

Impact of predation on establishment of the soybean aphid, *Aphis glycines* in soybean, *Glycine max*

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Abstract. The soybean aphid, *Aphis glycines* Matsumura is a new invasive pest of soybean in North America. We studied the ability of the existing predator community in soybean to reduce *A. glycines* establishment in field studies using either predator exclusion, open, or leaky cages that allowed aphid emigration but limited predation. Cages were infested with uniform initial densities of *A. glycines* adults and subsequent populations of aphids and predators were monitored over 24 h. The most abundant predators in these trials included the carabid beetles *Elaphropus anceps* (Le Conte), *Clavina impressifrons* Le Conte, *Bembidion quadrimaculatum* Say and spiders (Salticidae and Lycosidae). Foliar predators were less abundant and included; *Harmonia axyridis* Pallas, *Coccinella septempunctata* (L.), and *Orius insidiosus* (Say). Over the 2-year study, we found statistically significant predation on adult *A. glycines* in one out of six trials at 15 h and two out of six trials at 24 h. There was never significant evidence for predation of nymphs in any trial, however overall survival (adults + nymphs) was significantly reduced in one out of six trials at 15 h and three out of six trials at 24 h. Based on these results we suggest that generalist predators can be a significant but variable factor influencing the establishment of *A. glycines* populations in soybean. The impact of existing predator communities should be further investigated as a means of managing *A. glycines* populations in North American soybean production systems.

Key words: biological control, invasive species, predator communities

Introduction

The soybean aphid (*Aphis glycines* Matsumura) (Homoptera: Aphididae) is a new invasive insect herbivore in North America, first discovered in Wisconsin in 2000 infesting soybean (*Glycine max* L.) (Wedburg, 2000). In Asia, *A. glycines* feeding on soybean causes up to a

20 cm reduction in growth and a 27.8% reduction in seed yield (Wang et al., 1996). In Michigan, populations in excess of 13,000 aphids per plant and 40% losses in seed yield have been recorded (DiFonzo and Hines, 2002). Macedo et al. (2003) measured up to 50% reduction in photosynthetic rates for soybean leaves infested with *A. glycines*. Feeding by *A. glycines* can indirectly harm soybeans by vectoring alfalfa mosaic, soybean dwarf, soybean stunt, soybean mosaic, tobacco ring-spot, and bean yellow mosaic viruses (Van Den Berg et al., 1997; Clark and Perry, 2002; Wang and Ghabrial, 2002). Soybean aphids also cause indirect damage by excreting honeydew, promoting the growth of sooty molds that reduce photosynthetic capacity (Hirano et al., 1996). Development of effective management systems for this new invasive pest requires an understanding of the role of existing predator communities in *A. glycines* population dynamics (Rutledge et al., 2004).

Aphis glycines has a heteroecious holocyclic life cycle with overwintering occurring on various species of buckthorn (Rhamnaceae: *Rhamnus* spp.), and asexual stages on the secondary host plant, soybean (Wang et al., 1962; Ragsdale et al., 2004). In the spring, alate viviparous females migrate to soybean where they produce live young (Ragsdale et al., 2004). Soybean aphids reproduce parthenogenetically while on soybeans, and can increase their numbers rapidly. The survival of initial migrants and their offspring is critical to determining if infestations will develop early in the season.

Generalist predators can play a significant role in reducing herbivore populations in crop fields (Symondson et al., 2002). For example, ground beetles (Coleoptera: Carabidae) reduced populations of *Aphis fabae* Scopoli, a pest of sugar beet (Hance, 1987); and *Cantharis lateralis* L. (Coleoptera: Cantharidae) helped control establishing *A. fabae* in sugar beet fields in the Netherlands (Landis and Van der Werf, 1997). A complex of ground-dwelling predators, including carabids, and spiders, contributed to control of bird cherry–oat aphid, *Rhopalosiphum padi* (L.) in spring barley (Östman et al., 2001) and wheat aphids in winter wheat (Lang, 2002). While generalist predators are not as effective *per capita* as specialized predators, they can often compensate by being present earlier in the season (Chang and Kareiva, 1999), when pest densities are low and specialist predators scarce (Takagi, 1999).

In China, both predators and parasitoids contribute to *A. glycines* suppression (Liu et al., 2004) while during the initial years of *A. glycines* occurrence in the US, generalist predators dominated the natural enemy community (Fox, 2002; Fox and Landis, 2003; Rutledge et al., 2004). *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and *Orius insidiosus* (Say) (Heteroptera: Anthrocridae) were among the most

numerous and most commonly observed predators attacking aphids in the US during midseason (Fox, 2002; Rutledge et al., 2004). Previous studies have documented the importance of generalist predators in suppressing established populations of *A. glycines* (Fox et al., 2004), however, no studies have examined predation effects on initial *A. glycines* establishment. The first objective of this study was to determine the abundance and species composition of potential *A. glycines* predators in plots bordering natural enemy refuge habitats and control plots. Secondly we sought to determine if predation is a significant factor influencing early season *A. glycines* establishment in soybean.

