

Seasonal Phenology and Monitoring of the Non-Native *Halyomorpha halys* (Hemiptera: Pentatomidae) in Soybean

ANNE L. NIELSEN,^{1,2,3} GEORGE C. HAMILTON,¹ AND PETER W. SHEARER⁴

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ABSTRACT The introduction of an invasive species into an agroecosystem can alter both the interspecies dynamics and existing management practices. In the area of introduction, seasonality of *Halyomorpha halys* (Stål) in soybean fields was investigated by comparing monitoring efficiency of sweep net sampling and two sizes of pyramid traps baited with aggregation compound methyl (*E,E,Z*) 2,4,6-decatrienoate in 2006–2007. The large pyramid trap caught significantly higher densities of *H. halys* than the small pyramid trap and the sweep net samples each year. Adult males and females were detected in significantly higher densities in the large pyramid trap than other life stages. The pyramid traps caught *H. halys* adults and nymphs earlier than sweep net samples, during the R3 and R4 phenological stages of soybean growth. Peak abundances in the pyramid traps occurred during the R5–R6 stages, while the sweep samples were highest during the R6 stage. Soybean is sensitive to stink bug feeding damage from the R3–R6 stages. The occurrence of *H. halys* in soybean coincides with soybean's critical growth stage. *H. halys* has become the dominant stink bug species in the crop, indicating that damage thresholds need to be determined.

KEY WORDS seasonality, species composition, invasive species, population monitoring, stink bug

The introduction of an invasive species into an agroecosystem can alter both the interspecies dynamics and existing management practices. For an invasive insect species to become a pest, its seasonal phenology must match its host plants at growth periods where damage can occur. Truly invasive species can disrupt species composition and dominance (Pimentel et al., 2005; Hejda et al., 2009). In New Jersey and eastern Pennsylvania, stink bugs were not a significant economic concern in soybean before 2000. However, an introduced stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Hoebeke and Carter 2003), has become the dominant pentatomid species in this region in tree fruit and numerous ornamentals (Nielsen and Hamilton 2009a, b), establishing itself as an important member of the stink bug complex in the Mid-Atlantic States since its introduction around 1996. *H. halys* is now wide-spread in the Eastern United States with additional populations in California and Oregon. It has also been identified in Europe (Wermelinger et al. 2008), indicating that this is a successful invader in temperate climates.

H. halys is native to Asia where it is an occasional pest of legumes (including soybeans), tree fruits, and

many ornamentals (Kobayashi et al. 1972, Funayama 1996, Choi et al. 2000, Toyama et al. 2006). Since establishing in the United States, *H. halys* has attacked these crops and has caused economic losses in apples and pears in New Jersey and Pennsylvania (Nielsen and Hamilton 2009b). Over-wintering populations inside residences and businesses also makes *H. halys* a significant nuisance pest (Hamilton et al. 2008, Nielsen et al. 2008). Although populations have been observed in recent years in soybean, such observations have been anecdotal and no studies have examined the population dynamics of *H. halys* in soybean and its potential impact in the system. Impact of an invasive species can be measured by crop damage and a change in species dominance (Mack et al. 2000, Pimentel et al. 2005).

The life history of *H. halys* in the United States suggests that its seasonal dynamics will coincide with the susceptible periods in soybean. Where it is univoltine, reproductively immature adults ("spring" adults) gradually emerge from overwintering sites in early spring (March to April) but do not produce eggs until early June (Nielsen and Hamilton 2009a). These offspring undergo five instars during the summer months while feeding on a wide range of host plants. They feed primarily on plant reproductive structures, such as seed pods. Movement between host plants is believed to be associated with the phenological stage of the plant (Panizzi et al. 1980, Panizzi 1997). The resulting adults ("autumn" adults) feed until entering reproductive diapause in September and October.

¹Department of Entomology, Rutgers University, 93 Lipman Drive, New Brunswick, NJ 08901.

²Current address: Michigan State University, Department of Entomology, 205 Integrated Plant Systems, East Lansing, MI 48824.

³Corresponding author, e-mail: anielsen@msu.edu.

⁴Oregon State University, Mid-Columbia Agricultural Research and Extension Center, 3005 Experiment Station Drive, Hood River, OR 97031.

