

Development of Behaviorally-Based Monitoring Tools for the Brown Marmorated Stink Bug (Heteroptera: Pentatomidae) in Commercial Tree Fruit Orchards¹

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Abstract Captures of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), were significantly greater in pyramid traps baited with the known attractant, methyl (2*E*,4*E*,6*Z*)-decatrionoate, compared with unbaited traps. A dose-dependent response by adults to lures formulated with increasing amounts of methyl (2*E*,4*E*,6*Z*)-decatrionoate and deployed in association with black pyramid traps also was observed. Among pyramid traps representing different visual stimuli including black, green, yellow, clear, white and yellow, significantly greater captures were recorded in baited black pyramid traps for adults in 2009 and nymphs in 2010 compared with other trap types; the dark upright silhouette created by this trap likely represents a trunk-mimicking visual stimulus to foraging bugs. A ground-deployed baited black pyramid trap also captured significantly greater numbers of nymphs and adults compared with canopy-deployed commercially available baited traps from Japan. Based on semi-field cage studies, brown marmorated stink bug was confirmed to be bivoltine within the mid-Atlantic region. Thus, the need for a reliable monitoring tool to detect presence, abundance and seasonal activity of brown marmorated stink bug in tree fruit and other cropping systems is critical.

Key Words brown marmorated stink bug, *Halyomorpha halys* (Stål), methyl (2*E*,4*E*,6*Z*)-decatrionoate, pyramid traps

The brown marmorated stink bug, *Halyomorpha halys* (Stål), is an invasive pest species from Asia, now well established throughout the mid-Atlantic region. Officially, brown marmorated stink bug has been detected in 35 states and the District of Columbia. Brown marmorated stink bug is considered a polyphagous pest of many specialty crops in Asia (Panizzi et al. 2000) including tree fruit, vegetables, shade trees and leguminous crops with specific mention of apple, cherry, peach, and pear (Panizzi et al. 2000, Hoebeke and Carter 2003). Surveys conducted in the United States identified a number of tree fruit hosts for brown marmorated stink bug including apple, plum, peach, pear, and cherry (Bernon 2004, Nielsen and Hamilton 2009 a,b).

Native stink bugs have long been managed with broad-spectrum insecticides, but since the passage of the Food Quality Protection Act in 1996, many broad-spectrum materials have been lost or severely curtailed through regulatory measures, allowing

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populations of native stink bugs, considered to be secondary pests, to become more prevalent. Subsequently, as brown marmorated stink bug has become well established, populations have exerted tremendous season-long and unprecedented pest pressure complicating management for tree fruit growers, leading to devastating levels of fruit injury and replacing lepidopteran pests such as codling moth and oriental fruit moth as the key pest driving management decisions in the mid-Atlantic region (Leskey and Hamilton 2010). Although a number compounds were evaluated against brown marmorated stink bug in the laboratory (Nielsen et al. 2008a), no specific field-based management recommendations for any specialty crop were available during the 2010 season. Growers relied on recommendations made for native stink bugs, which unfortunately did not control brown marmorated stink bug in commercial orchards (Leskey and Hamilton 2010, U.S. Apple Association 2010).

Monitoring tools are used to assess the presence, abundance and seasonal activity of pests and natural enemies to determine the need for and timing of insecticide applications. Stink bug species are typically monitored in cropping systems using sweep nets, beating samples, pheromone-baited traps and/or black light traps. Among native stink bugs in tree fruit, baited yellow pyramid traps (Leskey and Hogmire 2005, Hogmire and Leskey 2006) and baited mullein plants (Krupke et al. 2001) were effective at monitoring native *Euschistus* spp. whereas *Chinavia halaris* (Say) was monitored in vegetable and row crops using black light traps (Kammainga et al. 2009). Black light traps have been evaluated for brown marmorated stink bug in Japan (Moriya et al. 1987) and in New Jersey (Nielsen and Hamilton 2009a).