Materials and methods

Sites

In 2001, studies were conducted at the Michigan State University Entomology Farm, Ingham County, MI. The site was established in 1994 (Carmona and Landis, 1999) and consisted of paired 30 m × 30 m plots arranged in a randomized complete block design with four replications. Refuge plots differed from their paired control plot in that they contained a central 3.2 m strip consisting of a combination of orchard grass (*Dactylis glomerata* L.), white clover (*Trifolium repens* L.), and several flowering perennial plants. With the exception of the central strip, both control and refuge plots were planted to soybean. One half of each plot was randomly selected and used for either predation studies or predator sampling during June of 2001. The outer 6 m of the field was not used to minimize edge effects. The crop area was managed using reduced primary tillage (chisel plow, disc) followed by secondary tillage (field cultivation). The herbicide metolachlor (Dual II) was applied at a rate of 2 l/ha. Potash was applied at a rate of 168 kg/ha to meet soil test requirements. Soybeans (Mycogen 5251RR) were planted on 5 May 2001 in 38 cm rows at a rate of 70,822 seeds/ha.

In 2002, studies were conducted at the Michigan State University Crop and Soil Sciences Research Farm, Ingham County, Michigan. The study site was arranged in five 12 m × 24 m blocks. The outer 6 m of the field was not used to minimize edge effect. Each block was divided in half, with each 12 m × 12 m area randomly designated for either predator sampling or predation studies. The crop area was managed using reduced tillage (fall chisel plow, secondary tillage with cultivator). Planting took place on 5 May, 2002 in 38 cm rows. The cultivar used

was Mycogen 5251RR at a population density of 68,825 seeds/ha. Alpine liquid fertilizer (6-24-6 Fortified) was applied on 5 May at a rate of 38 l/ha.

Insects

In both 2001 and 2002, *A. glycines* were obtained from a laboratory colony maintained by the USDA-APHIS-PPQ Invasive Pests Management Laboratory in Niles, Michigan, where they were reared on Asgrow #3303 cultivar soybeans at 26 °C and 60% RH, 16:8 (L:D). Upon arrival, aphids were held on the same cultivar in growth chambers at 25 °C, 70% RH, and 16:8 (L:D) before use.

Predator abundance

In both 2001 and 2002, ground dwelling predator abundance and composition was assessed using six 8.5 cm wide by 13 cm deep pitfall traps per plot (two rows of three traps). Ethylene glycol was used as the killing and preservative agent. Pitfalls were placed at a distance of 4 m and 8 m from the refuge or control strip, and 3.7 m distant from the previous trap with rims at the substrate level. Pitfall samples were collected every 48 h during trials. In addition, there was one 1.0 m × 0.3 m area in each sampling plot where both ground and foliar predator density and species composition were recorded during 5 min visual observations. The methodology was similar during 2002, except pitfall traps were placed in two rows in each block half, and the 1 m × 30 cm predator observation area was randomly placed over a pitfall trap in each block. Visual observations were all conducted between the hours of 10:00 and 14:00. In 2001, most predators were observed in the first few minutes, thus in 2002 visual observations were reduced to 3 min.

A. glycines survival and reproduction

Early season *A. glycines* survival and reproduction were studied by confining *A. glycines* on soybeans in clip cages allowing variable access to predators. Three predator exclusion treatments consisting of open, exclusion, and leaky clip cages were used. Clip cages were constructed of 1 cm (interior), 1.8 cm (outside) diameter Cresline® PVC pipe cut into 1 cm lengths. The top was covered with fine-mesh brass screen (33 threads/cm) which allowed air exchange but kept aphids enclosed. Leaky cages had a 3 mm hole in the cage wall that was initially closed with a small cork. Exclusion cages were intended to completely confine

aphids preventing all predator entry or aphid escape. With the cork removed, leaky cages allowed aphid emigration but greatly restricted predation. Finally, open cages allowed both emigration and full access by predators. Contrasting final aphid numbers in open vs. exclusion cages provides an estimate of total aphid loss to predation and emigration, while contrasting open vs. leaky cages provides an estimate of predation alone. While never observed, it is possible that small predators such as *Orius* may have occasionally entered leaky cages, thus the contrast of open vs. leaky cages is a conservative estimate of total predation.

Three trials were performed during 2001; 7–8 June (plants in V2 stage), 14–15 June (V3), and 24–25 June (V5), and three trials during 2002; 18–19 June (V3), 24–25 June (V5), and 2–3 July (V6). Natural *A. glycines* populations were first observed on 12 June 2001 at an initial density of 7.8 aphids/m² and on 14 June 2002 at 1.2 aphids/m². In 2001, three adult female *A. glycines* were placed in each clip cage for trial one, and four per cage during trials two and three. Four replicates of each cage type were placed in each of four blocks. In 2002, five adult *A. glycines* were used per cage with six replicates of each cage type placed in each of five blocks. For all treatments, aphids were initially transferred into clip cages using a fine camel-hair brush; then cages were clipped on the underside surface of soybean leaves. Cages were placed on the uppermost fully expanded trifoliolate of selected plants at 8:00 AM and *A. glycines* were given a 6 h acclimation period to settle. Once confined to soybean leaves, *A. glycines* settled and began to feed within several hours. At the end of the acclimation period, cages were completely removed from open treatments. Exclusion cages were briefly opened and then replaced, while leaky cages were briefly opened and replaced but had the cork removed. During 2001, cages were placed 4 m away from control or refuge strips, while in 2002 cages were randomly located within plots. In both years, at 8:00 AM (15 h) and 4:00 PM (24 h) the following day, data on the proportion of live adult *A. glycines* remaining and the number of nymphs produced in each cage were recorded.

