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Author(s): Wayne J. Ohnesorg, Kevin D. Johnson, and Matthew E. O'Neal
Source: Journal of Economic Entomology, 102(5):1816-1826. 2009.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/029.102.0512
URL: http://www.bioone.org/doi/full/10.1603/029.102.0512

Impact of Reduced-Risk Insecticides on Soybean Aphid and Associated Natural Enemies

WAYNE J. OHNESORG, KEVIN D. JOHNSON, AND MATTHEW E. O'NEAL[1]

Iowa State University, Department of Entomology, 113A Insectary, Ames, IA 50011

J. Econ. Entomol. 102(5): 1816–1826 (2009)

ABSTRACT Insect predators in North America suppress *Aphis glycines* Matsumura (Hemiptera: Aphididae) populations; however, insecticides are required when populations reach economically damaging levels. Currently, insecticides used to manage *A. glycines* are broad-spectrum (pyrethroids and organophosphates), and probably reduce beneficial insect abundance in soybean, *Glycine max* (L.) Merr. Our goal was to determine whether insecticides considered reduced-risk by the Environmental Protection Agency could protect soybean yield from *A. glycines* herbivory while having a limited impact on the aphid's natural enemies. We compared three insecticides (imidacloprid, thiamethoxam, and pymetrozine,) to a broad-spectrum insecticide (λ-cyhalothrin) and an untreated control using two application methods. We applied neonicotinoid insecticides to seeds (imidacloprid and thiamethoxam) as well as foliage (imidacloprid); pymetrozine and λ-cyhalothrin were applied only to foliage. Foliage-applied insecticides had lower *A. glycines* populations and higher yields than the seed-applied insecticides. Among foliage-applied insecticides, pymetrozine and imidacloprid had an intermediate level of *A. glycines* population and yield protection compared with λ-cyhalothrin and the untreated control. We monitored natural enemies with yellow sticky cards, sweep-nets, and direct observation. Before foliar insecticides were applied (i.e., before aphid populations developed) seed treatments had no observable effect on the abundance of natural enemies. After foliar insecticides were applied, differences in natural enemy abundance were observed when sampled with sweep-nets and direct observation but not with yellow sticky cards. Based on the first two sampling methods, pymetrozine and the foliage-applied imidacloprid had intermediate abundances of natural enemies compared with the untreated control and λ-cyhalothrin.

KEY WORDS *Glycine max*, biological control, insecticide regulation, conservation, nontarget impacts

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In North America, natural enemies, particularly foliage inhabiting predators, can suppress *Aphis glycines* Matsumura (Hemiptera: Aphididae) populations (Fox et al. 2004, 2005, Rutledge and O'Neil 2005, Mignault et al. 2006, Schmidt et al. 2007). Of the several species of predators that can be found in soybean, *Glycine max* (L) Merr. (Bechinski and Pedigo 1981, Schmidt et al. 2008), two have been identified as key predators of *A. glycines* in North America, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Rutledge et al. 2004, Rutledge and O'Neil 2005) and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Rutledge and O'Neil 2005, Desneux et al. 2006). Despite the natural control these predators provide, foliar insecticides are needed to prevent yield loss when *A. glycines* populations reach economically damaging levels (Ragsdale et al. 2007). Currently, growers are recommended to scout during July and August to determine whether the economic threshold of 250 aphids per plant (Ragsdale et al. 2007) has been exceeded. If the economic threshold is exceeded, then application of either an organophosphate or pyrethroid is recommended (Rice et al. 2005). Furthermore, there may be a need for soybean growers to apply an insecticide to manage additional arthropod pests before the occurrence of *A. glycines* outbreaks, such as the bean leaf beetle, *Cerotoma trifurcata* (Förster) and twospotted spider mite, *Tetranychus urticae* Koch. Many *A. glycines* predators are present in fields before arrival of *A. glycines* (e.g., *O. insidiosus*) (Rutledge et al. 2004); the application of a broad-spectrum insecticide may disrupt the natural control they provide (Johnson et al. 2008).

Given the role predators play in delaying and suppressing *A. glycines,* there may be benefit in replacing broad-spectrum insecticides with ones that have a limited impact on natural enemies (i.e., reduced-risk insecticides). The Environmental Protection Agency (EPA) defines reduced-risk insecticides as "insecticides that may reasonably be expected to accomplish one or more of the four following objectives: 1) reduce the risks of pesticides to human health; 2) reduce the

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[1] Corresponding author, e-mail: oneal@iastate.edu.

risks of pesticides to nontarget organisms; 3) reduce the potential for contamination of groundwater, surface water, or other valued environmental resources; and 4) Broaden the adoption of integrated pest management (IPM) strategies, or make such strategies more available or more effective" (EPA 1997). Insecticides that accomplish the first, second, and fourth objectives would be valuable for the management of *A. glycines.* Some insecticides that would fall within this category are approved for use for organic soybean production (Kraiss and Cullen 2008a, Kraiss and Cullen 2008b). To date, the potential for reduced-risk insecticides to manage *A. glycines* within conventional soybean production has not been explored. For such potential to be realized, the impact of putative reduced-risk insecticides on natural enemies should be assessed.

Our goal was to determine whether currently available insecticides considered reduced-risk by the EPA could be used to protect soybean yield from *A. glycines* herbivory while having a limited impact on its natural enemies. We tested several insecticides, within a soybean production system, to determine how well they fit portions two and four of the EPA definition for a reduced-risk insecticide.

Reduced-risk insecticides were selected based on known specificity to the target pest (pymetrozine) or systemic activity and thus encountered only when ingested by the herbivore (thiamethoxam, imidacloprid). Pymetrozine (Fulfill, Syngenta Crop Protection, Inc., Greensboro, NC) was included based on its novel mode of action (paralysis of the cibarial muscle) that subsequently prevents feeding in the hemipteran suborder Sternorrhyncha (Harrewijn and Kayser 1997, Wyss and Bolsinger 1997b, Sechser et al. 2002, Torres et al. 2003, Banks and Stark 2004). In addition to its selective mode of action, pymetrozine is plant systemic (symplasticly mobile) that results in a further reduction in exposure to nontarget organisms. Therefore, pymetrozine represents an insecticide that has been confirmed as having reduced impacts on nontarget, beneficial insects (i.e., portion two of the EPA definition for reduced-risk insecticides) in other cropping systems. We propose that comparing its activity against soybean aphids and its associated natural enemies provides a useful comparison for neonicotinoids that are expected to have a limited impact to natural enemies.

The neonicotinoids thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, Inc.) and imidacloprid (Gaucho 480FS, Bayer Crop Science, Research Triangle Park, NC) are plant systemic (apoplastically mobile) and when applied to seeds should result in reduced environmental exposure to nontarget organisms. A foliar formulation of imidacloprid (Trimax, Bayer Crop Science) was chosen to compare differences in application method and timing for this active ingredient. Although these seed treatments have been shown to increase aphid mortality in soybean to a limited degree (McCornack and Ragsdale 2006, Johnson et al. 2008), their impact on the natural enemy community in soybean has not been documented. These products are used for managing *C. trifurcata* (Bradshaw et al. 2008) as well as *A. glycines,* and if adoption increases and these products do reduce natural enemy abundance, the pest status of *A. glycines* could increase.