Aldrich et al. (2007) and Khrimian et al. (2008) confirmed that the aggregation pheromone of *Plautia stali* Scott, methyl (2*E*,4*E*,6*Z*)-decatrienoate (Sugie et al. 1996), is cross-attractive to brown marmorated stink bug, as reported in Asia (Tada et al. 2001 a,b, Lee et al. 2002). Adults are reported to be reliably attracted only early (Tada et al. 2001a) and late in the season (Tada et al. 2001a, Khrimian et al. 2008). Using this olfactory stimulus, we conducted studies toward development of effective behaviorally-based monitoring and management tools for brown marmorated stink bug in commercial tree fruit. Specifically, we evaluated responses of brown marmorated stink bug to traps representing different visual stimuli, compared the effectiveness of commercially available traps from Asia with a prototype monitoring trap for brown marmorated stink bug, compared relative attraction to different doses of methyl (2*E*,4*E*,6*Z*)-decatrienoate, and conducted a semi-field experiment designed to establish voltinism in the mid-Atlantic.

Materials and Methods

Visual stimuli associated with pyramid traps. Pyramid traps based on dimensions previously used for native stink bugs (Leskey and Hogmire 2005) were constructed of white Sintra (partially extruded PVC) sheets (Laird Plastics, Pittsburgh, PA). Each panel was 1.22 m high, 52 cm wide at the base and 7 cm wide at the top. Panels were painted with flat latex exterior paint in the following colors: black, green, yellow (in 2010 only), or white. Another set of traps were constructed of clear polycarbonate. Traps represented the following visual stimuli: standard black trunk mimic, foliar mimics (green and yellow), and no apparent visual stimulus (white Sintra and clear polycarbonate). Spectral reflectances of black, green, yellow, and white flat exterior latex paint and of clear polycarbonate were determined previously using a StellarNet EPP 2000C fiberoptic spectrometer (Hogmire and Leskey 2006).

Traps were baited with 45 mg of methyl (2*E*,4*E*,6*Z*)-decatrienoate formulated into rubber septa as described by Khrimian et al.(2008) or left unbaited in 2009. In 2010, traps were baited with lures containing 50 mg methyl (2*E*,4*E*,6*Z*)-decatrienoate (Ag-Bio Development Inc, Westminster, CO) or left unbaited. All lures were changed at 4-wk intervals and in 2010, a Hercon Vaportape II (Hercon Environmental, Emigsville, PA) was added as a killing agent to prevent escape from traps. In a preliminary study, addition of a killing agent increased trap captures ~250%. Four replicates of each baited and unbaited trap type were deployed 5 m from the border row of apple orchard blocks in 2009 and from apple and pear orchard blocks in 2010. Traps were spaced ~20 - 25 m apart and trap location was randomly assigned within each replicate. Traps were deployed from 7 October -17 November 2009 and from 23 July -14 October 2010. Data that were not normally distributed according to Levene's test were subjected to a square root transformation. Data were analyzed using a factorial ANOVA based on the GLM procedure (SAS Institute 2010) to evaluate the specific effects of visual stimulus, presence of bait and the interaction term. If the model indicated significant differences, multiple comparisons were calculated using Tukey's HSD ($P < 0.05$). All trap treatments were subsequently subjected to a one-way ANOVA followed by Tukey's HSD.