In 2001, a preliminary test was conducted to assess cage performance. Leaky and exclusion cages containing five aphids each were clipped onto wooden pot markers placed in the field. Comparison of *A. glycines* numbers after 24 h in these treatments allowed us to evaluate the propensity for *A. glycines* to escape or emigrate from the different cages. Under these conditions (i.e. no food or water resources) *A. glycines* readily dispersed from leaky cages but were unable to escape exclusion cages. In one trial, 100% of the *A. glycines* left the leaky cage

by 15 h, while in a second, 89.3% left by 15 h and 98.2% by 24 h. No aphids left exclusion cages and survival at 24 h was very low (0–7%).

Data analysis

Data from 2001 from predator observation areas were summarized as the mean number of predators per 5 min in refuge vs. control plots. Data from 2001 pitfall traps were analyzed as the mean ($n = 4$) number of predators/trap/day in refuge vs. control plots. A type III F -test for overall treatment effect determined statistical significance of treatment effect of pitfall and direct predator observation data. Data from 2002 predator observations were summarized as the mean ($n = 5$) number of predators per 3 min and pitfall data were summarized as the mean number of predators/trap/day. During 2001 and 2002, data on the proportion of live adult aphids and on the number of nymphs produced were analyzed by logistic and Poisson regression, respectively, using the GLIMMIX Macro of SAS (SAS Institute, 2000). A factorial analysis was applied using refuge (near or far), predation (exclusion, leaky and open cages) and time of observation (15 or 24 h) as fixed effects. Separate analyses were performed for each trial. When a significant treatment \times hour interaction existed, data were sliced to reveal individual significant comparisons with least square means (LS MEANS). The number of adults entering the trial (after acclimatization) differed between treatments and significantly affected the number of nymphs produced. Therefore, the number of adult *A. glycines* at the beginning of the trial was used as a covariate to nymph data to equalize treatments. Two factors suggested that a p value of 0.1 should be considered significant in this study. First, the relatively short 24 h exposure period coupled with the conservative estimate of total predation increased the chance of accepting the null hypothesis of no predator impact. Second, as the first test of its kind on *A. glycines* in North America, there was no preliminary data with which to predict the power of the tests used. All results are presented as means, standard errors and confidence intervals of untransformed data.

Results

Refuge habitat effects 2001

Proximity to refuge habitats did not significantly affect the number of predators observed in the 1.0 m \times 0.3 m plots during any of the three

trials ($F = 2.00$, $df = 1$, 18 , $p = 0.20$; $F = 0.10$, $df = 1$, 18 , $p = 0.90$; $F = 0.34$, $df = 1$, 18 , $p = 0.60$). Pitfall trap catches were significantly higher ($F = 2.90$, $df = 1$, 18 , $p = 0.04$) in control vs. refuge plots during trial one; however, this effect did not persist into trials two or three ($F = 0.08$, $df = 1$, 18 , $p = 0.93$; $F = 0.40$, $df = 1$, 18 , $p = 0.54$). Similarly, there was no significant effect of refuge on predation of *A. glycines* adults or nymphs during any trial during 2001. Though there was a significant interaction between refuge \times predation \times hour during trial three for adults ($F = 3.12$, $df = 2$, 168 , $p = 0.05$), slicing of the analysis showed that this interaction was not associated with refuge, therefore, refuge and control plot data were pooled for subsequent analysis.

Predator density 2001–2002

In 2001, predator density was greatest in trial one (4.1 ± 0.7 predators observed per 5 min), lowest in trial two (1.0 ± 0.3), and intermediate in trial three (2.5 ± 0.9) with ground dwelling predators predominant in all but trial three (Table 1). In trial one, the carabid beetle *Elaphropus anceps* (Le Conte) was the most abundant predator, accounting for 70.7% of the total number of predators observed. During trial two, *E. anceps* was again the most abundant predator, accounting for 55.6% of predators observed, with foliar-foraging predators making their first appearance. *Coccinella septempunctata* (L.) adults were the second most abundant taxa accounting for 27.8% of the predators. In contrast, during trial three, foliar-foraging predators were more abundant with *Harmonia axyridis* (Pallas) larvae (39.5%), *H. axyridis* adults (18.6%) and *Orius insidiosus* (Say) (14%) comprising most observed predators. Spiders in the families Salticidae and Lycosidae were also observed. However, in lab no-choice trials these spiders did not consume significant numbers of soybean aphid (Fox, unpublished data) and were thus not identified further. Predator density in 2002 was much lower than in 2001, peaking at 0.5 ± 0.2 predators per 3 min in trial two with densities of 0.8 ± 0.5 and 0.5 ± 0.3 in trials one and three, respectively (Table 1).

Pitfall captures 2001–2002

Pitfall captures in 2001 were greatest in trial one and lowest in trial three. Almost all of the ground-dwelling predators captured were carabid beetles. In trial one, *E. anceps* predominated in refuge and control plots accounting for 51.1% of the total catches (Table 2). In trial two, however, *Scarites quadriceps* Chd., 35.2%, was most

Table 1. Species composition, total number and percent of total potential *A. glycines* predators in 5-min observations in 1.0 m × 0.3 m areas of soybean during three trials in 2001 (7–8 June), (14–15 June), and (24–25 June) respectively; and 3-min observations during three trials in 2002 (18–19 June), (24–25 June), and (2–3 July), East Lansing, Michigan

