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Source: Florida Entomologist, 102(1) : 222-226

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.102.0136

Potential of *Melia azedarach* L. (Meliaceae) as a host plant for *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)

P. Glynn Tillman[1,], Ted E. Cottrell[2], and G. David Buntin[3]*

Abstract

Currently, the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is considered an agricultural and nuisance pest in Georgia. The invasive chinaberry tree, *Melia azedarach* L. (Meliaceae), commonly grows in dense thickets along roadsides, and in woodlands adjacent to agricultural crops across the southeastern USA. Thus, the objective of this study was to determine the potential of *M. azedarach* to serve as a host plant of *H. halys* by examining mortality and feeding of first and second instars on *M. azedarach* leaves vs. carrot (i.e., a control diet), and documenting presence of *H. halys* on *M. azedarach* in woodlands at 2 locations in Georgia where this stink bug has become established. Over all sampling dates and locations, the number of *H. halys* in chinaberry was very low (0.1 per tree), and only 3 late instars and 1 adult were observed feeding on *M. azedarach* at 1 field site late in the season. Percentage feeding by second instars of *H. halys* was lower for individuals given *M. azedarach* leaves vs. those provided with carrot, most likely indicating that compounds in *M. azedarach* have an antifeeding effect. In fact, mortality for second instars on *M. azedarach* leaves was very high, and thus we conclude that *M. azedarach* is an unsuitable host plant for *H. halys*.

Key Words: brown marmorated stink bug; chinaberry; antifeedant

Resumen

Actualmente, se considera el chinche invasor hediondo marmorino café, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), como una plaga agrícola y molesta en el estado de Georgia. El árbol de melio invasor, *Melia azedarach* L. (Meliaceae), comúnmente crece en matorrales densos a lo largo de los caminos y en los bosques adyacentes a cultivos agrícolas en todo el sureste de los EE. UU. Por lo tanto, el objetivo de este estudio fue determinar el potencial de *M. azedarach* para servir como una planta hospedera de *H. halys* mediante el examen de la mortalidad y la alimentación del primer y segundo estadios de *H. halys* sobre hojas de *M. azedarach* vs. zanahoria (una dieta de control) y documentando la presencia de *H. halys* sobre *M. azedarach* en bosques en 2 ubicaciones en Georgia donde se ha establecido este chinche hedionda. En todas las fechas y ubicaciones de muestreo, el número de *H. halys* sobre melio fue muy bajo (0,1 por árbol), y solo se observaron 3 estadios tardíos y 1 adulto alimentándose de *M. azedarach* en 1 sitio de campo al final de la temporada. El porcentaje de alimentación en los segundos estadios de *H. halys* fue más bajo para los individuos que recibieron hojas de *M. azedarach* en comparación con los que recibieron zanahoria, lo que probablemente indica que los compuestos en *M. azedarach* tienen un efecto antiagénico. De hecho, la mortalidad por segundos estadios en hojas de *M. azedarach* fue muy alta, y por lo tanto concluimos que *M. azedarach* es una planta hospedera inadecuada para *H. halys*.

Palabras Clave: chinche hediondo marmorino café; melio; anti-alimentación

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is native to China, Taiwan, Korea, and Japan. Unfortunately, this invasive insect pest has spread to the USA, Canada, Switzerland, and Europe (Lee et al. 2013). It is both an urban nuisance, due to its propensity for overwintering in structures (Inkley 2012), and a serious economic pest of orchard crops, including apples and peaches, row crops such as field corn and soybeans, and vegetable crops, including sweet corn, peppers, eggplant, and tomatoes (Leskey et al. 2012a, b; Rice et al. 2014). The first known *H. halys* populations in the USA were reported in 1996 from Allentown, Pennsylvania, USA. To date, *H. halys* is present in 44 states and 4 Canadian providences (Bergmann et al. 2018).