Coinciding with the appropriate phenological stage of soybean is a key factor for the pest's population growth and damage to the host plant (Smith et al. 2009). Typical stink bug feeding damage in soybean can range from deformed seeds, delayed maturity to reductions in yield and oil (Daugherty et al. 1964, McPherson 1996, Boethel et al. 2000, McPherson and McPherson 2000). Soybeans are susceptible to economic loss from stink bug feeding during the R3–R6 stages of development which encompasses the period when the seeds are beginning to develop (R3) to fully formed (R6). During seed hardening and full maturity (stages R7 and R8) the plant is not as susceptible to feeding damage (Schumann and Todd 1982).

Seasonality of stink bugs in this study is monitored using two types of pyramid traps and sweep net samples, which are compared for efficiency at monitoring *H. halys* in all life stages. The use of trapping methods, such as pyramid traps baited with aggregation pheromone, have had reasonable success capturing stink bugs and have been used as a successful integrated pest management (IPM) monitoring tool in tree fruits and tomato crops for other species (Leskey and Hogmire 2005, Cullen and Zalom 2006). Aggregation pheromones, unlike sex pheromones, are attractive to adult males and females and nymphs. Stink bug intraspecific communication occurs through a combination of pheromones and vibrational signals (Millar et al. 2002), so individuals are often attracted to the area, but lacking a short-range cue, may not actively move into the trap. Collection has been improved by incorporating color into the trap. Specifically, yellow has a positive attraction to many motile herbivorous insects, including stink bugs, because it is interpreted as an indicator of plant health (Mizell and Tedders 1995, Leskey and Hogmire 2005). Trap designs incorporating both visual and chemical cues have been successfully used to trap other species of insects (Prokopy and Owens 1983).

Traps baited with methyl (*E,E,Z*)-2,4,6-decatrienoate, the aggregation pheromone of a sympatric Asian species, *Plautia stali* Scott (Hemiptera: Pentatomidae), are highly attractive to *H. halys* (Khrimian 2005). This pheromone has been used in its native and non-native habitat for monitoring populations of *H. halys* (Lee et al. 2002, Adachi et al. 2007, Khrimian et al. 2008); however, no studies evaluating traps for *H. halys* have been done in soybean in either Asia or the United States. The objective of this study was to monitor the seasonality of *H. halys* in relation to soybean phenology while identifying the best monitoring method in the area of introduction in eastern Pennsylvania.

Methods

Location. The study was conducted at the Lichtenwalner Farm in Emmaus, PA near the area of *H. halys* introduction. This site had reported populations of *H. halys* for at least two years before the initiation of the study; it was bordered by sweet corn plantings on at least one side. The field was no-till indeterminate

soybean [Asgrow 3602 (2006), Seedway 50 (2007), planting date 30 May both years] with 30" row spacing and maintained without fertilizer or insecticide treatments. Treatments were set up in a randomized complete block design with six replicates with row as the blocking factor. Each block was 10m apart, or ≈13 rows. Each block contained one replicate of each treatment: a large pyramid trap, a small pyramid trap and a sweep net sample, each placed 10m apart (pheromone distance used by [Khrimian et al. 2008]). Sweep net samples were taken over a 10 m row distance, equal to ≈20 sweeps, using a 38 cm diameter sweep net (BioQuip, Rancho Dominguez, CA). Blocks began 10m from the field edge and extended 40 m into the field. A hedgerow containing various plants including multiflora rose (*Rosa multiflora* Thunberg), black locust (*Robinia pseudoacacia* Linnaeus), honeysuckle (*Lonicera* spp.), and wild grape (*Vitis* sp.) bordered the field ≈15 m from the eastern field edge.

Seasonal Phenology and Species Composition. Sweep net samples were taken at the beginning of R1 stage (containing one flower at any node), in mid-June. All samples were taken twice weekly until frost and the phenological stage of soybeans was recorded weekly according to Fehr et al. (1971). Sweep net sampling is a common sampling method in soybean for detecting or monitoring stink bugs and was used to monitor population densities and pentatomid species composition. In addition, a 110 volt blacklight trap (Gempler's, Madison, WI) was run from 1 May to 1 October to monitor flight activity and to supplement sweep net sampling assessments of stink bug population density. The blacklight trap was placed in an open area of the farm in front of a silo and emptied twice weekly. All phytophagous pentatomid captures in the sweep net samples, pyramid traps, and blacklight trap were identified to species and life stage.