Family	Species	Trial one		Trial two		Trial three	
		Total	%	Total	%	Total	%
2001							
Heteroptera: Anthocoridae	<i>Orius insidiosus</i> (Say) adults	–	–	–	–	6	14.0
Coleoptera: Carabidae	<i>Bembidion</i> spp.	3	4.6	–	–	1	2.3
	<i>Bembidion quadrimaculatum</i> Say	10	15.4	1	5.6	2	4.7
	<i>Elaphropus anceps</i> (Le Conte)	46	70.7	10	55.6	7	16.3
Coleoptera: Coccinellidae	<i>Coccinella septempunctata</i> (L.) adults	–	–	5	27.7	–	–
	<i>Harmonia axyridis</i> (Pallas) adults	–	–	–	–	8	18.6
	<i>Harmonia axyridis</i> (Pallas) larvae	–	–	–	–	17	39.4
	<i>Hippodamia convergens</i> Guerin adult	–	–	–	–	2	4.7
Other	Spiders ¹	6	9.3	2	11.1	–	–
Total		65	100.0	18	100.0	36	100.0
2002							
Heteroptera: Anthocoridae	<i>Orius insidiosus</i> (Say) adults	–	–	–	–	1	20.0
Coleoptera: Coccinellidae	<i>Coccinella septempunctata</i> (L.) adults	2	40.0	1	12.5	1	20.0
	<i>Hippodamia convergens</i> (L.) adults	1	20.0	–	–	–	–
Heteroptera: Nabidae	<i>Nabis</i> spp.	–	–	–	–	1	20.0
Other	Spiders ¹	2	40.0	7	87.5	2	40.0
Total		5	100.0	8	100.0	5	100.0

¹Primarily Salticidae and Lycosidae.

Table 2. Species composition, total number, and percent of total of predators collected in pitfall traps in refuge and control plots during three trials in 2001 (7–8 June), (14–15 June), and (24–25 June) respectively; and three trials in 2002 (18–19 June), (24–25 June), and (2–3 July), East Lansing, Michigan

Family	Species	Trial one		Trial Two		Trial Three	
		Total	%	Total	%	Total	%
2001							
Coloeptera: Carabidae	<i>Agonum cupripenne</i> Say	–	–	–	–	1	8.3
	<i>Amara aenea</i> (DeG.)	2	1.9	–	–	–	–
	<i>Anisodactylus santaecrusis</i> (F.)	3	2.9	–	–	–	–
	<i>Bembidion quadrimaculatum</i> Say	3	2.9	1	1.4	–	–
	<i>Bembidion</i> spp.	5	4.9	–	–	–	–
	<i>Clavina bipustulata</i> (F.)	1	1.0	8	11.3	2	16.7
	<i>Clavina impressifrons</i> Le Conte	12	11.7	10	14.1	3	25.0
	<i>Elaphropus anceps</i> (Le Conte)	53	51.5	9	12.7	1	8.3
	<i>Harpalus affinis</i> (Schrank)	1	1.0	4	5.6	–	–
	<i>Harpalus herbivigus</i> Say	–	–	1	1.4	–	–
	<i>Harpalus pensylvanicus</i> (DeG.)	–	–	1	1.4	–	–
	<i>Poecilus chalcites</i> (Say)	9	8.7	3	4.2	–	–
	<i>Poecilus lucublandus</i> (Say)	2	1.9	2	2.8	–	–
	<i>Pterostichus melanarius</i> (III.)	1	1.0	5	7.0	–	–
	<i>Scarites quadriceps</i> Chd.	8	7.8	25	35.2	1	8.3
	<i>Scarites subterraneus</i> F.	–	–	–	–	4	33.3
	<i>Stenolophus comma</i> (F.)	3	2.9	2	2.8	–	–
Total		103	100.0	71	100.0	12	100.0

Table 2. Continued.

Family	Species	Trial one		Trial Two		Trial Three	
		Total	%	Total	%	Total	%
2002							
Coleoptera: Carabidae	<i>Antisodactylus santacruis</i> (F.)	13	16.0	1	1.4	—	—
	<i>Bembidion quadrimaculatum</i> Say	—	—	—	—	1	8.3
	<i>Bembidion</i> spp.	2	2.5	—	—	—	—
	<i>Bembidion rapidum</i> (LeC.)	11	13.6	12	17.4	—	—
	<i>Clavina bipustulata</i> (F.)	12	14.8	5	7.2	—	—
	<i>Clavina impressifrons</i> Le Conte	19	23.5	22	31.9	6	50.0
	<i>Harpalus affinis</i> (Schrank)	3	3.7	6	8.7	1	8.3
	<i>Poecilus chalcites</i> (Say)	2	2.5	8	11.6	2	6.7
	<i>Poecilus lucublandus</i> (Say)	4	4.9	6	8.1	—	—
	<i>Pterostichus melanarius</i> (III.)	2	2.5	—	—	—	—
	<i>Scarites quadriceps</i> Chd.	3	3.5	5	7.2	1	8.3
	<i>Scarites subterraneus</i> F.	—	—	—	—	1	8.3
	<i>Stenolophus comma</i> (F.)	10	12.3	4	5.8	—	—
Total		81	100.0	69	100.0	12	100.0

abundant. During trial three, *Scarites subterraneus* F., 33.3%, was most abundant. In 2002, total trap captures were very similar to 2001; however, the species collected differed (Table 2). Notably, *E. anceps* was missing from 2002 collections and in all three trials. *Clavina impressifrons* Le Conte was the most abundant predator, comprising 23.5% of the catches in trial one, 31.9% in trial two, and 50% in trial three. Both *E. anceps* and *C. impressifrons* have been shown to be capable of consuming *A. glycines* in laboratory feeding assays (Rutledge et al., 2004).