In contrast to the previous insecticides, the pyrethroid λ-cyhalothrin (Warrior, Syngenta Crop Protection, Inc.) is a broad-spectrum insecticide that would have an impact on both soybean aphids and most other arthropods present within a soybean field. This product is commonly applied to soybean foliage to manage *A. glycines* and has been demonstrated to provide yield protection when applied within the context of an economic threshold (ET) (Ragsdale et al. 2007).

Although neonicotinoids are considered by EPA as a reduced-risk insecticide, we are not aware of published research that demonstrates a limited impact to natural enemies within soybean (i.e., portion two of the EPA definition) and performance against *A. glycines* that would increase adoption of IPM tactics (i.e., portion 4). Our objective was to determine whether the impact on natural enemy and *A. glycines* abundance of neonicotinoids are indistinguishable from a commonly used, broad-spectrum insecticide (λ-cyhalothrin) and a reduced-risk insecticide (pymetrozine). We report the natural enemy abundance, *A. glycines* density and soybean yield when these insecticides where applied to soybean exposed to naturally occurring *A. glycines* infestations.

Materials and Methods

Field Site. The field site for this experiment was located at the Iowa State University North East Research Farm in Floyd County, IA. In both 2005 and 2006, no-till production practices were used, with commercially available (NK S24-K4 RR in 2005 and NK S23-Z3 RR in 2006; NK brand Syngenta Seeds, Golden Valley, MN) varieties of soybean considered susceptible to *A. glycines.* In 2005, soybean was planted on 22 May in 5 by 30-m plots. In 2006, soybean were planted on 6 May in 10- by 15-m plots. In both years, soybean was planted at 76-cm row spacing and 470,000 seeds per ha.

Experimental Design. To evaluate the impact of reduced-risk insecticides on *A. glycines* and its associated natural enemies, we used a randomized complete block design with six insecticide treatments (Table 1) along with an untreated control; each treatment was replicated once within six blocks. Blocks were composed of six strips that ran perpendicular to the direction of planting. In 2006, a "zero aphid" treatment was added. The zero aphid treatment received an insecticide application whenever aphids were detected (more than one aphid per plant). As such, this treatment represents the maximum yield possible under existing field conditions in absence of aphid herbivory. Foliar insecticides were to be applied at the economic threshold (250 *A. glycines* per plant; Ragsdale et al. 2007). However, due to low populations of *A. glycines* during the 2006 growing season, foliar in-

Table 1. Insecticides and rates used during 2005 and 2006 field experiments

Treatment	Formulation	Rate[a]
Control	NA	NA
Zero-aphid control[b] λ-cyhalothrin	Warrior 1 CS	227 ml/ha
+ chlorpyrifos	Lorsban 4 E	273 ml/ha
Thiamethoxam	Cruiser 5 FS	50 g/100 kg
Thiamethoxam	Cruiser 5 FS	100 g/100 kg
Imidacloprid	Gaucho 480 F	62.5 g/100 kg
Imidacloprid[c]	Trimax 4 E	105 ml/ha
Pymetrozine[c]	Fulfill 50 WG	192.6 g/ha
λ-Cyhalothrin[c]	Warrior 1 SC	227 ml/ha

[a] Seed treatment rates are given as grams of formulated product per 100 kg of seed, and foliar treatment rates are given as milliliters of formulated product per hectare.

[b] Zero-aphid control was added in 2006 and was applied when aphids were detected (three applications, 5 June, 13 July, and 1 August).

[c] Foliar treatments were applied on 2 August 2005 and 1 August 2006 when *A. glycines* averaged 211 and 75 per plant, respectively.

secticides were applied during the same calendar period as in 2005. A backpack sprayer and hand boom were used to apply insecticides with TeeJet 11002 Twin Jet nozzles, 38.1-cm nozzle spacing, 275kPa pressure, and 187 liters/ha of carrier (water). Seed treatments were applied to soybean seeds before planting. All insecticide rates can be found in Table 1. Due to the lack of current soybean registration when the experiment was conducted, the foliar formulations of imidacloprid and pymetrozine were applied based on recommendations from their respective commercial sources.

Estimating Soybean Exposure to *A. glycines*. In both years, populations of *A. glycines* were estimated every 7 d beginning in June (20 June 2005 and 1 June 2006), with more frequent estimates made before and after foliar insecticides were applied. The estimates were based on the total number (whole plant counts) of *A. glycines* (both apterous and alate, adults and nymphs) on consecutive plants within each plot (Hodgson et al. 2004). Previous research has shown that as populations of *A. glycines* increase, the variability in number of *A. glycines* per plant decreases (Hodgson et al. 2004). Therefore, the number of consecutive plants counted ranged from five to 20, with the number of plants counted being determined by the percentage infested with aphids during the previous sampling date. When 0 to 80% of plants were infested with *A. glycines*, 20 plants were counted; when 81 to 99% of plants were infested, 10 plants were counted; at 100% infestation, five plants were counted (Ragsdale et al. 2007).

The seasonal exposure of soybean to *A. glycines* was estimated by calculating "aphid days," which are based on the number of aphids per plant counted on each sampling date. The seasonal exposure of soybean plants to *A. glycines* is then calculated with the following equation:

$$\sum_{n=1}^{\infty} = \left(\frac{x_{i-1} + x_i}{2}\right) \times t,$$

where x is the mean number of aphids on sample day i, x_{i-1} is the mean number of aphids on the previous sample day, and t is the number of days between samples $i - 1$ and i. Summing the aphid days accumulated during the growing season (cumulative aphid days) provides a measure of the seasonal aphid exposure that a soybean plant experienced (Ruppel 1983).

Yield. In 2005 and 2006, yields were measured by weighing grain with a grain hopper, which rested on a digital scale sensor custom designed for the harvester. The entire plot was used to measure yield in 2005 and the center six rows out of 12 in 2006. Yields were corrected to 13% moisture and reported as kilograms per hectare.

Natural Enemy Sampling. To determine the effect of each insecticide treatment on the abundance of the foliar-based natural enemy community, individual plots were monitored with three methods: direct observation, sweep-nets, and yellow sticky cards (YSCs; unbaited Pherecon AM Traps, Great Lakes IPM Inc., Vestaburg, MI). These methods were selected based on the portion of the total natural enemy community each method will sample (Schmidt et al. 2008). Sweep-net and YSC collect a greater portion of the active predators, such as adult syrphids and coccinellids; direct plant observations (i.e., in situ) provide a better estimate of more sessile predators such as *O. insidiosus* and coccinellid larvae.

