 also provides details on the number of trees (replicates) sampled from the different adjacent habitats, fields, and rows. These nurseries were planted with a wide diversity of trees, and this stratified sampling scheme was designed to minimize any biases associated with differences in cultivar attractiveness to *H. halys*, which will be reported elsewhere (E.J.B. unpublished data). Specifically, single cultivars were typically planted within a row, but fields differed in cultivar composition. Thus, favored cultivars, among the 146 unique cultivars we sampled, may have been present but were equally sampled at edges and core (see Table 1), and the adjacent habitat effect was documented across fields differing in composition.

For each tree, 1-min visual counts of *H. halys* abundance were conducted. This visual search included foliage, flowers, fruits/seeds, and bark to a height of up to 3 m. To ensure uniformity and consistency in the field protocols for data collection, the project directors (E.J.B. and M.J.R.) trained all the field personnel. *H. halys* abundance was recorded separately for three life stages: egg masses, nymphs, and adults. Each year, counts were conducted in early June, late June, mid-July, and early August. Abundance for each life stage

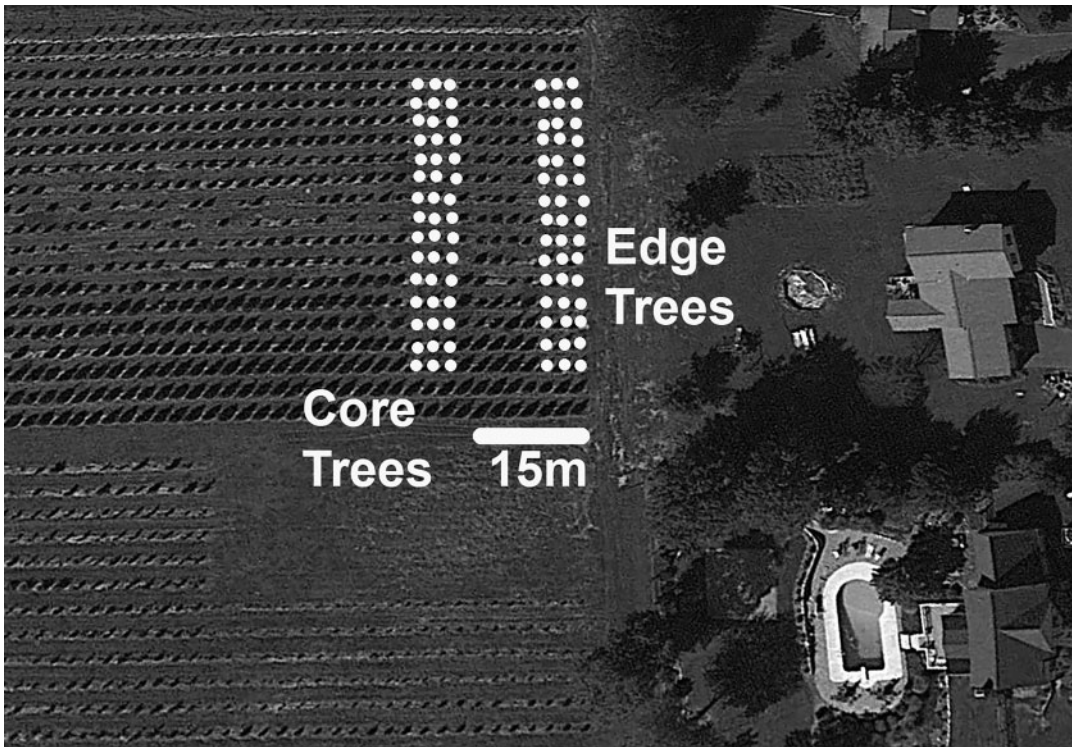


Fig. 1. A schematic representation of sampling strategy depicting the location of edge and core trees (white circles) within a tree nursery adjacent to residential areas. Image from Google Earth.

was summed within seasons for analysis; trees with missing data for any of the four visits within a year were omitted from the analysis.