Predator effects on A. glycines survival and reproduction

Adult survival 2001

Analysis of predator exclusion treatments revealed a significant treatment effect in trials one and two ($F = 3.31$, $df = 2$, 158, $P = 0.04$; $F = 4.66$, $df = 2$, 167, $p = 0.01$) (Figure 1). There was a significant treatment \times hour interaction in trials one and three ($F = 2.45$, $df = 2$, 158, $p = 0.09$; $F = 2.47$, $df = 1$, 168, $p = 0.09$), due to differential numbers remaining in open vs. exclusion treatments at 15 or 24 h. There was also a highly significant hour effect ($F = 27.63$, $df = 1$, 158, $p < 0.001$; $F = 16.33$, $df = 1$, 167, $p < 0.001$; $F = 26.51$, $df = 1$, 168, $p = 0.01$), with fewer adult *A. glycines* remaining at 24 h than at 15 h in all trials. In trial one, significantly fewer adult *A. glycines* remained in open vs. exclusion cages ($p = 0.03$) and open vs. leaky cages at 15 h ($p = 0.02$). In trial two, significantly fewer adult *A. glycines* remained in open vs. exclusion cages ($p = 0.01$) and leaky vs. exclusion cages at 15 h ($p = 0.04$). At 24 h in trial two there were significantly fewer adult *A. glycines* in open vs. exclusion cages ($p = 0.001$), and leaky vs. exclusion cages ($p = 0.07$). In trial three no significant effects occurred.

Nymphs produced 2001

In 2001, the greatest number of *A. glycines* nymphs per cage were generally produced in the exclusion treatments, however, there was no significant treatment effect during any trial or hour period (Figure 1). In contrast, all three trials showed a significant hour effect ($F = 24.86$, $df = 1$, 157, $p < 0.001$; $F = 40.90$, $df = 1$, 166, $p < 0.001$; $F = 32.84$, $df = 1$, 167, $p < 0.001$), with more nymphs present at 24 vs. 15 h. In trial two, there was a significant treatment \times hour interaction ($F = 10.53$, $df = 2$, 166, $p < 0.001$), but slicing of the data did not reveal further significance based on pairwise comparisons.

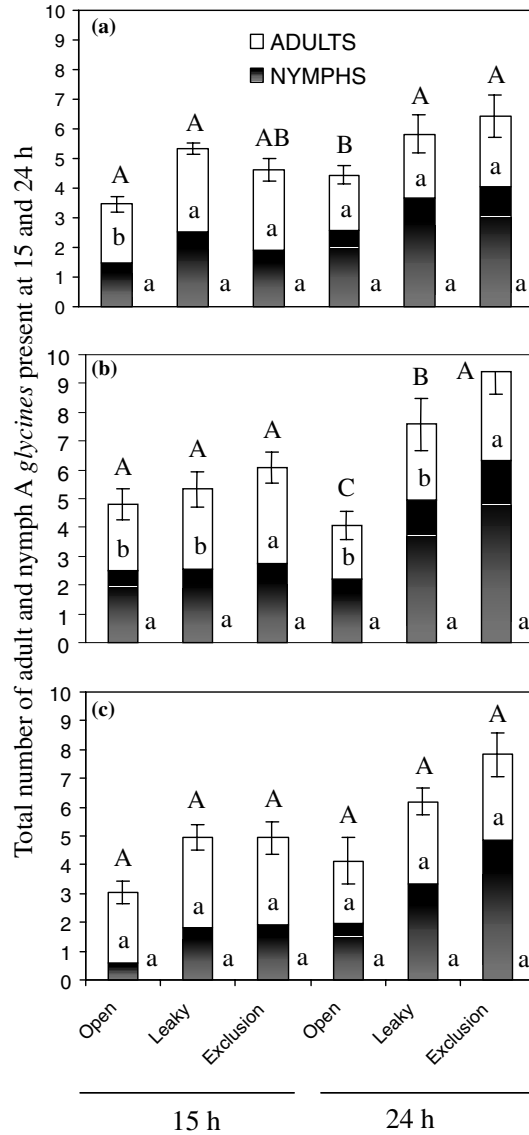


Figure 1. Adult, nymph and total *A. glycines* at 15 and 24 h for the open, leaky and exclusion treatments in refuge and control plots during (a) trial one (7–8 June 2001), (b) trial two (14–15 June 2001) and (c) trial three (24–25 June 2001), East Lansing, Michigan. Different letters above (total), in or beside (adult and nymph respectively) bars within an hour denote significant ($p \leq 0.1$) differences among open, leaky, and exclusion treatments (Poisson regression test).

Total aphids 2001

A comparison of total *A. glycines* number (adults + nymphs) remaining in the different treatments is shown in Figure 1. There was a significant effect of treatment in trials one and two ($F = 2.95$, $df = 2$, 157, $p = 0.06$; $F = 2.48$, $df = 2$, 166, $p = 0.09$) and there was also a significant treatment \times hour interaction in trial two ($F = 13.67$, $df = 2$, 166, $p < 0.001$). In all three trials, there was a significant hour effect ($F = 80.66$, $df = 1$, 157, $p = 0.004$; $F = 25.36$, $df = 1$, 166, $p < 0.001$; $F = 24.74$, $df = 1$, 167, $p < 0.001$), with fewer aphids remaining at 24 vs. 15 h. In trial one after 24 h, fewer total *A. glycines* were found in open vs. leaky treatments ($p = 0.03$), and in open vs. exclusion ($p = 0.007$) treatments. In trial two there were significantly fewer aphids in open vs. leaky ($p = 0.005$) and exclusion treatments ($p = 0.04$) at 24 h. In trial three, there were similar trends but no significant treatment effects.