Capture mechanism and deployment strategy. In 2010, we compared captures in a ground-deployed black pyramid (Fig. 1A) with 2 commercially available traps from Japan. These included a translucent pyramid-shaped trap with a 35.5 cm base and 15.24 cm tall collection device that used an interior 12.7 cm long entry tube with 2.54 cm diam. opening (CBC America, Shin-Etsu Chemical, Japan). This trap was different from the black pyramid trap in that it had no apparent visual stimulus and was designed to be hung within the canopy of a tree, but the capture mechanism was similar with bugs crawling up the pyramid base and being funneled into the collection jar (Fig. 1B). A 41.9 cm tall bucket-style water trap (Sankei Chemicals Co., Ltd., Kagoshima, Japan) also was deployed; it was considered to be visually stimulating based on the

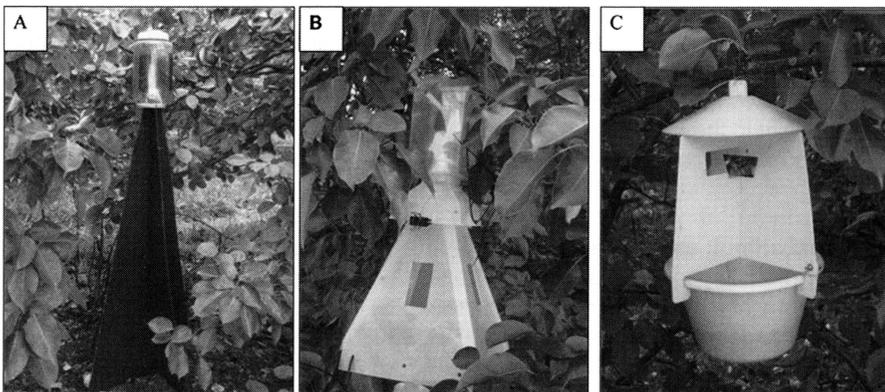


Fig. 1. Ground-deployed black pyramid trap (A) and commercially available canopy-deployed pyramid (B) and bucket trap (C) evaluated in 2010 as potential traps for capturing brown marmorated stink bug adults and nymphs.

foliar-mimicking yellow color and was also intended to be hung within the canopy of a tree. The capture mechanism required bugs to either walk, or in the case of adults, alight on the upper portions of the trap and then fall into the water bucket (Fig. 1C). All traps were baited with lures containing 50 mg methyl (2E,4E,6Z)-decatrienoate (Ag-Bio Development Inc, Westminster, CO) or left unbaited. All lures were changed at 4-wk intervals and Hercon Vaportape II strips, changed biweekly, were added to traps to prevent escape. Traps were spaced ~20 - 25 m apart within the border row of a minimally managed pear block at the Appalachian Fruit Research Station and trap location was randomly assigned within each replicate. Traps were deployed from 30 July -30 September 2010. Data that were not normally distributed according to Levene's test were subjected to a square root transformation. Data were analyzed using a one-way ANOVA followed by Tukey's HSD ($P < 0.05$).

Dose-dependent responsiveness. We conducted a dose response trial using methyl (2E,4E,6Z)-decatrienoate formulated into rubber septa. Black pyramid traps were baited with 450 mg, 45 mg, 5 mg or left unbaited. Three replicates of each were deployed 5 m from the border row of apple orchard blocks. Traps were spaced ~20 - 25 m apart and trap location was randomly assigned within each replicate. Traps were deployed from 9 October - 16 November 2009. Data that were not normally distributed according to Levene's test were subjected to a square root transformation. Data were analyzed using a one-way ANOVA followed by Tukey's HSD ($P < 0.05$).

In 2010, we deployed different doses of methyl (2E,4E,6Z)-decatrienoate directly in the canopies of apple trees located in a border row to determine if we could aggregate increasing numbers of brown marmorated stink bugs. Canopies of 9 trees comprising a 2 ha block of apples at the Appalachian Fruit Research Station were baited with either 500 mg, 50 mg, or left unbaited on 28 September 28, 2010. Lures were attached to central scaffold limbs in the inner third of each tree canopy and replicated 3 times per dose, treatment canopies were spaced ~50 m apart. After 3 d, the entire block was treated with oxamyl at 3 pints/acre per 100 gallons. Bugs were recovered from 1 m × 1 m areas beneath baited and unbaited canopies immediately after treatment.