Direct observations of natural enemies on soybean were made on five to 10 consecutive plants with identification and recording of natural enemies in the field. Sweep-net samples consisted of 20 pendulum sweeps per plot running in the direction of the row using a 38-cm-diameter net. Yellow sticky cards were placed four per plot and suspended on wooden stakes such that the base of the card was slightly higher than the plant canopy. Yellow sticky cards were replaced every 6–8 d. Direct observations and the collection of sweep-net samples were separated by several hours (2–4 h) with direct observations always preceding sweep-net sampling to minimize the effect of one sampling technique on the other, and samplers avoided exterior rows of the plots to minimize edge effects. Yellow sticky card were replaced after sweep-net samples were collected.

Sweep-net samples and YSCs were stored in a −20°C freezer in the laboratory before sorting and identification of natural enemies in the laboratory. Natural enemies from both methods were identified to several levels, with spiders identified to order and all insects to at least family. Damsel bugs (Hemiptera: Nabidae) were identified to genus, whereas lady beetles (Coleoptera: Coccinellidae) and predatory bugs (Hemiptera: Anthocoridae) were identified to species. Lady beetle larvae were identified to family when early instars were collected and to species when later instars were collected.

Direct observations were made in different rows from those in which sweep-net samples were taken. Sweep-net samples were collected when YCS where first deployed. Sampling was conducted for a 1-wk period beginning on 13 June in 2005 and 12 June in

2006, and repeated every other week until the foliar insecticides were applied. Those samples taken before *A. glycines* was present and before foliar insecticides were applied would allow for an account of potential differences in natural enemy abundance that could have occurred early in the season due to the seed-applied insecticides. To account for the impact of foliar insecticides later in the season, sampling methods were employed every 3–7 d after these insecticides were applied.

Analysis. To determine the impact of the various insecticides on the plant exposure to *A. glycines,* we reported the mean aphid days accumulated each week for each treatment throughout the growing season. The impact of treatments on the accumulation of aphid days was determined using natural log-transformed data to meet the assumptions of a one-way analysis of variance (ANOVA) by using PROC MIXED and a *F*-protected least-squares means test for mean separation in SAS (SAS Institute, Cary, NC).

Previous research suggests that the community of natural enemies collected in soybean can vary significantly by sampling method (Schmidt et al. 2008). Our results were consistent with this finding. Therefore, to investigate the effect of the selected insecticides on natural enemy abundance, we conducted separate ANOVA for each sampling method. A separate ANOVA was conducted for each of the following: total predators and parasitoids collected, all Coccinellidae, *H. axyridis,* and *O. insidiosus.*

Total natural enemy data from sweep-nets and direct observations were square root transformed to correct for heteroscadacity before analysis, and data collected from yellow sticky cards did not require transformation. Data for individual subsets of the total natural enemies were transformed using log base 10. Furthermore, we conducted a separate ANOVA for the period before and after the application of foliar insecticides. For each sampling method, data from both years were combined and analyzed for each sampling method. Data for all three sampling methods were analyzed separately for each sampling date in 2005 and 2006.

Due to an imbalance resulting from an additional replication of the untreated control within blocks and the addition of the zero aphid treatment in 2006, PROC MIXED was used for all analysis. Natural enemies were first analyzed for interactions between year, date, and treatment variables for each sampling method. This was accomplished using an ANOVA with PROC MIXED and repeated measures using a REPEATED statement, with the covariance structure defined by compound symmetry (type = cs; SAS Institute 2004). Due to the interaction of date × treatment for both sweep-net sampling and direct observations, data were analyzed separately for each sampling date without repeated measures. Differences in the abundance of natural enemies and abundance of key natural enemies were determined using an ANOVA with PROC MIXED with an *F*-protected least significant difference (LSD) test generated using the LSMEANS statement. All analyses were conducted for each year and each time period (i.e., before and after foliar insecticide application) individually to account for seasonal variation and differences in *A. glycines* population levels. This allowed differences due to seed treatments to be observed when those treatments would still be active.

Yields were estimated from seed samples collected at harvest and averaged across the treatments. Yield data were analyzed using a one-way ANOVA in PROC MIXED by using an F-protected students LSD test for means separation.

Results

Soybean Exposure to *A. glycines* and Yield. In 2005, foliar insecticides were applied on 2 August when *A. glycines* populations averaged 211 ± 48 per plant in non–seed-treated plots. However, *A. glycines* populations quickly surpassed the ET of 250 *A. glycines* per plant in the control treatment (266 ± 54 *A. glycines* per plant) by 4 August and peaked at 1,331 ± 323 *A. glycines* per plant on 25 August.

We observed a significant effect of the foliar-applied insecticides on *A. glycines* abundance ($F = 14.7$, df = 11, 43; $P = 0.0001$) and observed mean separation in soybean aphid exposure among all the treatments (Fig. 1a). Among the seven treatments, λ-cyhalothrin–treated soybean had the lowest exposure to *A. glycines* (<500 cumulative aphid days [CAD]), followed by the pymetrozine and the foliar applied imidacloprid (≈2,000 CAD). The untreated and seed-treated soybean experienced the highest exposure to *A. glycines* (>10,000 CAD) (Fig. 1a). This trend was not as consistent with regard to soybean yield. Although the greatest yield was recorded from plots treated with λ-cyhalothrin, this was not significantly different from plots treated with the highest rate of thiomethoxam or the two foliar-applied reduced risk insecticides (Fig. 2a). In general, seed treatments provided the lowest level of protection against *A. glycines.*

In 2006, insecticides were applied on 1 August when *A. glycines* populations averaged 75 ± 29 aphids per plant in nonseed treated plots. Unlike 2005, *A. glycines* populations did not surpass the ET and peaked at 114 ± 22 *A. glycines* per plant on 7 August. In 2006, we observed a significant decrease in soybean exposure to *A. glycines* after application of the foliar insecticides ($F = 14.0$, df = 10, 56; $P = 0.0001$). The lowest exposure to *A. glycines* was observed in plots treated with λ-cyhalothrin (<100 CAD), followed by the foliar applied reduced-risk insecticide and the untreated and seed-treated soybean that experienced the highest exposure to *A. glycines* (Fig. 1b). Unlike in 2005, in 2006 we did not observed a significant difference between the seed-applied and foliar-applied imidacloprid in terms of soybean exposure to *A. glycines.* Although insecticide applications reduced *A. glycines* populations, this did not result in significantly different soybean yield compared with the untreated control (Fig. 2b). This inference is reinforced by the lack of difference among any of the foliar-applied insecticide treatments, including the zero aphid control, which was