Aggregation Lures. The aggregation compound methyl (*E,E,Z*)-2,4,6-decatrienoate used in this study was synthesized by USDA-ARS in Beltsville, MD following the procedures outlined by Khrimian (2005). Rubber septa were impregnated with 2.5 mg of methyl (*E,E,Z*)-2,4,6-decatrienoate. In all trials, lures were replaced every 3 wk. Photoisomerization does not affect attractiveness to *H. halys* and thus the lures used in both studies were not shielded from UV exposure (Khrimian et al. 2008).

Monitoring Comparison. Effectiveness of *H. halys* monitoring methods in soybean near the area of introduction were evaluated in terms of density and abundance of life-stages. Two sizes of yellow pyramid traps baited with lures containing 2.5 mg of methyl (*E,E,Z*)-2,4,6-decatrienoate were compared with sweep net captures in 2006 and 2007. Both sizes of pyramid traps have been deployed to monitor native stink bug populations in other commodities. The small trap is cheaper to construct but the attraction distance may be decreased because of similarity between soybean canopy height and trap height. The pyramid traps were constructed using bright yellow sheets of Sintra plastic, 6 mm thick (Laird Plastics, Dayton,

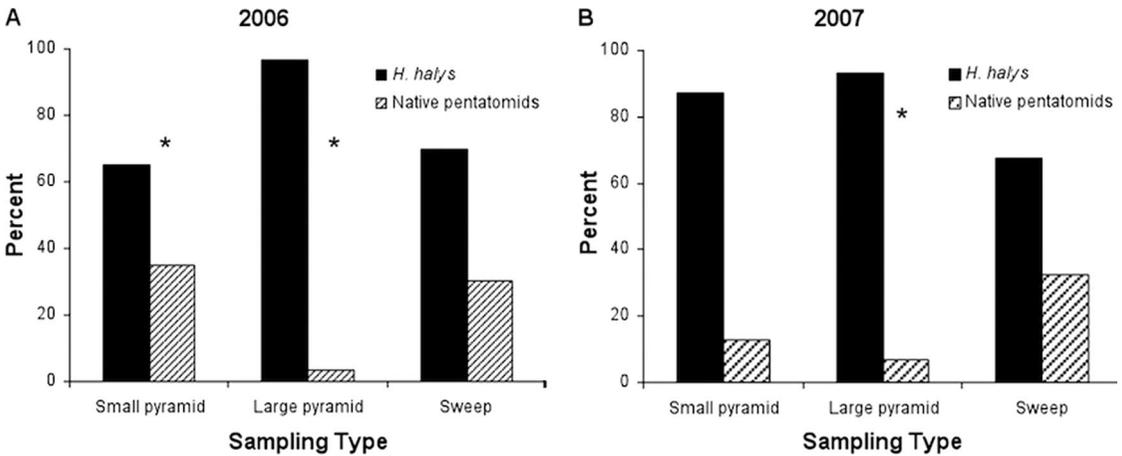


Fig. 1. Seasonal mean percentage of *H. halys* and all native pentatomid species found in each sample type in (A) 2006 and (B) 2007 in Emmaus, PA. Each significant parameter determined by χ^2 value ($P \leq 0.05$) is indicated by an asterisk.

OH). Small pyramid traps were 81.3 cm tall by 30.5 cm wide at the base (Leskey and Hogmire 2005). The large pyramid traps were 142 cm tall with a 56 cm wide base (Mizell and Tedders 1995). Trap tops were constructed out of 1 gallon PET clear plastic containers (25 cm h \times 14 cm w, United States Plastic Corps., Lima, OH) with the bottom removed and replaced with an inverted wire mesh cone, previously designed for boll weevil pyramid traps (Great Lakes IPM, Vestaburg, MI). Eight 3.8 cm holes were drilled in two columns around the clear plastic container and covered with flexible black plastic mesh for ventilation. The pheromone lure was hung from the removable lid by a metal hook with a metal binder clip to secure the lure.