Adult survival 2002

Analysis of predator exclusion treatments revealed a significant treatment effect in trials two and three ($F = 12.49$, $df = 2$, 83.9, $p < 0.001$; $F = 7.46$, $df = 2$, 73.2, $p < 0.001$) (Figure 2). There was a highly significant hour effect in all three trials ($F = 51.51$, $df = 1$, 99.2, $p < 0.001$; $F = 97.40$, $df = 1$, 97.4, $p < 0.001$; $F = 52.88$, $df = 1$, 80.4, $p < 0.001$) with more adults remaining at 15 than 24 h. During trial two, significantly fewer *A. glycines* adults were found in open vs. exclusion cages ($p = 0.002$) and leaky vs. exclusion cages ($p = 0.003$) at 15 h. At 24 h, significantly fewer adult *A. glycines* remained in open vs. exclusion ($p < 0.001$) and open vs. leaky cages ($p = 0.011$). During trial three there were significantly fewer adult *A. glycines* in open ($p = 0.002$) and leaky ($p = 0.006$) cages vs. exclusion at 15 h. At 24 h, open cages contained significantly fewer *A. glycines* adults than leaky ($p = 0.02$) or exclusion cages ($p = 0.003$).

Nymphs produced 2002

In 2002, the greatest number of nymphs were generally produced in leaky or exclusion treatments, but differences were not consistent (Figure 2). There was no significant treatment effect in trial one or three, but there was a significant treatment effect in trial two ($F = 6.48$, $df = 2$, 81.8, $p = 0.002$). There was a significant hour effect in trials one and two ($F = 24.23$, $df = 1$, 87.9, $p < 0.001$; $F = 6.46$, $df = 1$, 86, $p = 0.01$) with more nymphs at 15 than 24 h. In trial two there were significantly more nymphs in exclusion vs. open ($p = 0.03$, $p = 0.10$) or leaky cages ($p = 0.002$, $p = 0.007$) at 15 and 24 h,

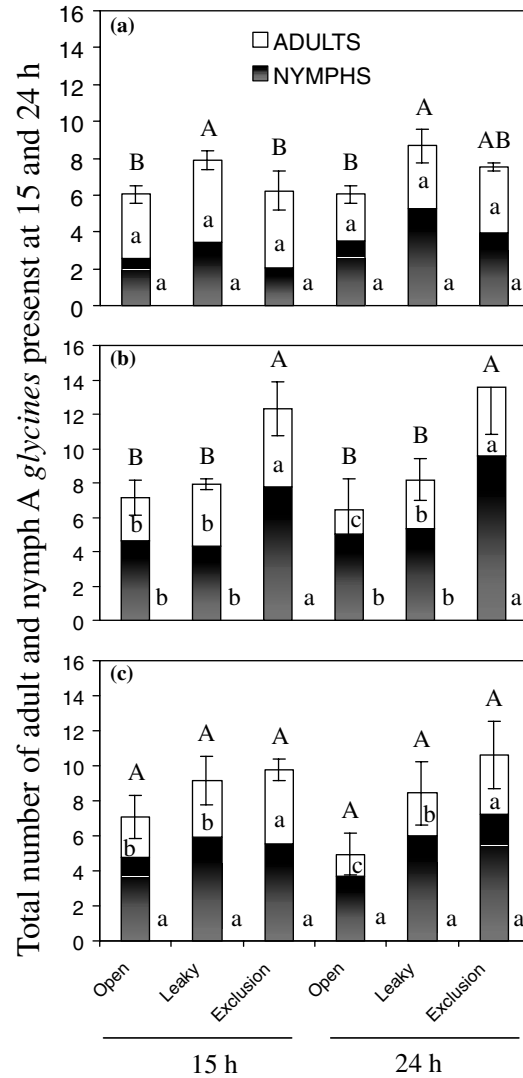


Figure 2. Adult, nymph and total *A. glycines* at 15 and 24 h for the open, leaky and exclusion treatments in refuge and control plots during (a) trial one (18–19 June 2002), (b) trial two (24–25 June 2002) and (c) trial three (2–3 July 2002), East Lansing, Michigan. Different letters above (total), in or beside (adult and nymph respectively) bars within an hour denote significant ($p \leq 0.1$) differences among open, leaky, and exclusion treatments (Poisson regression test).

respectively. There was no significant treatment effect in trial three, though there was a significant treatment \times hour interaction ($F = 24.23$, $df = 1, 83.9$, $p < 0.001$) but slicing this interaction did not reveal significant pairwise comparisons.

Total aphids 2002

In 2002, total *A. glycines* numbers per cage (adults + nymphs) were again higher in leaky and exclusion cages vs. open cages (Figure 2). In trials one and two, there was a significant treatment effect ($F = 2.41$, $df = 2$, 79.1 , $p < 0.09$; $F = 7.13$, $df = 2$, 81.2 , $p = 0.001$). There was a significant hour effect in trials one and two ($F = 3.16$, $df = 1$, 87.9 , $p = 0.08$; $F = 0.77$, $df = 2$, 84.8 , $p = 0.08$) with more aphids at 15 than 24 h. A significant treatment \times hour interaction was found in trials two and three ($F = 2.78$, $df = 2$, 84.8 , $p < 0.07$; $F = 2.9$, $df = 2$, 76.7 , $p = 0.06$). In trial one, there were significantly more *A. glycines* in leaky vs. exclusion and open treatments ($p = 0.06$) at 15 h, and leaky vs. open treatments ($p = 0.03$) at 24 h. In trial two, there were significantly more *A. glycines* in exclusion vs. open ($p = 0.01$) and exclusion vs. leaky cages ($p = 0.003$) at 15 and 24 h. In trial three, there were no significant treatment effects.