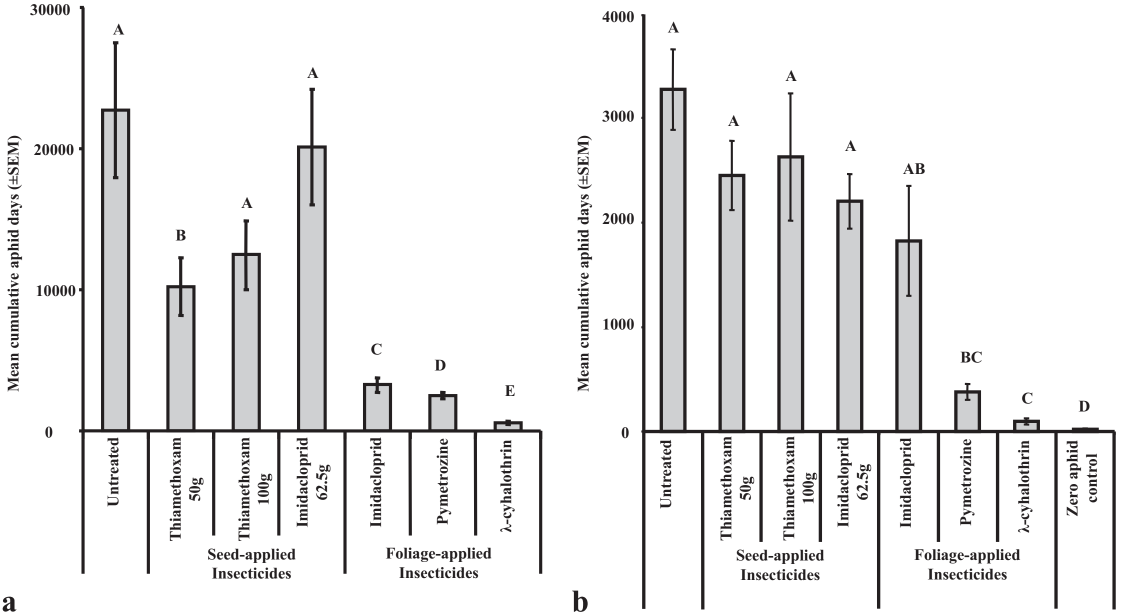

Fig. 1. Comparison of soybean exposure to *A. glycines* based on average cumulative aphid days between soybean grown with seed or foliar-applied insecticides in 2005 (a) and 2006 (b). Soybean was planted on 22 May in 2005 and 6 May in 2006. Thiamethoxam (Cruiser) and imidacloprid (Gaucho) were applied to seeds before planting. Imidacloprid (Trimax), pymetrozine (Fulfill), and λ-cyhalothrin (Warrior) were applied to foliage on 2 August in 2005 and 1 August in 2006 when aphid populations averaged 211 and 75 *A. glycines* per plant, respectively. Means labeled with a unique letter were significantly different ($P < 0.05$).

essentially free of aphids. This is not unexpected, as the density of *A. glycines* in the untreated plots would not be expected to have a significant impact on soybean yield (Ragsdale et al. 2007).

Natural Enemies. Natural enemies collected in 2005 and 2006 are summarized in Table 2. Sweep-nets collected the greatest abundance of natural enemies, with a greater abundance of natural enemies collected in 2005 than in 2006. In 2005, Coccinellidae was the most abundant natural enemy (60.7%), with *H. axyridis* being the single most abundant natural enemy species (55.2%) collected with sweep-nets. In 2006, *O. insidiosus* was the most abundant natural enemy in sweep-net samples (29.1%). Natural enemy abundance estimated from direct observations were the lowest of the three methods. *O. insidiosus* was the most abundant natural enemy encountered in direct observation during both years, 35.9 and 53.9% in 2005 and 2006, respectively. Microhymenoptera were the most abundant natural enemy sampled using YSC in both 2005 and 2006, 71.7 and 62.1%, respectively.

In general, we observed a significant decrease in the abundance of natural enemies due to the application of foliar insecticides. This decrease varied by natural enemy, sampling method, and active ingredient. This variation is explained below. However, a consistent trend was the lack of a significant response in natural enemy abundance to any of the insecticides when natural enemies were collected with YSC (Table 3). In both years for all natural enemies sampled, individually or in subsets, we did not observe a significant treatment effect for these data collected with YSC. Therefore, we do not report any further data from the YSC.

Preapplication of Foliar Insecticides. The impact of the various treatments on the abundance of natural enemies was not observed until after the foliar insecticides were applied (Table 3). In both 2005 and 2006, we did not observe any differences ($P < 0.05$) in mean total abundance of natural enemies collected with sweep-net, direct observation, and YSC methods in soybean planted with seed-applied insecticide (seed treatment) and those left untreated. Although this trend was apparent in all the sampling methods, we report only the sweep-net sampling both before and after application of foliar insecticides (Fig. 3a and b). Furthermore, we observed no effect of seed treatment on subgroups and individual members of the natural enemy community, including the Coccinellidae, *O. insidiosus*, and *H. axyridis* across all three sampling methods.

Postapplication of Foliar Insecticides. After foliar insecticides were applied, during both 2005 and 2006, we did observe differences ($P < 0.05$) in natural enemy abundance among the various treatments (Table 3). These differences were observed in total natural enemies, *O. insidiosus*, Coccinellidae, and *H. axyridis.*

Using a sweep-net, we observed significant ($P < 0.05$) differences among the treatments (Table 4) in the abundance of total natural enemies, *O. insidiosus*, Coccinellidae, and *H. axyridis.* After the foliar insecticides were applied, there were no significant differences ($P < 0.05$) in natural enemy abundance between seed treatments