In Georgia, urban pest management professionals began reporting overwintering *H. halys* aggregations in the metropolitan Atlanta area in 2011. In late Aug 2014, *H. halys* adults and nymphs were collected from a pheromone-baited stink bug trap at the University of Georgia Horticulture Farm near Watkinsville, Georgia, USA (Tillman et al. 2017). When the immediate area was searched, *H. halys* nymphs and adults were found on catalpa, pecan, and ornamental hibiscus. In the summer of 2015, *H. halys* was collected feeding and reproducing on soybean in Pike County, Georgia (Piedmont region), and on peaches and pecan in Peach County, Georgia (Coastal Plain region). Currently, *H. halys* is considered an agricultural and nuisance pest in this state (Bergmann et al. 2018).

The chinaberry tree, *Melia azedarach* L. (Meliaceae), is a deciduous tree that is native to southeast Asia and northern Australia (Swearingen & Bargeron 2016). The plant was introduced in the mid-1800s as an ornamental in the United States. It invades disturbed areas, and in Georgia, it commonly grows in dense thickets along roadsides and in woodlands adjacent to agricultural crops. Leaf and fruit extracts of *M. azedarach* elicit a variety of effects in insects, including antifeedant, growth retardation, reduced fecundity, molting disorder, and mortality

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(Ascher et al. 1995; Schmidt et al. 1998; Abou-Fakhr Hammad et al. 2001; Defagó et al. 2009). Extracts from *M. azedarach* have been suggested for use in integrated pest management (IPM) programs due to selectivity against parasitoids and predators of insect pests (Jazzar & Abou-Fakhr Hammad 2003; Abou-Fakhr Hammad & McAuslane 2006; Defagó et al. 2011).

Over 100 host plants have been reported for *H. halys* (Bergmann et al. 2018), but *M. azedarach* currently is not considered a host plant of this pest. With the expansion of *H. halys* into Georgia, there is concern that chinaberry will become a host plant for this invasive insect pest. Therefore, the objective of this study was to determine the potential of chinaberry to be a host plant of *H. halys*. Specifically, we examined feeding behavior and mortality of first and second instars on *M. azedarach* leaves and carrots in no-choice tests, and determined levels of *H. halys* on chinaberry in an orchard and a field crop site in Georgia locations where this stink bug has become established.

Materials and Methods

For a plant species to be a reproductive host plant, stink bugs must be able to develop successfully on the plant. Therefore, no-choice feeding tests were conducted in the laboratory to determine the mortality of first and second instar *H. halys* feeding on leaves of *M. azedarach*. The 2 treatments were *M. azedarach* leaves and a carrot slice (i.e., a control diet, a known suitable host). For the *M. azedarach* treatment, stems collected from insecticide-free chinaberry were washed gently in a 10% solution of dish detergent and water, rinsed in water, and patted dry with a paper towel. Leaflets were cut from the 10.2 cm tip a chinaberry stem; the bottom 2 sets of leaflets were removed. Then the leaflets were inserted into a hole in the lid of a 1.5 ml microcentrifuge tube filled with deionized water. Parafilm was placed around the stem at the hole in the lid to prevent water from leaking from the tube. The leaflets in the tube were placed on filter paper in the bottom of a 150 ´ 25 mm Petri dish. Deionized water was replenished every 2 to 3 d. For the carrot treatment, a slice of carrot obtained from a grocery store, approximately 3 cm^2 in size was placed in the bottom of Petri the same size as above. For each treatment, a *H. halys* egg mass was placed on the food source in a Petri dish just before egg hatch. Ten samples of each treatment were used for each of 4 blocks (replicates). The number of dead nymphs and the number of nymphs observed feeding on *M. azedarach* leaves and carrots were determined for each Petri dish daily. Feeding (i.e., proboscis inserted into food source) by first and second instars was observed using a dissecting microscope. Feeding and mortality data for first instars were taken the d they hatched from eggs (beginning 4–6 h after hatching), and then every d for a total of 7 d. For second instars, these data were taken from the d they ecdysed (observed exuviae) into second instars (4–6 h after ecdysis) and then every d for a total of 8 d.