Discussion

Previous studies have shown that the presence of refuges can increase densities of generalist predators (Bowie et al., 1999; Carmona and Landis, 1999; Landis et al., 2000; Lee et al., 2001; Lee and Landis, 2002). However, in this study, refuge plots did not alter the abundance or species composition of either foliar-foraging or ground-dwelling predators, or predation of *A. glycines*. One possible reason for lack of a significant refuge effect was that plot size was relatively small compared to the mobility of the dominant aphid predators encountered.

Examining adult, nymph and total *A. glycines* survival in 2001 revealed several trends. First, the number of aphids surviving to 15 h was generally rather high. Although a trend of reduced numbers surviving in the more exposed treatments (leaky and open) was observed, it was seldom significant. More pronounced differences are seen at 24 h where a consistent trend for reduced numbers of aphids remaining in open treatments was observed. The best evidence of predation occurred when the aphid numbers in open treatments were significantly less than the leaky treatments. In this case open field losses are corrected for possible emigration effects and hereafter referred to as strong evidence that predation occurred. For this rather sedentary aphid, the case for predation is weaker but not excluded in cases where there was no significant difference between open and leaky cages but there was a difference between exclusion and open cages. It should be noted that potential microclimate differences exist between cages and open treatments. The short test duration was in part selected to minimize such effects.

Over the two years of the study, there was strong evidence for predation on adult *A. glycines* in one out of six trials at 15 h and two out of six trials at 24 h (i.e. numbers in open cages significantly less than leaky cages). There was never strong evidence for predation of nymphs in any trial, suggesting that young nymphs may be less preferred or more difficult for predators to locate. Finally, based on the total number of live *A. glycines* which established (adults + nymphs), there was strong evidence for predation in one out of six trials at 15 h and three out of six trials at 24 h.

Our studies also suggest which predators may be responsible for reductions in *A. glycines* establishment. Significant predation occurring at the 15 h mark can be attributed to the activity of nocturnal predators. While many carabid beetles were present in the plots and our no-choice tests show that most of the carabid species present could consume *A. glycines* (Fox, 2002), the field results show this predation to be limited. This is in contrast to other studies that found carabids significantly contribute to aphid suppression in cereal grains (Hance, 1987; Östman et al., 2001; Lang, 2002). Other investigators have noted an interaction between foliar predators that cause aphids to drop and predation by ground-dwelling predators (Losey and Denno, 1998). A preliminary study indicated that *A. glycines* do not drop in response to disturbance, thus ground-dwelling predators would have to climb plants to encounter most *A. glycines*. However, this study is in agreement with the findings of Chang and Kareiva (1999), and Takagi (1999), who suggest that generalist predators may be most effective by reducing initial pest populations before they reach outbreak levels. Even a low level of nocturnal feeding may thus reduce initial numbers so that diurnal predators foraging on soybean leaves would be better able to reduce populations.

During the later spring trials, foliar searching aphid predators became predominant and were responsible for aphid reduction. During the second and third trials in both years aphids were reduced the most at 24 h. During these studies predators were observed consuming *A. glycines* on eight separate occasions in the open treatment. This included observations of predation by early instar *H. axyridis* larvae and *O. insidiosus* nymphs. Considering the very low density of *A. glycines* in the field at this time, these observations of early season predation on *A. glycines* should not be underestimated and are consistent with the known ability of aphidophagous predators to search for and find prey at low densities (Grasswitz and Burts, 1995; Starý, 1995; Van Den Berg et al., 1997; Obrycki and Kring, 1998). Due to their importance as generalist predators in many systems (Symondson et al., 2002),

the potential impact of spiders on soybean aphid should be further studied.

These studies establish that predation is a significant but variable factor influencing the establishment of *A. glycines* populations in soybean. A complex of predators including both generalist and more specialized natural enemies contributed to these effects. Establishment success was greatest in the first trial each spring and lowest in the last. This may indicate a potentially important interaction between crop planting date, *A. glycines* arrival, and conservation of predators in the field. Further research should concentrate on means to improve the predictability of predators in reducing early season *A. glycines* survival and establishment.

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References

- Bowie, H.M., G.M. Gurr, Z. Hossain, L.R. Baggen and C.M. Frampton, 1999. Effects of distance from field edge on aphidophagous insects in a wheat crop and observations on trap design and placement. *Intl. J. Pest Manage.* 45: 69–73.
- Carmona, C.M. and D.A. Landis, 1999. Influence of refuge habitats and cover crops on seasonal activity-density of ground beetles (Coleoptera: Carabidae) in field crops. *Environ. Entomol.* 28: 1145–1153.
- Chang, G.C. and P. Kareiva, 1999. The case for indigenous generalists in biological control. In: B.A. Hawkins and H.V. Cornell (eds), *Theoretical Approaches to Biological Control*. Cambridge University Press, UK. pp. 103–115.
- Clark, A.J. and K.L. Perry, 2002. Transmissibility of field isolates of soybean viruses by *Aphis glycines*. *Plant Dis.* 86: 1219–1222.
- DiFonzo, C. and R. Hines, 2002. Soybean aphid in Michigan: update from the 2001 season. MSU Extension Bulletin E-2748. Michigan State University, E. Lansing MI.