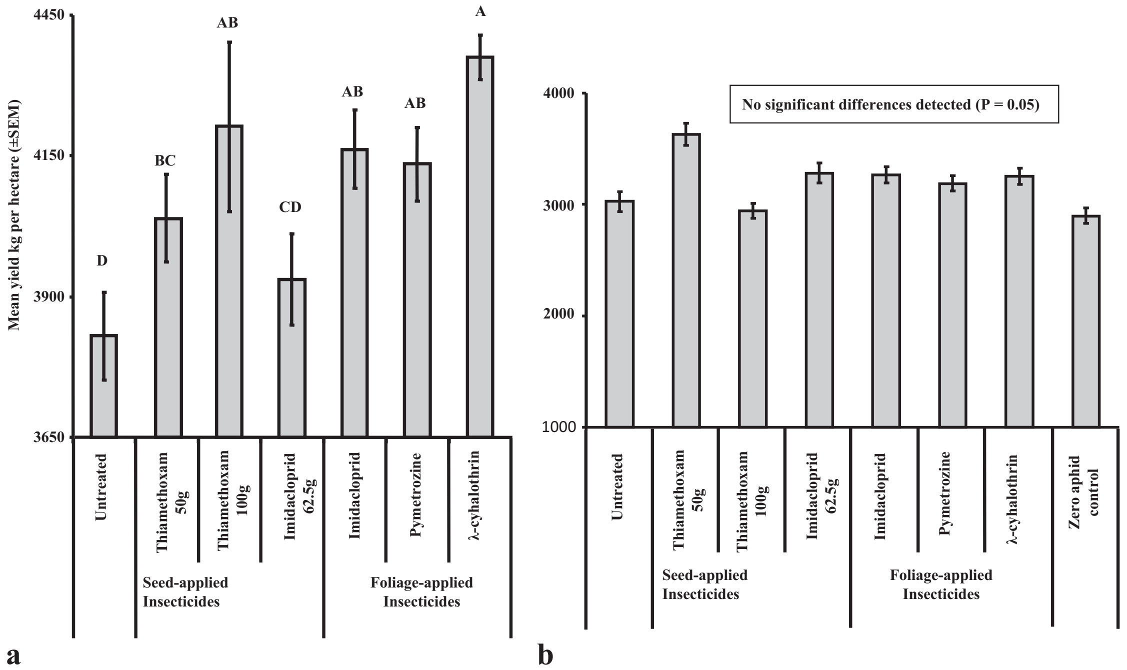

Fig. 2. Comparison of soybean yield (kilograms per hectare) between soybean grown with seed or foliar-applied insecticides in 2005 (a) and 2006 (b). Soybean was planted on 22 May in 2005 and 6 May in 2006. Thiamethoxam (Cruiser) and imidacloprid (Gaucho) were applied to seeds before planting. Imidacloprid (Trimax), pymetrozine (Fulfill), and λ-cyhalothrin (Warrior) were applied to foliage on 2 August in 2005 and 1 August in 2006 when aphid populations averaged 211 and 75 *A. glycines* per plant, respectively. Means labeled with a unique letter were significantly different ($P < 0.05$).

and the untreated control (Table 4). We did observe an occasional difference in the abundance of natural enemies between a putative (imidacloprid) and a confirmed (pymetrozine) reduced-risk insecticide at various times after foliar insecticides were applied (days after treatment, DAT). Between these two insecticides, only during four sampling dates (Table 4) were there significant differences in the abundance of all natural enemies (6 and 21 DAT in 2006), *O. insidiosus* (13 DAT in 2005 and 3 DAT in 2006), coccinellids (21 DAT in 2006), and *H. axyridis* (21 DAT in 2006). The foliar application of imidiacloprid and pymetrozine often resulted in intermediate abundance of natural enemies between the untreated control and λ-cyhalothrin. The foliar application of the insecticide λ-cyhalothrin consistently had the lowest abundance of any natural enemy category (Table 4).

Table 2. Natural enemies collected[a] in soybean during 2005 and 2006

Order	Family	Species
Coleoptera	Coccinellidae *Coleomegilla maculata* *Harmonia axyridis* *Hippodamia convergens* *Hippodamia parenthesis* Unidentified[c]	*Coccinella septumpunctata*
Diptera	Syrphidae	
Hemiptera	Anthocoridae	*Orius insidiosus*
	Nabidae	*Nabis* spp.
	Pentatomidae	*Podisus maculiventris*
Hymenoptera[b]	Aphelinidae[b] Braconidae Ichneumonidae	
Neuroptera	Chrysopidae Hemerobiidae	
Araneae		
Opiliones		

[a] Collected with a sweep-net, yellow sticky trap or direct observation of plant.

[b] Identified only as mummies found on plants.

[c] Larvae in early instars that lack characteristic coloration used for identification.

Treatment differences in natural enemy abundance from direct observations (Table 5) in 2005 were similar to those from sweep-nets in 2005 after application of foliar insecticides. Seed treatments were commonly grouped with the untreated control, and the foliar applications of imidacloprid and pymetrozine typically were intermediates between the untreated control and λ-cyhalothrin in the abundance of total natural enemies, *O. insidiosus,* total coccinellid, and *H. axyridis.*

Discussion

The impacts of the reduced-risk insecticides in reducing *A. glycines* populations were mixed, with the foliar applications (timed with larger populations of *A. glycines*) of both imidacloprid and pymetrozine, providing greater *A. glycines* population reductions (sometimes equal to λ-cyhalothrin) than the neonicotinoid seed treatments (imidacloprid and thiamethoxam). Not surprisingly, the neonicotinoid seed

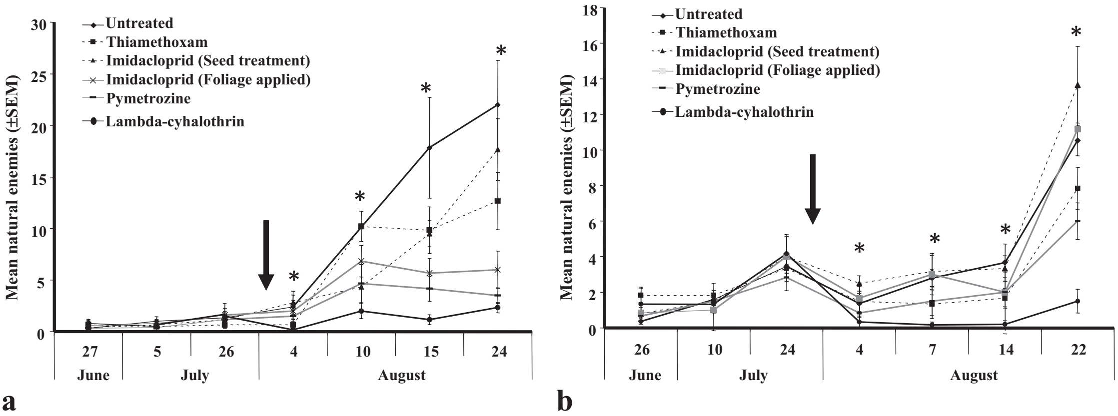

Fig. 3. Seasonal abundance of the total natural enemy community in soybean collected with a sweep-net in 2005 (a) and 2006 (b). Soybean was planted on 22 May in 2005 and 6 May in 2006 with seed treatments of imidacloprid and thiomethoxam were applied as seed treatments at planting. Foliar treatments of imidacloprid, pymetrozine, and λ-cyhalothrin were applied on 2 August in 2005 and 1 August in 2006 and are denoted by an arrow. Significant differences ($P < 0.05$) are denoted by an asterisk (*) more detail is given in Table 5.

treatments (imidacloprid and thiamethoxam) provided limited, inconsistent yield protection to soybean that was on occasion not significantly different from the untreated control. This lack of yield protection and the limited effect on *A. glycines* population from seed applied neonicotinoids are consistent with other published research (McCornack and Ragsdale 2006, Johnson et al. 2008). Foliar applied imidacloprid and pymetrozine provided soybean yield protection indistinguishable from the broad-spectrum insecticide (λ-cyhalothrin), although the performance of foliar imidacloprid was inconsistent from 2005 to 2006. The efficacy of the foliar-applied imidacloprid compared with the seed-applied imidicloprid illustrates the importance of timing the application of an insecticide for optimal soybean aphid management.