The field study was conducted at the 83 ha University of Georgia Bledsoe Research Farm (33.1774°N, 84.4076°W) in Pike County, and the 486 ha USDA, ARS, Southeastern Fruit & Tree Nut Research Laboratory (32.3910°N, 83.4321°W) in Peach County, in 2018. Wheat, corn, soybean, and sorghum were grown at the Bledsoe Research site, and peach, plum, and pecan were grown at the Fruit & Tree Nut Research site. Twenty *M. azedarach* trees were sampled at each site. The entire plant was visually examined for stink bugs. When necessary, a 1.5 m hooked cattle show stick (Valley Vet Supply, Marysville, Kansas, USA) was used to gently pull down a branch within reach to check for stink bugs. Species and developmental stages of stink bugs were identified and recorded in the field using a HP iPAQ pocket personal computer (Hewlett-Packard Co., Palo Alto, California, USA). At the Bledsoe site, chinaberry was sampled on 7 and 21 Aug, and 4 Sep. At the Byron site, this shrub was sampled on 20 Jul, 17 and 31 Aug, and 7 Sep. Stink bug behavior (i.e., sitting, walking, or feeding) and plant part (i.e., leaf, stem, or fruit) fed on by stink bugs were recorded for each sampling date. Also, for comparison with a known host tree, 17 peach trees with maturing fruit were sampled visually for stink bugs at the Byron site on 20 and 24 Jul.

Also at each field site, black ground-deployed pheromone-baited pyramid traps were established along woodlands to document the presence of *H. halys* at the 2 field sites during the period of time chinaberry trees were examined for this stink bug. The insect-collecting device was the pyramid trap, made from a 3.8 L clear plastic PET® jar (US Plastic Corp., Lima, Ohio, USA) with a screw-cap lid (10.2 mm in diam), and seated atop a 1.22 m tall pyramid base (Cottrell et al. 2000). The dual lure for *H. halys* (Trécé Inc., Adair, Oklahoma, USA) with 2 aggregation pheromones, methyl (*E,E,Z*)-2,4,6-decatrienoate and a mixture of (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol was placed in the collecting device. An insecticidal ear tag (10% λ-cyhalothrin and 13% piperonyl butoxide) (Saber extra insecticide ear tags, Sagebrush Tags, De Smet, South Dakota, USA) was placed in this device to kill stink bugs only at the Bledsoe field site. At the Bledsoe site, lures were changed and insects collected from traps on a biweekly basis from 7 to 28 Aug. At the Byron site, lures were changed on a weekly basis from 20 to 31 Aug, and collected from traps on 20, 24, and 27 Jul, and 2, 14, 20, and 31 Aug.

All data were analyzed using SAS statistical software (SAS Institute 2010). For first and second instars, daily percentage feeding data were modeled by a Beta distribution. For second instars, percentage mortality (accumulative) data were modeled by a Beta distribution. Because mortality did not occur for second instars on the first 2 d of the experiment, only data for days 3 to 8 were analyzed. Analyses were done using PROC GLIMMIX. The LINK=LOGIT function was used in the model statement. Model fit was evaluated by use of the chi-square and df statistic provided by PROC GLIMMIX (Littell et al. 2006). Fixed effects were treatment, d, and treatment by d. Random effects were replicate and residual error. For first instars, the treatment by data interaction was insignificant for feeding data; the interaction was dropped from the model which was rerun. Means were back transformed using the ILINK option in the LSMEANS statement and compared using Tukey's honestly significant difference (HSD).

Results

The percentage of first instars feeding was not significantly influenced by treatment ($F_{1, 213}$ = 1.97; P < 0.1623), but was significantly affected by day ($F_{1, 213}$ = 1.97; P < 0.1623). Mean (± SE) percentage feeding was 10.4% (± 2.4) for carrot, and 9.1% (± 2.2) for chinaberry. Percentage feeding was higher for d 3 compared to d 1 and 4 (Fig. 1). Only 5 first instars (4 on carrot and 1 on *M. azedarach*) died during the experiment.