Statistical Analysis. Annual stink bug relative-abundance data were summed and analyzed using χ^2 test. If values are significant from expected, to identify which frequency value (stink bug abundance per sampling type) is driving the overall departure from expected values, the residuals were compared and those with an absolute value >2.0 were considered a major contributor to a significant χ^2 value. Abundance of each life stage was calculated as the proportion to the seasonal sum of *H. halys* and was arcsine transformed and analyzed with two-way analysis of variance (ANOVA) to test the interaction of year and treatment. Seasonality and monitoring comparison were analyzed by taking the weekly average of the twice-weekly captures of stink bugs in traps (sum of each stink bug species/trap/date). Data points were restricted to the soybean phenology stage R3 until frost (that occurred during R8), which was when individuals were present in the fields. Sampling methodologies for 2006 and 2007 were assessed using repeated measures MANOVA. The data did not conform to assumptions of sphericity and the G-G epsilon values are reported. All analyses were done in JMP v.8 (SAS Institute 2008).

Results

Seasonal Phenology and Species Composition. Native pentatomids and *H. halys* were caught in both sizes of pyramid traps and in sweep net samples. However, *H. halys* comprised 70% of the pentatomid species in the sweep net samples, indicating it is the most abundant pentatomid species in soybeans at this location, 10 yr after its suspected introduction. The following native species were identified: *Euschistus servus*, *E. tristigmus* (Say), *E. variolarius* (Palisot de Beauvois), *Acrosternum hilare*, and *Thyanta accera* McAtee. The seasonal mean density of *H. halys* was significantly higher compared with native species in all sampling types for both years (2006: $\chi^2 = 21.32$, $P < 0.0001$; 2007: $\chi^2 = 9.21$, $P < 0.01$). In both years, the low frequencies of native pentatomids in both sizes of pyramid trap were major contributors to a significant χ^2 (Fig. 1).

Seasonality of *H. halys* from June through October was measured by sweep net and blacklight samples. *H. halys* adults were mobile in the landscape from late June through early September (Fig. 2). Blacklight traps were used to monitor flight activity and as an additional measure of species biodiversity. Peak flight activity occurred during late July/early August in 2006 and 2007, coinciding with the imaginal eclosion of fall adults (as indicated by Degree Day requirements) that are likely moving between host plants (Nielsen and Hamilton 2009a). Peak flight activity occurred during the R4 stage of soybean, before *H. halys* populations build-up in soybean. Regardless, *H. halys* development occurs in soybean fields as occasional nymphs were found in sweep net samples beginning in late July, during the R3 soybean phenological stage. *H. halys* egg masses and successive instars (discussed below) were also found both years. Peak abundance (based upon sweep net samples) occurred in mid-August though mid-September, coinciding with the R5–R7 stages, developing seed through seed hardening. While *H. halys* is present during stages R3–R8, it