Table 3. ANOVA revealing response of natural enemy abundance to insecticides to soybean in 2005 and 2006

Method	Period[a]	Variable	*F*	Df[b]	*P*
Sweep-net	Pre	Yr	34.25	1, 75.3	<0.0001
		Date	27.41	6, 147	<0.0001
		Treatment	0.23	5, 72.9	0.9460
		Date × treatment	0.79	10, 156	0.6410
		Yr × treatment	0.49	5, 75	0.7804
	Post	Yr	25.52	1, 95.8	<0.0001
		Date	54.20	3, 213	<0.0001
		Treatment	20.24	5, 90.8	<0.0001
		Date × treatment	2.17	15, 200	0.0081
		Yr × treatment	2.02	5, 95.8	0.0821
Direct observation	Pre	Yr	0.01	1, 94.7	0.9084
		Date	70.2	2, 158	<0.0001
		Treatment	0.84	5, 96.3	0.5266
		Date × treatment	0.43	10, 170	0.9291
		Yr × treatment	2.03	5, 94.4	0.0815
	Post	Yr	16.11	1, 64.7	0.0002
		Date	35.02	1, 81.9	<0.0001
		Treatment	7.53	5, 64.2	<0.0001
		Date × treatment	1.93	5, 82.5	0.0981
		Yr × treatment	2.03	5, 64.2	0.0864
Yellow sticky cards	Pre	Yr	99.23	1, 63	<0.0001
		Date	56.52	2, 124	<0.0001
		Treatment	1.61	5, 70.8	0.1684
		Date × treatment	2.06	10, 144	0.0312
		Yr × treatment	1.55	5, 63	0.1871
	Post	Yr	146.67	1, 97.5	<0.0001
		Date	9.91	3, 201	<0.0001
		Treatment	0.96	5, 95	0.4491
		Date × treatment	0.47	15, 226	0.9529
		Yr × Treatment	0.42	5, 97.3	0.8307

[a] Data analyzed from pre- or postapplication of foliar insecticides.

[b] Degrees of freedom, numerator followed by denominator degrees of freedom.

Table 4. Mean ± SEM of all natural enemies (NE), and *O. insidiosus*, Coccinellidae, **and *H. axyridis* collected in soybean by using a sweep-net after application of foliar insecticides**

Yr	DAT[a]	Treatment[b,c]	Total NE	*O. insidiosus*	Coccinellidae	*H. axyridis*
2005	2	Untreated	2.4 ± 1.2 AB	0.0 ± 0.0 NS	1.2 ± 0.6 A	0.8 ± 0.6 NS
		Thiomethoxam	0.7 ± 0.5 BC	0.0 ± 0.0 NS	0.3 ± 0.3 AB	0.3 ± 0.3 NS
		Imidacloprid (ST)	2.8 ± 1.1 A	0.0 ± 0.0 NS	0.8 ± 0.4 AB	0.2 ± 0.2 NS
		Imidacloprid	2.0 ± 1.0 AB	0.0 ± 0.0 NS	1.3 ± 1.0 AB	0.8 ± 0.8 NS
		Pymetrozine	1.5 ± 0.6 ABC	0.2 ± 0.2 NS	0.7 ± 0.2 AB	0.5 ± 0.2 NS
		λ-Cyhalothrin	0.2 ± 0.2 C	0.0 ± 0.0 NS	0.0 ± 0.0 B	0.0 ± 0.0 NS
	8	Untreated[d]				
		Thiomethoxam	10.2 ± 1.5 A	3.4 ± 1.2 A	3.6 ± 0.5 A	2.8 ± 0.6 A
		Imidacloprid (ST)	4.3 ± 2.1 BC	1.5 ± 0.8 B	1.8 ± 1.0 BC	1.7 ± 0.8 AB
		Imidacloprid	6.8 ± 1.5 ABC	2.5 ± 0.9 AB	2.7 ± 0.8 AB	2.3 ± 0.9 A
		Pymetrozine	4.7 ± 1.9 BC	2.0 ± 1.0 B	2.5 ± 0.9 ABC	2.3 ± 0.9 A
		λ-Cyhalothrin	2.0 ± 0.7 C	0.3 ± 0.3 C	0.8 ± 0.5 C	0.3 ± 0.2 B
	14	Untreated	17.8 ± 4.9 A	0.5 ± 0.2 AB	14.0 ± 4.2 A	13.7 ± 4.1 A
		Thiomethoxam	9.8 ± 2.2 AB	1.5 ± 0.6 A	5.2 ± 1.7 BC	4.2 ± 1.5 BC
		Imidacloprid (ST)	9.5 ± 1.3 AB	1.3 ± 0.8 A	6.0 ± 0.4 B	5.3 ± 0.6 B
		Imidacloprid	5.7 ± 1.4 BC	1.5 ± 0.6 A	2.8 ± 0.7 CD	2.5 ± 0.6 CD
		Pymetrozine	4.2 ± 1.2 CD	0.0 ± 0.0 B	1.3 ± 0.6 DE	1.3 ± 0.6 D
		λ-Cyhalothrin	1.2 ± 0.5 D	0.0 ± 0.0 B	0.2 ± 0.2 E	0.2 ± 0.2 E
	22	Untreated	22.0 ± 4.3 A	0.0 ± 0.0 B	19.2 ± 4.2 A	18.8 ± 4.1 A
		Thiomethoxam	12.7 ± 2.8 B	0.0 ± 0.0 B	11.8 ± 2.4 B	11.3 ± 2.3 B
		Imidacloprid (ST)	17.7 ± 3.0 AB	1.0 ± 0.5 A	14.0 ± 2.9 AB	13.8 ± 2.9 AB
		Imidacloprid	6.0 ± 1.8 C	0.3 ± 0.2 A	4.0 ± 1.5 C	3.7 ± 1.3 C
		Pymetrozine	3.5 ± 0.8 C	0.7 ± 0.3 AB	1.8 ± 0.5 CD	1.8 ± 0.5 C
		λ-Cyhalothrin	2.3 ± 0.5 C	0.2 ± 0.2 AB	0.7 ± 0.3 D	0.5 ± 0.3 D
2006	3	Untreated	1.4 ± 0.6 BC	0.0 ± 0.0 C	0.5 ± 0.3 NS	0.3 ± 0.2 NS
		Thiomethoxam	1.5 ± 0.6 AB	0.8 ± 0.5 AB	0.0 ± 0.0 NS	0.0 ± 0.0 NS
		Imidacloprid (ST)	2.5 ± 0.4 A	0.5 ± 0.3 BC	0.0 ± 0.0 NS	0.0 ± 0.0 NS
		Imidacloprid	1.7 ± 0.7 AB	1.0 ± 0.4 A	0.5 ± 0.3 NS	0.5 ± 0.3 NS
		Pymetrozine	0.8 ± 0.5 AB	0.2 ± 0.2 BC	0.5 ± 0.5 NS	0.5 ± 0.5 NS
		λ-Cyhalothrin	0.3 ± 0.3 C	0.0 ± 0.0 BC	0.0 ± 0.0 NS	0.0 ± 0.0 NS
	6	Untreated	2.8 ± 0.5 AB	0.5 ± 0.2 AB	0.7 ± 0.3 A	0.5 ± 0.2 NS
		Thiomethoxam	1.3 ± 0.8 BC	0.2 ± 0.2 AB	0.7 ± 0.7 AB	0.7 ± 0.7 NS
		Imidacloprid (ST)	3.2 ± 1.0 A	1.0 ± 0.6 A	0.3 ± 0.3 AB	0.3 ± 0.3 NS
		Imidacloprid	3.0 ± 1.1 A	0.7 ± 0.4 AB	1.0 ± 1.0 AB	1.0 ± 1.0 NS
		Pymetrozine	1.5 ± 0.8 BC	0.0 ± 0.0 B	0.5 ± 0.3 AB	0.3 ± 0.2 NS
		λ-Cyhalothrin	0.2 ± 0.2 C	0.0 ± 0.0 B	0.0 ± 0.0 B	0.0 ± 0.0 NS
	13	Untreated	3.7 ± 1.0 A	0.0 ± 0.0 NS	1.0 ± 0.3 A	0.8 ± 0.3 A
		Thiomethoxam	1.7 ± 0.6 B	0.2 ± 0.2 NS	0.5 ± 0.2 AB	0.5 ± 0.2 AB
		Imidacloprid (ST)	3.3 ± 0.7 AB	0.0 ± 0.0 NS	1.3 ± 0.6 A	0.8 ± 0.4 A
		Imidacloprid	2.0 ± 1.1 BC	0.0 ± 0.0 NS	0.6 ± 0.5 AB	0.6 ± 0.5 AB
		Pymetrozine	2.0 ± 0.8 AB	0.0 ± 0.0 NS	0.7 ± 0.4 A	0.5 ± 0.4 AB
		λ-Cyhalothrin	0.2 ± 0.2 C	0.0 ± 0.0 NS	0.0 ± 0.0 B	0.0 ± 0.0 B
	21	Untreated	10.5 ± 0.9 AB	4.3 ± 0.8 A	3.6 ± 0.8 A	3.3 ± 0.7 A
		Thiomethoxam	7.8 ± 1.2 AB	3.5 ± 0.8 A	2.8 ± 0.4 A	2.7 ± 0.4 A
		Imidacloprid (ST)	13.7 ± 2.2 A	6.0 ± 1.8 A	4.2 ± 0.3 A	3.3 ± 0.3 A
		Imidacloprid	11.2 ± 2.3 AB	4.0 ± 1.4 AB	3.8 ± 0.8 A	3.3 ± 0.9 A
		Pymetrozine	6.0 ± 1.0 C	1.7 ± 0.5 B	0.2 ± 0.1 B	0.2 ± 0.1 B
		λ-Cyhalothrin	1.5 ± 0.7 D	0.3 ± 0.2 C	0.2 ± 0.1 B	0.2 ± 0.1 B