Statistical Analysis. The influence of distance from field edge on the abundance of *H. halys* was analyzed by generalized linear mixed models (GLMMs) based on Laplace approximation, with a Poisson-lognormal error distribution and log link function (Elston et al. 2001, Bolker et al. 2009).

Model building and model selection for the mixed effects modeling followed the procedures suggested by Zuur et al. (2009). First, several candidate models, each with different combinations of random effects (site, year, site within year, and adjacent habitat), but identical fixed effect (distance from edge), were tested to choose the optimal random effect model using a combination of Akaike information criteria and Bayesian information criteria values for selection criteria (Pinheiro and Bates 2000). For GLMM analyzing the adult dataset, study sites sampled nested with each study year, the adjacent habitats, and a variable to account for overdispersion (see Bolker et al. 2009) were chosen as random effects in the final model. For GLMM analysis of both nymphs and egg masses, only the variable accounting for overdispersion was chosen in the final model. The summed count of *H. halys* observed at an individual tree within a season (summed over four visits to each tree) was used as the response variable. Analyses to determine the influence of distance from edge (two levels: edge and core) on

H. halys abundance were performed on three separate datasets: egg masses, nymphs, and adults. For the adult dataset, we further compared the abundance among each of the sampled trees (tree positions from the nursery edge: 1, 2, 3, 9, 10, and 11) using a model with log-transformed tree position as a fixed effect to test an exponential decline model. For each of these analyses, the statistical significance of the fixed effects was determined by Wald chi-square tests and post hoc model-estimated means comparisons were performed using Tukey's honestly significant difference (HSD) test. Models were evaluated for assumption appropriateness by testing for over-dispersion and correlations among random effect terms, and by visualizing variances in a location-scale plot with superimposed loess fit (Bolker et al. 2009).

Trees included in this study were located in nursery fields adjacent to four types of habitat: low-density residential, production sod, field corn, and soybeans. We provide a graphical comparison of overall *H. halys* abundance (nymphs and adults together) on nursery trees adjacent to these habitats. Detailed statistical analysis comparing abundance among adjacent habitats was not performed because of limited availability of spatial and temporal replications for adjacent habitats.

All statistical analyses were performed in R program (version 3.1.1; R Development Core Team 2014, Vienna, Austria) and associated statistical packages. GLMMs were performed with package "lme4" (Bates et al. 2014), significance tests with package "car"

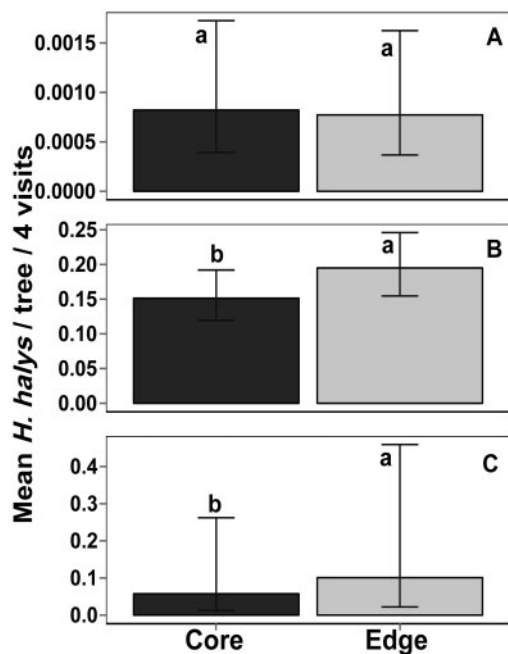


Fig. 2. Cumulative mean nymph *H. halys* abundance in tree nurseries in central and western Maryland, in relation to distance from the field edge. Estimates derived from Poisson-lognormal GLMMs are compared among distance from edge for three different datasets: Egg masses (A), nymphs (B), and adults (C). Values presented here have been back-transformed from the original log link function estimated model coefficients. Vertical lines represent upper and lower 95% CIs and are, hence, not symmetrical around the estimated means. For each set of comparisons, categories with the same letter above them are not statistically different ($\alpha = 0.05$; Tukey's HSD).