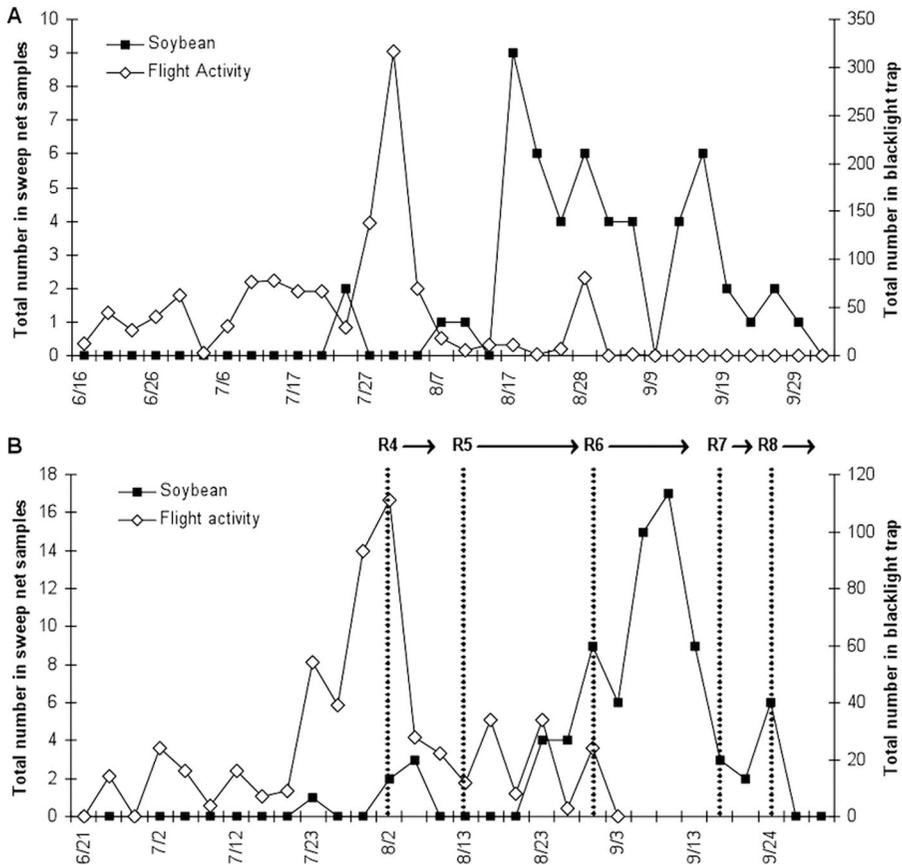


Fig. 2. Seasonal occurrence of *H. halys* in Pennsylvania soybean field collected with a sweep net and a blacklight trap which indicates flight activity in (A) 2006 (B) 2007. Soybean phenological stages are indicated where stink bug feeding can cause economic damage (R4–R8) in 2007.

is very prevalent during R5 and R6 stages of soybean growth which are the most susceptible to stink bug damage (McPherson and McPherson 2000).

Soybean phenology, particularly before the R5 stage when beans begin to develop, also influenced the results, with both pyramid trap sizes capturing *H. halys* adults and nymphs before detection in sweep samples (Table 1; Fig. 3). The number of *H. halys* in the pyramid traps was highest during soybean R3–R6 stages. The large pyramid trap was attractive earliest in the season. Individuals were collected in traps 60 m into the field before they were present in sweep net samples. Densities in the sweep net samples were highest

in the R6 stage. When the canopy became dense, numbers of *H. halys* captured in the small pyramid traps decreased. A positive nymph:adult ratio indicates population density growth, numbers ≥ 1 suggest high population growth (Smith et al. 2009). High nymph:adult ratios were indicated by both pyramid traps throughout the season although, this could have been biased by pheromone attraction. The sweep net had a nymph:adult ratio of 9.50 during the R6 soybean growth phase indicating positive population growth.

Sampling Method Comparison. The small and large pyramid traps baited with methyl (E,E,Z)-2,4,6-decatrienoate and sweep net sampling of *H. halys* in soy-

Table 1. Abundance of *H. halys* by phenological stage of soybean in Pennsylvania

Soybean stage	Small pyramid			Large pyramid			Sweep net (20 sweeps)		
	Maximum	Mean \pm SE	Nymph/adult ratio	Maximum	Mean \pm SE	Nymph/adult ratio	Maximum	Mean \pm SE	Nymph/adult ratio
R3	4	0.21 \pm 0.17	4.00	33	2.96 \pm 1.57	6.10	1	0.04 \pm 0.04	0.00
R4	13	2.39 \pm 0.85	13.33	10	2.67 \pm 0.65	3.00	3	0.28 \pm 0.18	0.00
R5	6	0.79 \pm 0.35	0.00	11	2.13 \pm 0.60	1.32	3	0.17 \pm 0.13	0.00
R6	3	0.33 \pm 0.12	2.50	17	3.48 \pm 0.68	0.47	12	1.50 \pm 0.39	9.50
R7	1	0.17 \pm 0.11	0.00	6	2.67 \pm 0.73	0.23	3	0.67 \pm 0.31	0.33
R8	1	0.10 \pm 0.06	0.50	4	0.80 \pm 0.26	0.60	0	0.00 \pm 0.00	0.00