Voltinism. In 2010, we conducted semi-field trials to document the number of generations completing development in Kearneysville, WV. Three cages (1.83 × 1.83 m) were erected in an open field planted with mixed fescue. The lower edge of each cage was buried in the soil to a depth of 5 cm to prevent entry from the outside or escape from bugs contained within. Provisioned cages were set up between 14 April -21 May 2010. Each cage received known hosts of brown marmorated stink bug including 2 newly leafed-out *Paulownia tomentosa* (Thunb.) (~1 m tall), a dwarf nectarine tree (5 yr old), and a single pea plant prior to pod set and then a soybean plant at full pod set. All plants were maintained throughout the trial with water and necessary pruning to accommodate field cage dimensions. On 19 May, 2010, 5 male and 5 female adults also were added to each cage with no-see-um nylon bags (Quest Outfitters, Sarasota, FL) covering individual *Paulownia* leaves. When egg masses were detected in each field cage, adults were removed. Nymphal development was subsequently observed until a new generation of adults was present. After new generation adults reproduced, they were removed from cages and nymphal development was followed to the adult stage. Observations continued until 23 September 2010. In addition, degree day accumulations also were calculated based on developmental data reported by Nielsen et al. (2008b).

Results

Visual stimuli. In 2009, the factorial ANOVA for adult trap captures indicated significant effects ($F = 14.14$; $df = 7, 160$; $P < 0.001$) of presence of lure ($F = 83.39$; $df = 1, 160$; $P < 0.001$); visual stimulus ($F = 3.90$; $df = 3, 160$; $P = 0.047$); but not the interaction term ($F = 0.50$; $df = 3, 160$; $P = 0.0685$). Throughout the trapping period, baited traps captured 31.70 ± 5.43 SE adults per trap per week, whereas unbaited traps captured 1.20 ± 0.23 SE. Captures in black traps were significantly greater (26.54 ± 8.46) than white (11.57 ± 4.71), with captures in clear (10.64 ± 2.85) and green traps (17.07 ± 6.05) being intermediate. Among all baited and unbaited traps, greatest captures were recorded in black baited traps (Fig. 2A).

In 2010, the factorial ANOVA of adult trap captures indicated significant effects ($F = 3.74$; $df = 9, 390$; $P < 0.001$) of presence of lure ($F = 29.56$; $df = 1, 390$; $P < 0.001$), but not visual stimulus ($F = 0.64$; $df = 4, 390$; $P = 0.632$) or the interaction term ($F = 0.38$; $df = 4, 390$; $P = 0.819$). Throughout the trapping period, baited traps captured 58.25 ± 6.79 SE adults per trap per week whereas unbaited traps captured 20.42 ± 3.20 SE. Among all baited and unbaited traps representing different visual stimuli, more adults were captured in baited green and black traps (Fig. 2B).

The factorial ANOVA for nymphal captures in 2010 indicated significant effects ($F = 2.30$; $df = 9, 390$; $P = 0.016$) of presence of lure ($F = 6.59$; $df = 1, 390$; $P = 0.011$) and visual stimulus ($F = 3.14$; $df = 4, 390$; $P = 0.015$), but not the interaction term ($F = 0.38$; $df = 4, 390$; $P = 0.821$). Throughout the trapping period, baited traps captured 97.71 ± 15.82 SE nymphs per trap per week whereas unbaited traps captured 49.00 ± 6.42 SE. Captures in black traps were significantly greater (108.32 ± 25.21) than clear (37.14 ± 9.05), with captures in yellow (85.52 ± 22.97), green (93.14 ± 21.34) and white (42.64 ± 11.03) traps being intermediate. Among all baited and unbaited traps, greatest captures were recorded in black baited traps (Fig. 2C).