(Fox and Wiesberg 2011), and the multiple comparisons of means were computed with the package “multcomp” (Hothorn et al. 2008). GLMM estimated coefficients were extracted through package “effects” (Fox 2003) and plotted using “ggplot2” (Wickham 2009).

Results

Over the 3-yr study period, 222 egg masses and 15,229 stink bugs were observed. More nymphs than adults were observed, with nymphs comprising 65.0% of the *H. halys* population. The maximum number of egg masses, nymphs, and adults recorded (summed across the timed counts within a season) from an individual tree was 6, 100, and 164, respectively.

There was not a significant influence of edge distance on the abundance of *H. halys* egg masses (Wald $\chi^2 = 0.38$; $df = 1$; $P = 0.85$; Fig. 2A). The abundance of *H. halys* nymphs was significantly influenced by distance from edge (Wald $\chi^2 = 4.139$; $df = 1$; $P = 0.041$), with higher abundance at the field edge than in the core (Fig. 2B).

The abundance of *H. halys* adults was significantly influenced by distance from edge (Wald $\chi^2 = 51.52$;

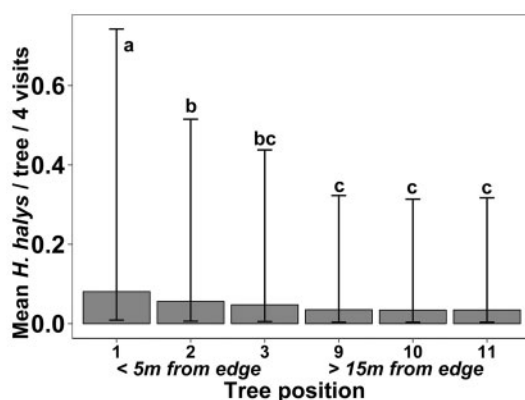


Fig. 3. Cumulative mean adult *H. halys* abundance in tree nurseries in central and western Maryland, in relation to distance from the field edge. Estimates derived from Poisson-lognormal GLMM are compared among each tree position, representing various distances from the field edge. Values presented here have been back-transformed from the original log link function estimated model coefficients. Vertical lines represent upper and lower 95% CIs and, hence, are not symmetrical around the estimated means. For any given comparison, categories with the same letter above them are not statistically different ($\alpha = 0.05$; Tukey's HSD).

$df = 1$; $P < 0.001$), with higher abundance at the field edge than in the core (Fig. 2C). Particularly, adult abundance was highest at the tree closest to the edge (tree position 1) and significantly greater than other tree positions 2, 3, 9, 10, and 11 (Fig. 3; Tukey's HSD, $P < 0.05$), declining with tree position into the field. Adult *H. halys* abundance was not significantly different among trees at positions 3, 9, 10, and 11 (Fig. 3).

Preliminary comparison of abundance among adjacent habitats revealed the highest abundance of *H. halys* (nymphs + adults) in nursery trees abutting soybean fields, with up to three times more active stages than production sod, field corn, or residential areas (Fig. 4).

Discussion

Overall, our results demonstrate a clear dependence of *H. halys* abundance on the location of trees within a nursery. This has strong implications for management of this pest. First, if growers are concerned about *H. halys* at their nursery, they should focus monitoring efforts along the perimeters of their fields. Searching trees in the core of the nursery will be a poor use of time. The timely detection of adults colonizing fields and subsequent monitoring for nymphs that are highly susceptible to insecticide sprays (Bergmann and Raupp 2014 and references therein) can guide the timing of insecticidal intervention. Most important is the finding that *H. halys* concentrate in a narrow band at the edges of fields. This provides growers an opportunity to greatly reduce pesticide use by targeting trees and shrubs in boarder rows and at row edges. Consequently, the pesticide use may be dramatically reduced with the added benefit of conserving natural enemies