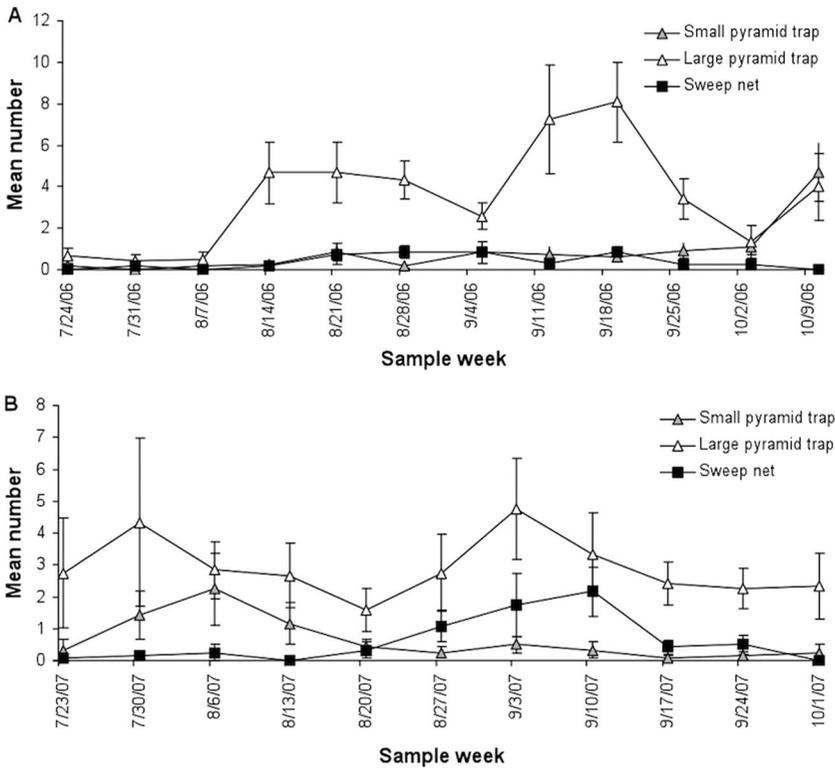


Fig. 3. Comparison of sampling methods for *H. halys* in soybean in Emmaus, PA in (A) 2006 and (B) 2007.

bean during 2006 and 2007 revealed that all sampling methods caught *H. halys* and native stink bug species (Fig. 4). Each year, a significant difference between sampling methods was observed (2006: $F = 8.46$, $df = 2, 15$, $P = 0.0035$; 2007: $F = 6.88$, $df = 2, 15$, $P = 0.0076$). Univariate contrast tests indicate significant differences between the large pyramid trap and both the small pyramid trap and sweep net samples ($P < 0.05$) each year. In both years, the small pyramid trap and sweep net samples captured lower densities of *H. halys*. In 2006, repeated measures MANOVA indicated a significant effect of sample date ($F = 9.11$; $df = 3.72$; 55.84 ; $P < 0.0001$). There was also a significant interaction between treatment and sample date ($F = 6.80$; $df = 7.45$; 55.84 ; $P < 0.0001$). The changing suitability and attractiveness of the crop at different phenological stages is the reason for this interaction. In 2007, no effect of sample date ($F = 2.38$; $df = 2.68$; 40.23 ; $P = 0.09$) or the interaction of treatment and sample date effect ($F = 1.09$; $df = 5.36$; 40.23 ; $P = 0.38$) was found. The ability to detect populations and life-stages of *H. halys* by each trapping method varied with the large pyramid trap detecting significantly larger populations of *H. halys* (Figs. 3 and 4).

Stage-Specific Occurrence. Pyramid traps containing aggregation pheromone were attractive to second, fifth instars and both adult sexes. These life stages were also captured in sweep net samples (Fig. 4). Nymphs were attracted to the pyramid traps throughout the season and were detected in the traps earlier in the

season than were found with sweep net samples. Specifically, the pyramid traps caught higher numbers of small nymphs than sweep samples which had a high density of fifth instars. Males and females were found at significantly greater proportions in the large pyramid trap than the sweep net, but no significant differences occurred between sampling methods for the nymphal stages (Fig. 5) (Male: $F = 3.29$, $df = 2, 30$, $P = 0.05$; Female: $F = 3.46$, $df = 2, 30$, $P = 0.04$; second: $F = 1.02$, $df = 2, 30$, $P = 0.37$; third: $F = 0.71$, $df = 2, 30$, $P = 0.50$; fourth: $F = 1.24$, $df = 2, 30$, $P = 0.30$; fifth: $F = 2.41$, $df = 2, 30$, $P = 0.11$). There was no significant interaction between treatment and year for any life-stage, thus data were averaged between years.