Capture mechanism and deployment strategy. For nymphal captures, there were significant differences among trap types ($F = 28.75$; $df = 2, 78$; $P < 0.001$) with significantly greater captures of nymphs (606.48 ± 113.73 nymphs per trap per week) in ground-deployed black pyramid traps compared with the canopy-deployed translucent pyramid trap (61.37 ± 12.11) and yellow bucket trap (6.63 ± 1.37). Similarly, significant differences were detected among trap types for adult captures ($F = 23.84$; $df = 2, 78$; $P < 0.001$) with significantly greater captures of adults in black pyramid traps (123.83 ± 30.43 adults per trap per week) compared with the other two trap styles. In addition, captures in the canopy-deployed translucent pyramid (49.33 ± 13.28) were significantly greater than yellow bucket traps (0.51 ± 0.17).

Dose-dependent responsiveness. Among traps baited with different doses of methyl (2E, 4E, 6Z)-decatrienoate or left unbaited, there were significant differences among captures ($F = 12.50$; $df = 3, 68$; $P < 0.001$). Significantly more adults were captured in traps baited with 450 mg compared with those baited with 5 mg and the unbaited control (Table 1). For the baited canopy trial, the one-way ANOVA was not significant. However, numerically greater captures were recovered in canopies baited with 500 mg (23.25 ± 18.68 adults per canopy) compared with 50 mg (2.25 ± 1.31) or the unbaited control (2.50 ± 0.50).

Voltinism. In 2010, we found that brown marmorated stink bug was bivoltine in Kearneysville, WV; two full generations were completed based on presence of eggs and newly-molted adults in 3 field cages. The summer generation preoviposition and developmental period averaged 224.7 ± 5.6 DD and 569.5 ± 22.8 DD, respectively.