The percentage of second instars feeding was significantly influenced by treatment ($F_{1, 267}$ = 9.24; P < 0.003) but not d ($F_{7, 267}$ = 1.51; P = 0.164) or the interaction between treatment and d ($F_{7, 267}$ = 0.4; P = 0.903). Mean (± SE) percentage feeding was 11.7% (± 0.9) for carrot and 6.5% (± 1.3) for chinaberry. Because an interaction was not detected between treatment and d, percentage feeding followed a similar pattern for nymphs on carrot and *M. azedarach* over time (Fig. 2A).

Percentage mortality was significantly affected by treatment ($F_{1, 104}$ = 147.42; P < 0.001), d ($F_{5, 104}$ = 7.21; P = 0.001), and the interaction be-

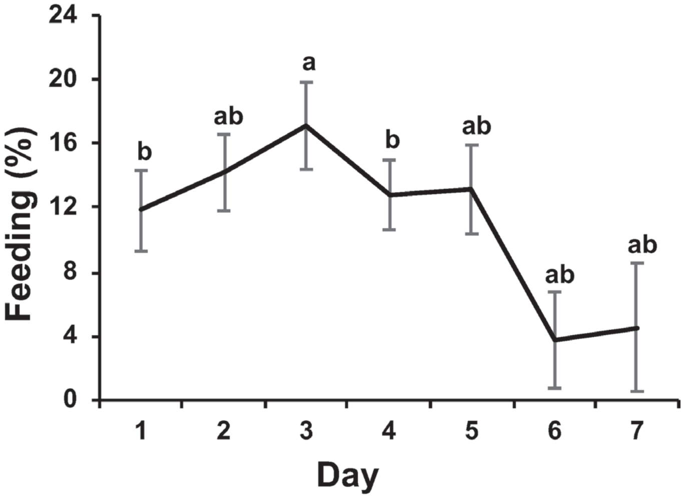

Fig. 1. Percentage feeding of of *H. halys* first instars on chinaberry leaves and carrots combined. Days with the same letter are not significantly different from each other ($P > 0.05$). Error bar represents standard error of the mean.

tween treatment and d ($F_{5, 104} = 5.69$; $P = 0.001$). Mortality was higher for second instars feeding on *M. azedarach* than feeding on carrot for d 4 through 8 (Fig. 2B). After d 3, second instar mortality on *M. azedarach* leaves increased over time, reaching a high of 85.4% by d 8. For both treatments, none of the second instars died during the process of molting into third instars.

Melia azedarach trees existed in woodland areas adjacent to crops at both field sites. Fruit was present on the trees during the time they were examined for *H. halys*. In general, principally early instars (second and thirds) were captured in traps near these woodland areas in mid-Jul to early Aug (Fig. 3A, B). Trap capture of late instars (fourth and fifths) increased around mid-to-late Aug. Thereafter, mainly adults were captured in traps. The mean number of adults captured in traps peaked at 35.3 per trap at the Bledsoe site, and at 8.4 per trap at the Byron site near the end of Aug.

Even though capture of *H. halys* nymphs and adults in pheromone-baited traps was consistent, and at times occurred in relatively high numbers, the number of *H. halys* nymphs and adults detected on *M. azedarach* generally was extremely low at both field sites (Fig. 3A, B). At the Bledsoe site, no *H. halys* nymphs were found on chinaberry on 7 Aug, but a male was detected sitting on a leaf on each of 2 *M. azedarach* trees (Fig. 3A). By 21 Aug, 2 fifth instars were observed sitting on a leaf on each of 2 trees, and 1 male was sitting on a leaf of another tree. At the Byron site, *H. halys* early instars and adults were captured in traps on 20 and 24 Jul, indicating they were reproducing on host plants at the site at that time (Fig. 3B). Two egg masses along with early instars and adults were detected on peach trees, but no *H. halys* were found on *M. azedarach* on these dates. A second instar was observed walking on a leaf on a *M. azedarach* tree, and a fourth instar was sitting on a green fruit on a second tree on 14 Aug. On 31 Aug, a fourth instar was present on a leaf of a tree, and a male and 3 fifth instars were detected on another tree. A fifth instar was observed feeding on green fruit on 1 tree, a fourth instar was feeding on stem, and a fifth instar was feeding on green fruit on another tree. In summary, only 15 *H. halys* were detected on 11 *M. azedarach* trees for a total of 120 trees sampled (i.e., mean of 0.1 per tree) over all sampling dates and field sites. Only 3 late instars were observed feeding on *M. azedarach* at 1 site late in the season when capture of *H. halys* late instars in pheromone-baited traps was relatively high.