Discussion

The research presented here is the first to document the occurrence of *H. halys* in United States soybean. As with native pentatomids, *H. halys* seasonality coincided with the development of soybean seed pods. Peak abundance found by sweep samples was during the R5–R7 stages when the seed pods begin to develop through maturity. Positive population growth was indicated during the R3 and R4 stages by the capture of early instar *H. halys* in pyramid traps. Sweep net samples also showed positive population growth later in the season, during the R6 stage because of the high densities of late instars that were detected. High *H.*

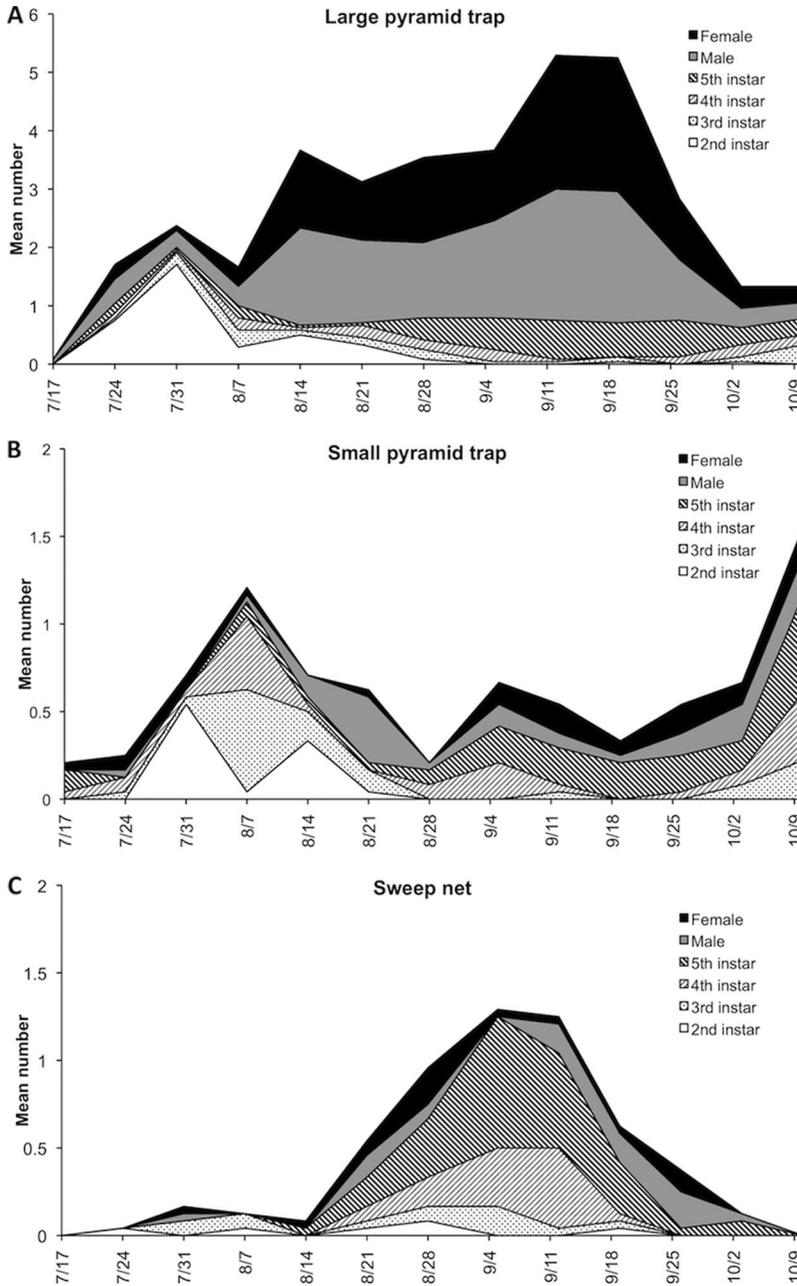


Fig. 4. Seasonal dynamics of *H. halys* in Pennsylvania soybean field collected by (A) large pyramid trap, (B) small pyramid trap, and (C) sweep net.

halys densities and nymph:adult ratios in the pyramid traps during R3–R6 soybean stages suggest that the traps are more efficient at detecting nymphal populations than sweep net samples. However, pyramid traps are continuously present in the landscape and are able to attract insects during all active periods. The R3–R6 phenological stages coincide with the time when economic loss is greatest from stink bug feeding (McPherson and McPherson, 2000). Although damage estimates were not conducted during this study,

the development of treatment thresholds is warranted. To prevent high levels of damage, a successful monitoring program should be used.