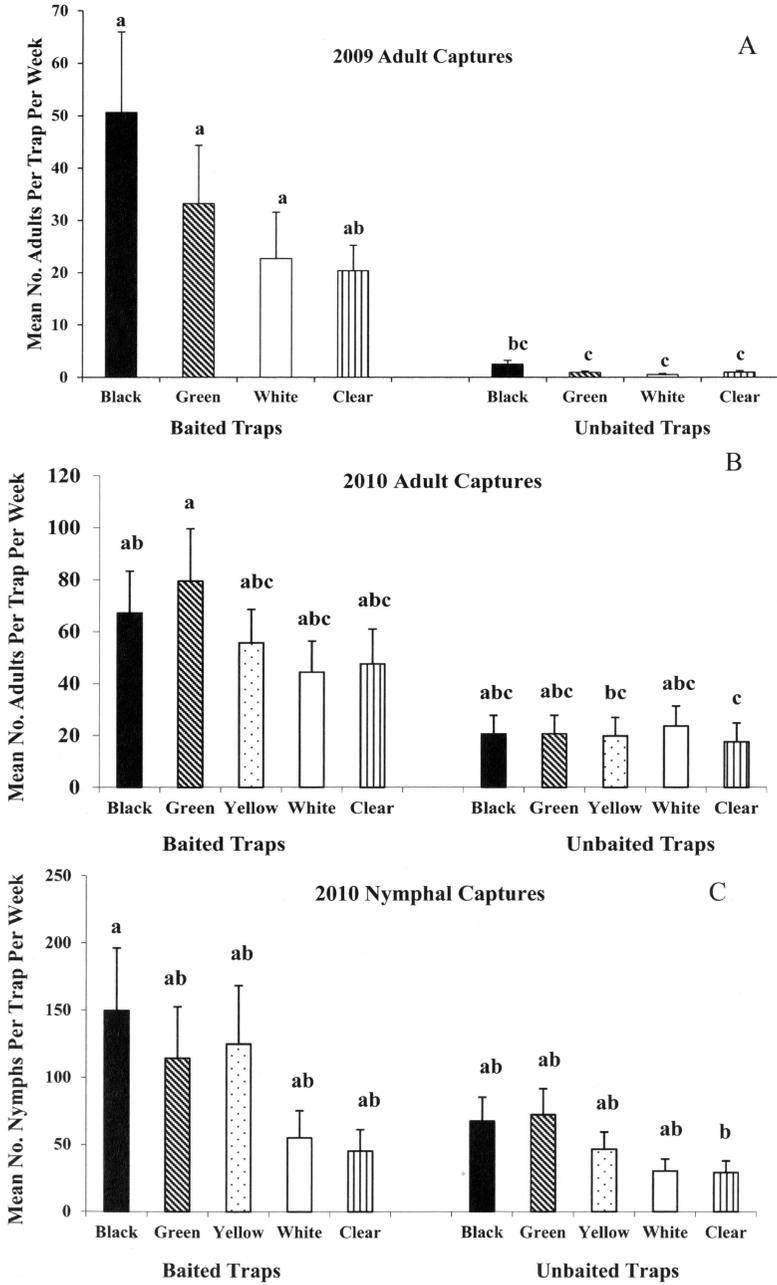


Fig. 2. Mean number of adult captures (\pm SE) in 2009 (A) and 2010 (B) and nymphal (C) captures (\pm SE) in 2010 in baited and unbaited pyramid traps representing different visual stimuli.

Table 1. Mean number of adult brown marmorated stink bug captured per trap per week in black pyramid traps baited with different doses of methyl (2E, 4E, 6Z)-decatrienoate or left unbaited.

Dose-Dependent Treatment	Mean \pm SE
450 mg	20.94 \pm 6.36 a
45 mg	9.88 \pm 3.38 ab
5 mg	2.83 \pm 0.92 bc
Control (unbaited)	0.55 \pm 0.27 c

The second generation preoviposition and developmental period averaged 136.7 ± 6.0 DD and 666.3 ± 20.8 DD, respectively.

Discussion

In our studies, we evaluated pyramid traps representing different visual stimuli as potential tools for monitoring brown marmorated stink bug populations. Traps were either unbaited or baited with aggregation pheromone of *Plautia stali* Scott, methyl (2E,4E,6Z)-decatrienoate (Sugie et al. 1996). This compound was found to be cross-attractive to brown marmorated stink bug in Asia (Tada et al. 2001 a,b, Lee et al. 2002), with the same response confirmed here in the United States (Aldrich et al. 2007, Khrimian et al. 2008). We found that traps baited with this compound captured significantly more adults and nymphs compared with unbaited traps during the late season as well. Among visual stimuli, traps with darker visual stimuli, particularly black, appear to be more visually stimulating with significantly greater captures of adults in 2009 and nymphs in 2010 in baited black pyramid traps. This result is very different than that for native stink bugs in which yellow pyramid traps were found to be most visually stimulating for *Euschistus* spp. (Mizell and Tedders 1995, Hognire and Leskey 2006). Prokopy and Owens (1983) noted that a large number of phytophagous insects respond to yellow; this particular pigment is considered to be a supernormal foliage-type visual stimulus. However, unlike *E. servus*, which typically reproduces on broadleaf weed hosts (McPherson and McPherson 2000), brown marmorated stink bug appears to commonly use arboreal hosts (Hoebeke and Carter 2003, Bernon 2004, Nielsen and Hamilton 2009a), possibly explaining why a dark, upright “trunk-mimicking” stimulus may be more appealing than more generalized foliar cues.

Ground-deployed black pyramid traps captured significantly more adults and nymphs than canopy-deployed commercially available traps for brown marmorated stink bug. Although all traps were baited, the capture mechanism and deployment strategy of a pyramid trap appears to be more compatible with brown marmorated stink bug movement patterns, as brown marmorated stink bug have a natural tendency to climb up vertical surfaces. Indeed large numbers of brown marmorated stink bug and elevated injury are often found in the upper third of the canopy of deciduous fruit trees (Leskey et al., unpubl. data). Our results also likely reflect the fact that ground-deployed traps exploit major points of entry by brown marmorated stink bug to trees prior to arrival in the canopy with adults flying directly to the trunk and both

adults and nymphs walking to and up the trunk. Although the translucent tree-deployed pyramid trap has a similar design and has been evaluated against the canopy-deployed bucket trap in Japan (Adachi et al. 2007), it captured significantly fewer brown marmorated stink bugs. The lower captures likely reflect a poorer capture mechanism associated with the collection jar and the deployment location. Because traps are deployed in the canopy, brown marmorated stink bugs have already bypassed a single major point of entry, i.e., the trunk, and can forage and avoid the trap itself.