[a] DAT, days after treatment of foliar insecticides.

[b] Foliar insecticides were applied on August 2 and August 1 in 2005 and 2006, respectively.

[c] Seed treatments were applied at planting on May 22 and May 6 in 2005 and 2006, respectively.

[d] Data lost due to mechanical failure of storage equipment.

Not only do *A. glycines* populations respond in a density-dependent manner to natural enemies, so do natural enemies, whose density in soybean increase based on the density of *A. glycines* (Donaldson et al. 2007). This relationship confounds our ability to determine the impact of the insecticides tested on natural enemy abundance. We observed variation in *A. glycines* abundance between 2005 and 2006, with an almost 2 order of magnitude difference in the peak population of *A. glycines.* Despite the variation between years, we noted very similar trends in natural enemy abundance among the insecticide treatments in 2005 as in 2006. We did not observe a significant difference in natural enemy abundance between the seed treatments and the untreated control. In general, seed treatments had a reduced impact on the abundance of natural enemies when compared with the foliar applied insecticides. This observation is consistent with more controlled studies where the impact of imidacloprid on predatory hemipterans was shown to be less than that of a broad-spectrum insecticide (cyfluthrin; Elzen 2001).

In general, as *A. glycines* density declined after the foliar insecticides were applied, so too did the abundance of natural enemies. This decline was greatest for soybean treated with λ-cyhalothrin. However, the abundance of natural enemies in plots treated with two reduced-risk insecticides (pymetrozine and imi-

Table 5. Mean ± SEM of all natural enemies (NE), *O. insidiosus*, Coccinellidae, and *H. axyridis* from direct observations of soybean after application of foliar insecticides

Yr	DAT[a]	Treatment[b,c]	Total NE	*O. insidiosus*	Coccinellidae	*H. axyridis*
2005	8	Untreated	2.0 ± 1.0 A	0.3 ± 0.3 NS	1.5 ± 1.0 A	1.0 ± 0.6 A
		Thiomethoxam	0.0 ± 0.0 AB	0.2 ± 0.2 NS	0.3 ± 0.3 AB	0.3 ± 0.3 AB
		Imidacloprid(ST)	1.0 ± 0.7 AB	0.2 ± 0.2 NS	0.2 ± 0.2 AB	0.2 ± 0.2 B
		Imidacloprid	0.7 ± 0.4 AB	0.3 ± 0.3 NS	0.2 ± 0.2 AB	0.0 ± 0.0 B
		Pymetrozine	0.7 ± 0.5 AB	0.0 ± 0.0 NS	0.7 ± 0.5 AB	0.2 ± 0.2 AB
		λ-Cyhalothrin	0.3 ± 0.2 B	0.0 ± 0.0 NS	0.0 ± 0.0 B	0.0 ± 0.0 B
	14	Untreated	4.5 ± 1.8 AB	0.8 ± 0.4 AB	2.5 ± 1.2 A	0.5 ± 0.3 AB
		Thiomethoxam	1.5 ± 0.6 BC	0.6 ± 0.4 AB	1.0 ± 0.7 ABC	0.2 ± 0.2 BC
		Imidacloprid(ST)	2.8 ± 0.7 A	2.2 ± 1.6 A	1.5 ± 0.8 ABC	1.2 ± 0.8 A
		Imidacloprid	2.4 ± 0.9 C	1.0 ± 1.0 AB	0.5 ± 0.4 BC	0.3 ± 0.2 ABC
		Pymetrozine	1.6 ± 0.9 ABC	0.5 ± 0.5 B	1.2 ± 0.4 AB	0.3 ± 0.2 ABC
		λ-Cyhalothrin	6.0 ± 1.8 C	0.2 ± 0.2 B	0.3 ± 0.2 C	0.0 ± 0.0 C
2006	3	Untreated	3.2 ± 1.0 AB	1.8 ± 0.5 AB	0.3 ± 0.3 B	0.0 ± 0.0 NS
		Thiomethoxam	2.3 ± 0.6 AB	1.0 ± 0.5 ABC	0.0 ± 0.0 B	0.0 ± 0.0 NS
		Imidacloprid(ST)	4.7 ± 1.0 A	2.2 ± 0.7 A	0.8 ± 0.4 A	0.2 ± 0.2 NS
		Imidacloprid	1.5 ± 0.6 BC	0.7 ± 0.3 BC	0.3 ± 0.2 AB	0.0 ± 0.0 NS
		Pymetrozine	1.0 ± 0.4 BC	0.2 ± 0.2 C	0.3 ± 0.2 AB	0.0 ± 0.0 NS
		λ-Cyhalothrin	0.7 ± 0.3 C	0.0 ± 0.0 C	0.0 ± 0.0 B	0.0 ± 0.0 NS
	6	Untreated	5.8 ± 1.2 A	3.2 ± 0.7 A	0.8 ± 0.4 AB	0.1 ± 0.1 NS
		Thiomethoxam	1.7 ± 0.6 B	1.5 ± 0.6 A	0.0 ± 0.0 C	0.0 ± 0.0 NS
		Imidacloprid(ST)	3.7 ± 1.0 AB	2.3 ± 0.6 A	0.2 ± 0.2 BC	0.2 ± 0.2 NS
		Imidacloprid	3.8 ± 1.4 AB	1.5 ± 0.8 A	1.2 ± 0.8 A	0.2 ± 0.2 NS
		Pymetrozine	1.8 ± 0.5 B	1.8 ± 0.5 A	0.0 ± 0.0 C	0.0 ± 0.0 NS
		λ-Cyhalothrin	0.2 ± 0.2 C	0.0 ± 0.0 B	0.0 ± 0.0 C	0.0 ± 0.0 NS
	13	Untreated	6.4 ± 1.4 AB	4.3 ± 0.9 AB	0.2 ± 0.1 NS	0.0 ± 0.0 NS
		Thiomethoxam	9.0 ± 1.9 A	5.2 ± 1.0 A	0.0 ± 0.0 NS	0.0 ± 0.0 NS
		Imidacloprid(ST)	6.8 ± 2.0 AB	2.5 ± 0.6 AB	0.2 ± 0.2 NS	0.2 ± 0.2 NS
		Imidacloprid	5.5 ± 2.2 BC	4.3 ± 1.7 B	0.2 ± 0.2 NS	0.0 ± 0.0 NS
		Pymetrozine	2.8 ± 0.7 C	3.2 ± 1.1 B	0.0 ± 0.0 NS	0.0 ± 0.0 NS
		λ-Cyhalothrin	0.3 ± 0.3 D	0.0 ± 0.0 C	0.0 ± 0.0 NS	0.0 ± 0.0 NS