We were successful at employing pyramid traps as a monitoring tool in soybean for *H. halys* adults and nymphs during the susceptible period for yield loss. The seasonality of all life stages suggests that development occurs in soybean but the high population densities of adult stages for an extended period also suggests that some of the population dispersed there

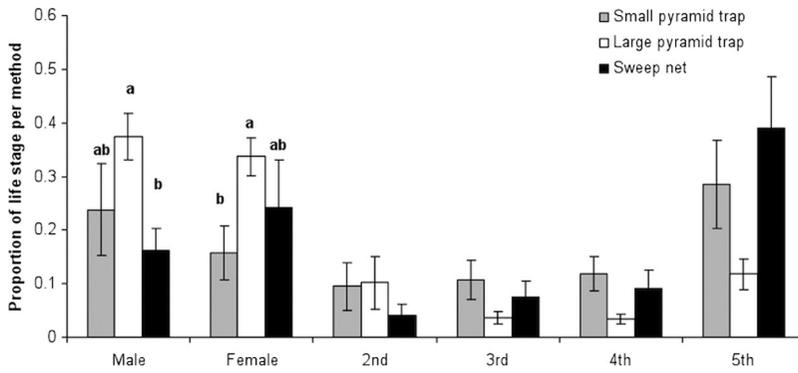


Fig. 5. Comparison of seasonal abundance of *H. halys* by life-stage as indicated by sampling method in Emmaus, PA. Values are mean \pm SE. For each significant parameter determined by ANOVA, sampling means with the same letter are not significantly different ($P \leq 0.05$, Tukey's honestly significant difference).

after completing development on other host plants. We observed that canopy cover appears to impact the effectiveness of the trap or pheromone plume as the canopy closed over the small pyramid trap during the R5 stage in our study and levels of *H. halys* captured decreased.

Two trends emerged that highlight the potential of using yellow pyramid traps for monitoring *H. halys* in soybeans: (1) successful capture of adults and nymphs at population peaks when soybean is susceptible to damage; (2) traps captured adults and nymphs earlier in the season than they were detected in sweep net samples. While we acknowledge that these results are based from one study site, this research documents the presence of *H. halys* in soybean and describes its seasonality at that site, where it is univoltine. The large pyramid trap consistently was the most effective monitoring tool, especially for detecting early populations in soybean.

Monitoring of invasive species can identify shifts in species abundance. While the previous populations densities of native pentatomids are unknown, *H. halys* was considerably more abundant than all native species combined in the sweep net, which represents an unbiased sample. It was expected that densities of *H. halys* in the pyramid traps would be higher than levels of native species because of differential response to chemical cues. However, the action of *P. stali* pheromone (methyl (E,E,Z) 2,4,6-decatrienoate) or other aggregation pheromones as an attraction to other stink bug species is not uncommon and has been suggested to act as a kairomone (Aldrich et al. 2007). *Thyanta* spp. produce methyl (E,Z,Z) 2,4,6 decatrienoate as a sex pheromone and were occasionally found in the pyramid traps. Field observations of late-summer *H. halys* aggregations often have *A. hilare* present suggesting that *H. halys* pheromone may be used this stink bug species for host finding and identifying overwintering locations. In natural populations, aggregation pheromone is likely produced early in the season to identify food resources (and possibly mates) and late in the season just before diapause (Aldrich 1988).

In the area of introduction (PA), *H. halys* has become the predominant species of stink bug detected

in our sampling studies and its phenology closely follows soybean development. Laboratory studies have shown that *H. halys* requires 538 DD (50°F base temperature) to complete development from egg to adult (Nielsen et al. 2008) and climate data supports that *H. halys* can have multiple generations per year in the warmer parts of the United States (Nielsen et al. 2008). As *H. halys* expands its range and becomes established in the other regions of the United States, it will likely become a significant pest of soybean and other crops. These results could easily be applied to other field crops, such as cotton. Our results suggest that in a three year period (2005–2007) the invasion of *H. halys* into a habitat substantially changed pentatomid species dominance. Total impact of *H. halys* in soybean still needs to be determined by assessing damage and developing economic and treatment thresholds.

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