Interestingly, we observed increasing responsiveness by brown marmorated stink bug adults to increasing doses of methyl (2*E*,4*E*,6*Z*)-decatrienoate. We evaluated 5, 45, and 450 mg of material per trap (based on the number of septa included per trap) and found that increasing numbers of adults were captured with increasing dose. A similar aggregation response was recorded for *E. servus* to commercially available pheromone lures containing methyl (2*E*,4*Z*)-decadienoate deployed in association with yellow pyramid traps; adults aggregated in significantly greater numbers located on mullein plants located 1 m from baited traps compared with plants at greater distances (Leskey and Hogmire 2007). Thus, this aggregation response could be used as part of a spatially precise mass trapping or attract and kill approach (as indicated by our baited canopy results) for management of adult populations. Unfortunately, methyl (2*E*,4*E*,6*Z*)-decatrienoate does not reliably attract brown marmorated stink bug adults season-long, as responses are principally recorded during the late season (Khrimian et al. 2008). Thus, identification of a brown marmorated stink bug pheromone could provide a much more sensitive tool for monitoring season-long populations of brown marmorated stink bug.

Table 2. Julian dates, mean accumulated and range of degree days for brown marmorated stink bug preoviposition and developmental periods in 2010.

Biological Period	Julian Date	Mean DD ± SE (Range)*
Overwintered Generation Preoviposition	104 - 124	224.7 ± 5.6
Overwintered adults placed in field cage	146 - 158	(216.0 - 235.2)
Eggs deposited		
Summer Generation Developmental	146 - 158	569.5 ± 22.8
Eggs deposited Summer generation	195 - 208	(536.8 - 613.4)
Adults present		
Summer Generation Preoviposition	195 - 208	136.7 ± 6.0
Summer generation adults present	200 - 208	(130.8 - 142.7)
Eggs deposited		
Second Generation Developmental	200 - 208	666.3 ± 20.8
Eggs deposited Second generation	256 - 266**	(624.7 - 687.1)
Adults present		

*Nielsen et al. 2008b indicated that brown marmorated stink bug require 147.65 DD and 537.63 DD to complete preoviposition and developmental periods, respectively.

**Adults were present in one field cage on day 256. The other two cages were not checked daily, but adults were present 10 days later. Therefore, we used day 266 as a conservative developmental estimate.

A sensitive monitoring tool likely will be very important for many tree fruit growers, particularly because we have documented bivoltine populations in Kearneysville, WV. Although Nielsen and Hamilton (2009a) reported univoltine populations in eastern Pennsylvania, it appears that in more southerly locations within the mid-Atlantic, bivoltine populations are present. Based on degree day requirements for preoviposition (147.65 DD) and developmental (537.63 DD) periods reported by Nielsen et al. (2008b), we found that brown marmorated stink bug easily completed two generations in semi-field cages (Table 2). Indeed total degree day accumulations (preoviposition and developmental periods) for the summer and second generations at a minimum totaled 752.8 DD and 755.5 DD, respectively. This is well within the total 685.28 DD reported by Nielsen et al. (2008b). Because brown marmorated stink bug is bivoltine in parts of the mid-Atlantic and could have up to 5 generations in more southerly locations (Hoffman 1931), the threat posed by this invasive species is profound. Thus, the development of a sensitive monitoring tool to detect presence, abundance and seasonal activity is paramount.

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