[a] DAT, days after treatment of foliar insecticides.

[b] Foliar insecticides were applied on August 2 and August 1 in 2005 and 2006, respectively.

[c] Seed treatments were applied at planting on May 22 and May 6 in 2005 and 2006, respectively.

dacloprid) was often indistinguishable from each other and an intermediate between the untreated control and λ-cyhalothrin. So, although the abundance of *A. glycines* varied significantly between the 2 yr of this study, the effect of the insecticide treatments was consistent.

Interestingly, we did not observe differences among the treatments when natural enemies were sampled with yellow sticky cards. The abundance of natural enemies continued to increase on yellow sticky cards, even after foliar insecticides were applied (data not shown). This observation is probably due to the type of sampling tool used. Schmidt et al. (2008) compared the natural enemy community in soybean captured with the sampling methods used within our study. Overall, yellow sticky cards described a community of natural enemies that was dominated by large, mobile predators. For individual species of predators such as *H. axyridis* and other coccinellids, yellow sticky cards were more likely to capture mobile predators than sweep-nets and collect more than what was observed directly. Given the relatively small size of our experimental units (150-m^2 plots) and the propensity of yellow sticky cards to capture mobile predators, it is likely that these visually attractive traps collected more mobile natural enemies (such as *H. axyridis* and other coccinellids) from adjacent crop land or the heavily aphid infested untreated controls. We suggest that the lack of treatment differences when natural enemy abundance was measured with yellow sticky cards in this study was an artifact of the sampling technique and not an accurate representation of the natural enemy community's response to insecticides. We recommend against using sampling methods such as a yellow sticky trap that sample predominantly one type of natural enemy (i.e., large, mobile versus small, sessile). Future studies of nontarget impacts from insecticides within soybean, and probably other crops, should use a combination of sampling methods that describe the entire community of insects (Schmidt et al. 2008).

Although we measured natural enemy abundance, and collected predators considered important sources of *A. glycines* mortality, we did not measure biological control of *A. glycines.* However, the negative impact of the natural enemy community within soybean on *A. glycines* has been well documented (Fox et al. 2004, 2005; Gardiner et al. 2009). This community is comprised mostly of predators (Schmidt et al. 2008) that can respond in a density-dependent manner to *A. glycines* population growth (Donaldson et al. 2007). Both *O. insidiosus* (Desneux et al. 2006) and coccinellids (Costamagna et al. 2008) have been identified as important components of this community. However, it is not known whether these predators interact in a positive or negative manner as it relates to *A. glycines* mortality. Although it may be possible to estimate the potential for biological control by calculating predator:prey ratios, without knowing how these predators interact, it is not clear how valuable this estimate would be. For the sake of designating an insecticide as reduced-risk, it does not have to increase biological control of the target pest. Rather, these products have to demonstrate a reduced impact on nontarget organisms and lead to greater adoption of IPM. In our experiment we have observed a reduced impact to one class of nontarget organisms. What is not clear from our results is how this would relate to the latter part of the EPA's definition for classification of a reduced-risk insecticide.

The use of broad-spectrum insecticides may interfere with an importation biological control program targeting *A. glycines* (Heimpel et al. 2004). The impacts of land use (Gardiner et al. 2009) and agricultural intensification (Landis et al. 2008) have been documented as contributing to the frequency and intensity of *A. glycines* outbreaks. Because growers use broad-spectrum insecticides more frequently to manage *A. glycines,* our data would indicate additional impacts to the natural enemy community within soybean, lowering the capacity of natural enemies to suppress *A. glycines* outbreaks. For example, *A. glycines* mortality on their overwintering host (*Rhamnus* spp.) has been attributed to natural enemies (Nielsen and Hajek 2005, Welsman et al. 2007). Components of the natural enemy community on *Rhamnus cathartica* L. are shared with soybean, including a key predator *H. axyridis.* Our results suggest that reduced-risk insecticides would allow for less disruption of the natural enemy community that suppresses soybean aphid outbreaks. Unfortunately, to date pymetrozine and imidicloprid are not available to soybean growers in a form that can be applied to soybean foliage. One product that includes imidicloprid (Leverage, Bayer CropScience) is available to farmers; however, this product also contains cyfluthrin, a broad-spectrum insecticide that would probably remove existing natural enemies (Elzen 2001). Thus, the benefits from the use of reduced-risk insecticides, as it relates to pest management, may be at risk if these products are sold in combination with a broad-spectrum insecticide. We observed significantly higher *A. glycines* populations when imidicloprid and pymetrozine were applied to soybean foliage compared with λ-cyhalothrin (Fig. 2a). Therefore, growers may need education regarding the role reduced-risk insecticides can play within an IPM program if these products are to be adopted.

Acknowledgments

In addition to the state of Iowa, we thank the Iowa Soybean Association and North Central Soybean Research Program for financial support of our research. We also thank both Bayer CropScience and Syngenta Crop Protection for supplying both insecticides and for additional financial support of our research. This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 5032, was supported by Hatch Act and State of Iowa funds.

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Received 2 July 2008; accepted 16 June 2009.