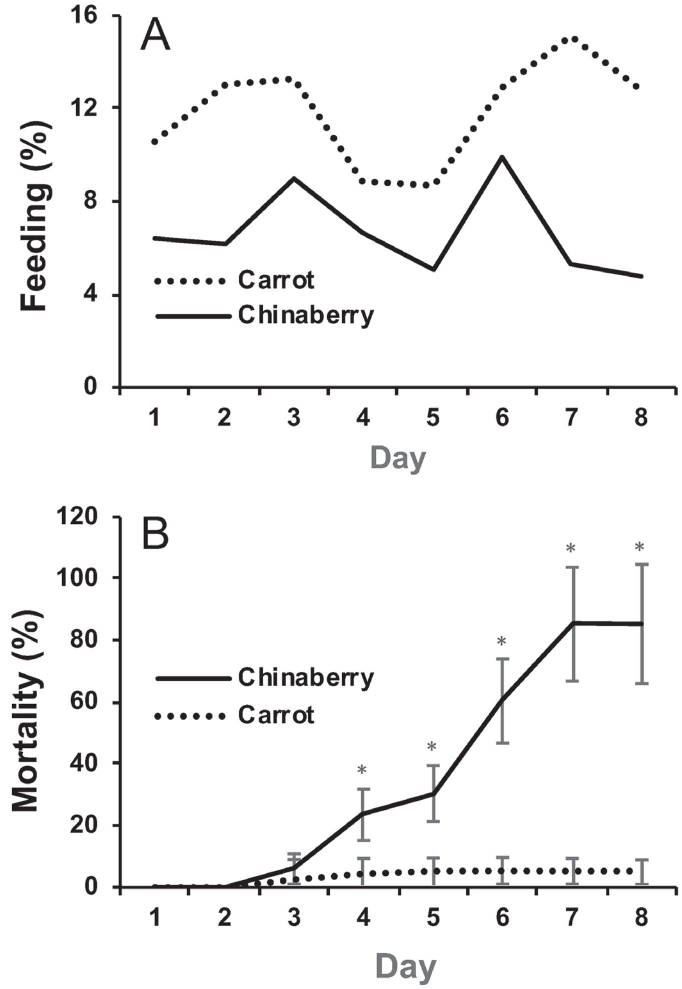

Fig. 2. Percentage feeding of *H. halys* second instars on chinaberry leaves and a carrot slice (i.e., control) (A), and percentage mortality of these second instars feeding on chinaberry leaves and carrot (B). Asterisk (*) indicates significant difference ($P < 0.05$) between the 2 treatments at the same point in time. Error bar represents standard error of the mean.

Discussion

Our results strongly suggest that *M. azedarach* is not a reproductive host plant for *H. halys*. As evidenced by capture of *H. halys* nymphs and adults in pheromone-baited traps over time, this invasive stink bug pest was present and developing in woodlands with *M. azedarach* at 2 different field sites, 1 in row crop production and the other in orchard production. However, *H. halys* nymphs and adults rarely were present on *M. azedarach* trees in these woodland areas, even when relatively high numbers of nymphs and adults were captured in traps at the Bledsoe site. Even more rarely were *H. halys* observed feeding on *M. azedarach*, and only by late instars at a time when late instars were peaking at the Byron location.