- Fox, T.B., 2002. Biological control of the soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae). M.S. Thesis, Department of Entomology, Michigan State University.
- Fox, T.B. and D.A. Landis, 2003. Impact of habitat management on generalist predators of the soybean aphid, *Aphis glycines* Matsumura. In: *Proceedings, International Symposium on Biological Control of Arthropods*. Jan 14–18, 2002, Honolulu, HI. FHTET-03-05. United States Department of Agriculture, Forest Service, Forest Health Enterprise Team, Morgantown, WV. pp. 250–255.
- Fox, T.B., D.A. Landis, F.F. Cardoso and C.D. DiFonzo, 2004. Predators suppress *Aphis glycines* Matsumura population growth in soybean. *Environ. Entomol.* 33: 608–618.
- Grasswitz, T.R. and E. Burts, 1995. Effect of native natural enemies and augmentative releases of *Chrysoperla rufilabris* Burmeister and *Aphidoletes aphidimyza* (Rondani) on the population dynamics of the green apple aphid, *Aphid pomi* De Geer. *Intl. J. Pest Manage.* 41: 176–183.
- Hance, T., 1987. Predation impact of carabids at different population densities on *Aphis fabae* development in sugar beet. *Pedobiologia* 30: 251–262.
- Hirano, K., K. Honda and S. Miyai, 1996. Effects of temperature on development, longevity and reproduction of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). *Appl. Entomol. Zool.* 31: 178–180.
- Landis, D.A. and W. Van der Werf, 1997. Early-season aphid predation impacts establishment and spread of sugar beet yellows virus in the Netherlands. *Entomophaga* 42: 499–516.
- Landis, D.A., S.D. Wratten and G. Gurr, 2000. Habitat manipulation to conserve natural enemies of arthropod pests in agriculture. *Annu. Rev. Entomol.* 45: 173–199.
- Lang, A., 2002. Intraguild predation and biocontrol effects of generalist predators in a winter wheat field. *Oecologia* 134: 144–153.
- Lee, J.C. and D.A. Landis, 2002. Noncrop habitat management for carabid beetles. In: J. Holland (ed), *Carabid Beetles and Agriculture*, Intercept, Adover. pp. 279–303.
- Lee, J.C., F.D. Menalled and D.A. Landis, 2001. Refuge habitats modify insecticide disturbance on carabid beetle communities. *J. Appl. Ecol.* 38: 472–483.
- Liu, J., K. Wu, K.R. Hopper and K. Zhao, 2004. Population dynamics of *Aphis glycines* (Homoptera Aphididae) and its natural enemies in Northern China. *Ann. Entomol. Soc. Am.* 97: 235–239.
- Losey, J.E. and R.F. Denno, 1998. The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. *Ecol. Entomol.* 23: 53–61.
- Macedo, T.B., C.S. Bastos, L.G. Higley, K.R. Ostlie and S. Madhavan, 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *J. Econ. Entomol.* 96: 188–193.
- Obrycki, J.J. and T.J. Kring, 1998. Predaceous Coccinellidae in biological control. *Annu. Rev. Entomol.* 43: 285–321.
- Östman, Ö., E. Ekborn and J. Bengtsson, 2001. Landscape heterogeneity and farming practice influence biological control. *Basic Appl. Ecol.* 2: 365–371.
- Ragsdale, D.W., D.J. Voegtlin and R.J. O'Neal, 2004. Soybean aphid biology in North America. *Ann. Ent. Soc. Am.* 97: 204–208.
- Rutledge, C.E., R.J. O'Neil, T.B. Fox and D.A. Landis, 2004. Soybean aphid predators and their use in IPM. *Ann. Ent. Soc. Am.* 97: 240–248.
- SAS Institute, 2000. *SAS/STAT user's guide*, release 8.11 edn. SAS Institute, Cary, NC.

- Stary, P., 1995. Natural enemy spectrum of *Aphis spiraephaga* (Hom: Aphididae), an exotic immigrant aphid in central Europe. *Entomophaga* 40: 29–34.
- Symondson, W.O.C., K.D. Sunderland and M.H. Greenstone, 2002. Can generalist predators be effective biocontrol agents? *Annu. Rev. of Entomol.* 47: 561–594.
- Takagi, M., 1999. Perspective of practical biological control and population theories. *Res. Pop. Ecol.* 41: 121–126.
- Van Den Berg, H., D. Ankasah, A. Muhammad, R. Rusli, H.A. Widayanto, H.B. Wirasto and I. Yully, 1997. Evaluating the role of predation in population fluctuations of the soybean aphid, *Aphis glycines* in farmers' fields in Indonesia. *J. Appl. Ecol.* 34: 971–984.
- Wang, S.Y., X.Z. Boa, Y.J. Sun, R.L. Chen and B.P. Zhai, 1996. Study on the effects of the population dynamics of soybean aphid (*Aphis glycines*) on both growth and yield of soybean. *Soybean Sci.* 15: 243–247.
- Wang, R.Y. and S.A. Ghabrial, 2002. Effect of aphid behavior on efficiency of transmission of *Soybean mosaic virus* by the soybean-colonizing aphid, *Aphis glycines*. *Plant Dis.* 86: 1260–1264.
- Wang, C.L., N.I. Siang, G.S. Chang and H.F. Chu, 1962. Studies on the soybean aphid, *Aphis glycines* Matsumura. *Acta Entomol. Sin.* 11: 31–44.
- Wedburg, J., 2000. Important update on aphids in soybeans. Wisconsin Crop Manager. August 17, 2000. <http://ipcm.wisc.edu/wcm/pdfs/2000/00-22insect1.html>.