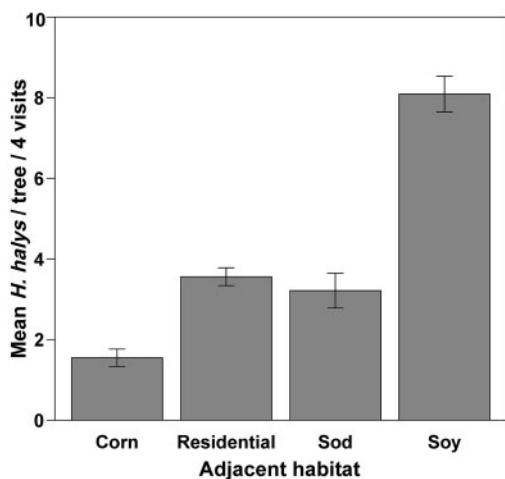


Fig. 4. Raw means of overall stink bug abundance (nymphs and adults) among different habitats adjacent to the tree nursery. The vertical lines represent the standard error around the means.

found in the core of the nursery. The edge effects exhibited by *H. halys* in tree nurseries in our study conform to earlier reports of *H. halys* in other cropping systems in the United States, including fruit and field crops (Leskey et al. 2012a, Rice et al. 2014, Venugopal et al. 2014) as well as for other native stink bug species (Toscano and Stern 1976; Tillman et al. 2009; Pease and Zalom 2010; Reay-Jones 2010; Reeves et al. 2010; Tillman 2010, 2011).

As with findings of other studies on stink bugs, our preliminary results also identify the association between adjacent cultivated crops (mainly soybean) and high stink bug populations. Notably, the abundance of *H. halys* in nursery trees adjacent to soybeans was three times the abundance observed on trees adjacent to corn, soy, and residential habitats (Fig. 4). Previous reports show adjacent fields of alfalfa, field corn, and other cultivated borders as a source contributing to higher densities of stink bugs in tomato, cotton, sorghum, and peanut fields (Toscano and Stern 1976, Toews and Shurley 2009, Reeves et al. 2010, Tillman 2011). Adjacent habitat, particularly wooded areas and other crops, are associated with high *H. halys* abundance in fruit and field crops in the mid-Atlantic region (Leskey et al. 2012a, Rice et al. 2014, Venugopal et al. 2014). Soybean is a preferred host for *H. halys* (Lee et al. 2013, Rice et al. 2014) and stink bugs are considered important pests in soybean production areas of the world (Panizzi and Slansky 1985).

A constraint faced during our study was the lack of availability of replicate adjacent habitats in our nurseries. This lack of spatial replication precluded us from using formal statistical tests to compare *H. halys* abundance among the adjacent habitats. We suggest that future sampling efforts in woody plant nurseries focus on additional land use types, especially woods, and include replicate fields of tree nurseries abutting different habitats. On the other hand, the patterns we describe here in relation to edge effects should be robust, as our

sampling effort spanned 3 yr, several sites, 146 cultivars of woody plants, and 2,016 individual trees.

Together, the influence of both distance from edge and adjacent habitat indicates that monitoring efforts for *H. halys* in nurseries can be prioritized at nursery edges bordered by field crops, and we would additionally recommend scouting efforts adjacent to woodlots based on previous research in other cropping systems (Leskey et al. 2012a, Rice et al. 2014, Venugopal et al. 2014, Blaauw et al. in press). Where edge effects are well-documented, management interventions including pesticide applications can likewise be spatially targeted. In a pilot study in soybeans, for example, insecticide applications limited only to the field edges (0–12 m into the field) reduced subsequent *H. halys* colonization of fields and required 85–95% less insecticide compared with full-field sprays (Leskey et al. 2012a, Rice et al. 2014).

Efforts are ongoing to determine whether direct feeding by *H. halys* through the bark damages underlying tissues. We are also investigating if the impact of feeding on fruit and leaves of common nursery plants results in reduced growth and loss of aesthetic quality that translate into economic losses for the grower. While strategies for *H. halys* management in tree nurseries are currently being developed, when insecticidal treatment for *H. halys* in nurseries are necessary, edge-only applications should be sufficient for effective control.

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