Leaf and fruit extracts of *M. azedarach* have been reported to inhibit feeding activity of insects in various orders, including Hemiptera, Coleoptera, Orthoptera, Lepidoptera, and Diptera (Valladares et al. 1997, 1999; Abou-Fakhr Hammad et al. 2001; Banchio et al. 2003; Carpinella et al. 2003; Defagó et al. 2009). Lower body weight and delayed development, as well as reduced food consumption, were recorded in no-choice tests. In our study, percentage feeding by *H. halys* second instars was lower for individuals given *M. azedarach* leaves vs. carrot. Thus, as previously determined for other insect species, *M. azedarach* likely has an antifeeding effect on *H. halys*.

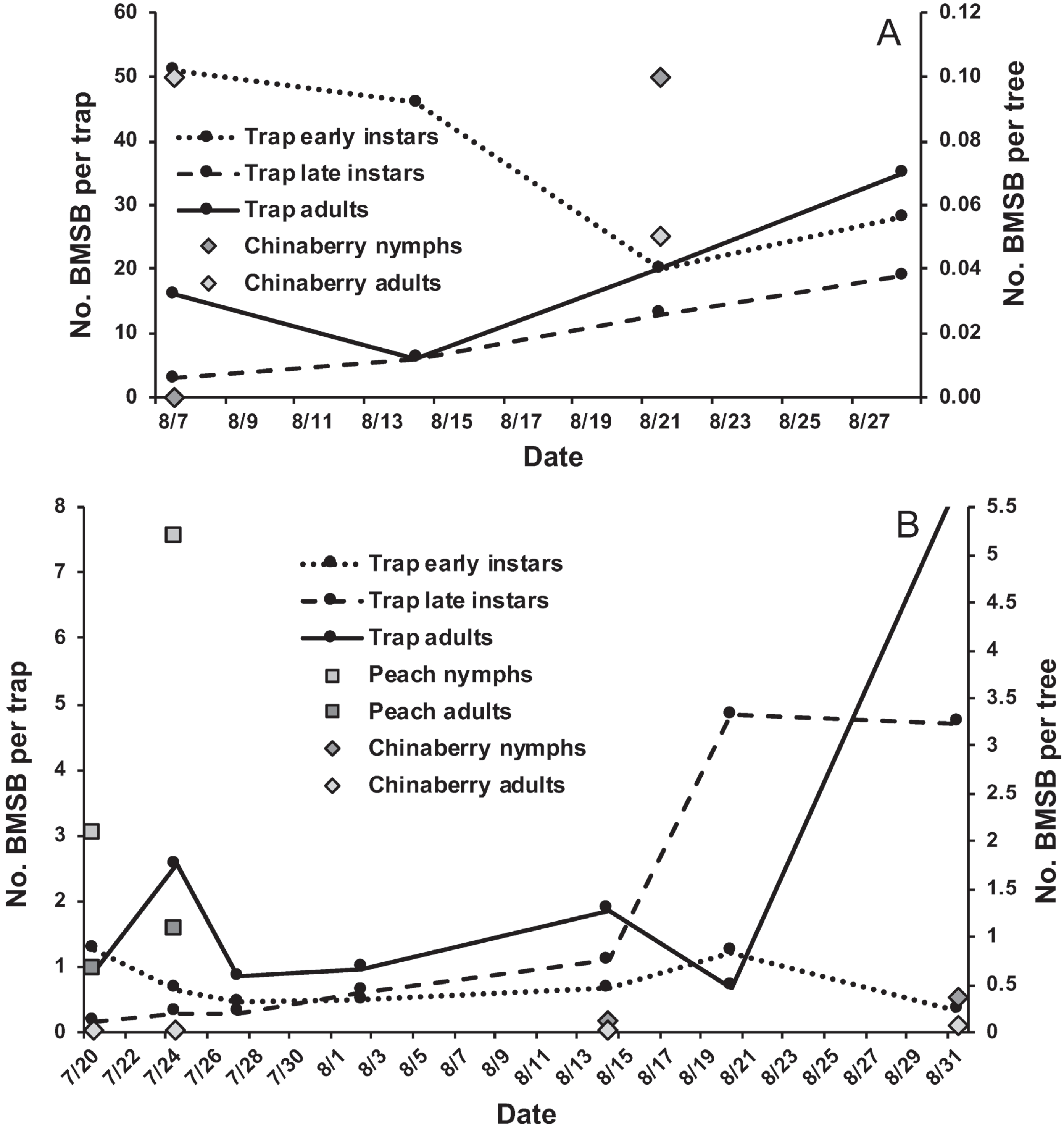

Fig. 3. Mean trap capture for early instars (second and thirds), late instars (fourth and fifths), and adults, and number of nymphs and adults per chinaberry and peach tree for brown marmorated stink bug (BMSB) at the Bledsoe (A) and Byron (B) field sites in Georgia in 2018.

In the current study, mortality was very high for second instars feeding on *M. azedarach* leaves. For other phytophagous insects, mortality appeared to be caused mainly by starvation resulting from the strong antifeedant effects of compounds from *M. azedarach* that reduce food intake (Valladares et al. 1997; Carpinella et al. 2003; Defagó et al. 2009). Mortality of *H. halys* second instars likely was related, as least in part, to the antifeeding activity of *M. azedarach*. However, toxic effects were observed for larvae and adults of *Epilachna paenulata* Germar (Coleoptera: Coccinellidae) fed pumpkin leaves treated with *M. azedarach* fruit extract (Defagó et al. 2009). Further studies are needed to completely understand the cause of the observed mortality of *H. halys*. The triterpenoids that inhibit food intake in phytophagous insects are present in all parts of the *M. azedarach* plant but are concentrated in ripe fruit (Botha & Penrith 2009). In general, young second instar stink bugs feed on leaves of crop and non-crop host plants in the field (Tillman 2008, 2015; Tillman & Cottrell 2016), but if *H. halys* second instars ever fed on *M. azedarach* fruit, mortality likely would be higher, not lower, than mortality determined for second instars on leaves in the current study. For example, leaf extracts of *M. azedarach* killed larvae of *Anopheles stephensi* Liston (Diptera: Culicidae), but only at higher doses than that for fruit extracts (Nathan et al. 2006).

First and second instar *H. halys* fed on *M. azedarach* leaves and carrots. Kiritani (1964) reported that *Nezara viridula* L. (Hemiptera: Pentatomidae) first instars remained on the egg shells throughout the stage without feeding on rice. Using the presence of stylets to confirm feeding, Bowling (1979) determined that *N. viridula* first instars did not feed on rice panicle stems and grain, or on soybean pods. Many stink bug species, including *H. halys*, oviposit egg masses on leaves (Tillman 2008; Rice et al. 2014). Therefore, their first instars are more likely to feed on leaves than on fruit. Plant feeding by first instar stink bugs was first reported for *N. viridula* and *Euschistus servus* (Say) (Hemiptera: Pentatomidae) on peanut leaves in the laboratory (Tillman 2008) and later affirmed for *N. viridula* using a marked bacterial pathogen of cotton (Esquivel & Medrano 2014). Unlike second instars, first instar *H. halys* were observed feeding equally on *M. azedarach* leaves and carrot, and feeding on these leaves did not result in mortality. Perhaps the first instars fed mainly on xylem sap, and thus avoided ingesting the antifeedant compounds in *M. azedarach*. According to Spiller et al. (1990) and Powell & Hardie (2002), xylem sap ingestion may be a strategy used for maintaining water balance and avoiding dehydration, which could be essential for the survival of *H. halys* first instars.

In summary, density of *H. halys* was very low on *M. azedarach* in 2 sites with populations of this insect present in woodlands, and feeding on *M. azedarach* in the laboratory results in the mortality of second instars. We conclude that *M. azedarach* is an unsuitable host plant for *H. halys*.

Acknowledgments

The authors thank Xeulin Luo (University of Georgia, Tifton, Georgia) for her statistical support, and Kristie Graham (USDA, ARS, Crop Protection & Management Research Laboratory, Tifton, Georgia), Rebekah Hartley and Merry Bacon (USDA, ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, Georgia), and Colby Nordstrom, University of Georgia, Entomology, Griffin, Georgia) for their technical assistance.

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