

The role of natural enemy guilds in *Aphis glycines* suppression

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Abstract

Generalist natural enemy guilds are increasingly recognized as important sources of mortality for invasive agricultural pests. However, the net contribution of different species to pest suppression is conditioned by their biology and interspecific interactions. The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), is widely attacked by generalist predators, but the relative impacts of different natural enemy guilds remains poorly understood. Moreover, low levels of *A. glycines* parasitism suggest that resident parasitoids may be limited through intraguild predation. During 2004 and 2005, we conducted field experiments to test the impact of different guilds of natural enemies on *A. glycines*. We contrasted aphid abundance on field cages with ambient levels of small predators (primarily *Orius insidiosus*) and parasitoids (primarily Braconidae), sham cages and open controls exposed to large predators (primarily coccinellids), and cages excluding all natural enemies. We observed strong aphid suppression (86- to 36-fold reduction) in treatments exposed to coccinellids, but only minor reduction due to small predators and parasitoids, with aphids reaching rapidly economic injury levels when coccinellids were excluded. Three species of resident parasitoids were found attacking *A. glycines* at very low levels (<1% parasitism), with no evidence that intraguild predation by coccinellids attenuated parasitoid impacts. At the plant level, coccinellid impacts resulted in a trophic cascade that restored soybean biomass and yield, whereas small natural enemies provided only minor protection against yield loss. Our results indicate that within the assemblage of *A. glycines* natural enemies in Michigan, coccinellids are critical to maintain aphids below economic injury levels.

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1. Introduction

There is increasing recognition that established generalist predators can provide pest suppression, due to their voracity and earlier presence in the field (Chang and Kareiva, 1999; Symondson et al., 2002; Stiling and Cornelissen, 2005). However, within these multiple enemy assemblages interactions among the natural enemy species may result in very different outcomes (Sih et al., 1998). Within-guild enemy interactions may result in either enhancement of pest suppression due to predator facilitation (Losey and Denno, 1998; Cardinale et al., 2003), or a reduction in pest

control due to negative interactions such as predator interference, cannibalism, predator avoidance behavior, and intraguild predation (Snyder and Wise, 1999; Prasad and Snyder, 2006). Thus, it is important to identify the relative contribution of different natural enemy groups within multiple species assemblages to pest suppression, in order to focus management efforts on the most efficient species (Chang and Snyder, 2004).

Intraguild predation (IGP hereafter) involves trophic interactions among members of the same predator guild, i.e. predator species that share the same resource (Polis and McCormick, 1987; Polis et al., 1989; Polis and Holt, 1992; Rosenheim et al., 1995). IGP has been shown to be widespread among food webs, where it is postulated to confer stability in trophic relationships (Polis et al., 1989, 2000; Rosenheim et al., 1995; Rosenheim, 1998; Brodeur and Rosenheim, 2000; Müller and Brodeur, 2002). Since

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generalist predators are prone to engage in IGP, they have the potential to provide pest suppression, but also to disrupt control by other natural enemies. In contrast, parasitoids that are specialized on herbivores typically do not have the potential to attack other members of the guild, with the exception of other parasitoid species sharing the same host (Brodeur and Rosenheim, 2000; Borer, 2002). Instead, parasitoids are themselves frequently susceptible to asymmetric IGP by predators in both immature and adult stages, and this has been suggested as a potential explanation for the failure of parasitoids to suppress their hosts (Rosenheim et al., 1995; Ferguson and Stiling, 1996; Heimpel et al., 1997; Rosenheim, 1998; Brodeur and Rosenheim, 2000; Müller and Brodeur, 2002).

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is a pest of soybeans that is originally from Asia and has been found in North America since 2000 (Ragsdale et al., 2004; Venette and Ragsdale, 2004). Several studies have shown significant impacts of natural enemies on *A. glycines* in Asia (Van den Berg et al., 1997; Liu et al., 2004; Wu et al., 2004) and North America (Fox and Landis, 2003; Fox et al., 2004, 2005; Rutledge et al., 2004; Costamagna and Landis, 2006; Costamagna et al., 2007a). In Asia, the complex of natural enemies attacking *A. glycines* includes the predators *Propylaea japonica* (Thunberg), *Harmonia axyridis* (Pallas), and *H. arcuata* (Fabricius) (Coleoptera: Coccinellidae), and several species of syrphids and lacewings (Van den Berg et al., 1997; Wu et al., 2004). In addition, 10–53% parasitism by the parasitoid *Lysiphlebia japonica* (Ashmead) has been reported in China (Wu et al., 2004). In North America, the assemblage of *A. glycines* natural enemies is dominated by generalist predators, mainly the coccinellids *H. axyridis* and *Coccinella septempunctata* L., and the anthorid *Orius insidiosus* (Say) (Fox et al., 2004, 2005; Rutledge and O'Neil, 2005; Costamagna and Landis, 2006, 2007; Mignault et al., 2006; Costamagna et al., 2007a). *Orius insidiosus* is typically present in the field before the arrival of soybean aphid, due to its ability to feed on alternative small prey, and on the soybean plant itself (Coll and Guershon, 2002; Rutledge and O'Neil, 2005). Therefore, it has been suggested that *O. insidiosus* can provide substantial suppression of soybean aphid (Rutledge et al., 2004; Rutledge and O'Neil, 2005; Desneux et al., 2006). In contrast, other studies suggest that coccinellids play a leading role in suppressing soybean aphid population increase (Fox et al., 2004; Costamagna and Landis, 2006, 2007; Costamagna et al., 2007a).

Reports of field parasitism of *A. glycines* in North America indicate generally null to very low levels, with only a handful of exceptions reporting parasitism levels above 10% (Landis and Brewer, 2003; Nielsen and Hajek, 2005; Costamagna and Landis, 2006; Baute, 2007; Noma and Brewer, 2008). Due to the lack of effective parasitoids, efforts are currently underway to introduce parasitoids from Asia (Heimpel et al., 2004). One potential explanation for the lack of parasitism of *A. glycines* in North America is

the occurrence of asymmetric IGP on parasitoids. In a recent survey, Kaiser et al. (2007) detected 6 parasitoid species from sentinel *A. glycines* in Michigan, suggesting that North American parasitoid assemblages have the potential to attack soybean aphid. In addition, results of a 2003 field cage study revealed higher number of the native parasitoid *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) in cages in which predators were excluded, supporting the hypothesis of IGP limitation of parasitism (Costamagna and Landis, 2006).

Here we report the results of field experiments using different types of exclusion cages to test the impact of ambient levels of different groups of *A. glycines* natural enemies. Specifically, we examined (1) the relative impact of the whole assemblage of natural enemies, versus small predators and parasitoids, and controls with all natural enemies excluded, on aphid abundance, (2) the impact of large predators as intraguild predators on resident parasitoids, and (3) the indirect impact of the different natural enemy groups on plant biomass and yield. Assessing the role of small versus large natural enemy suppression on *A. glycines* population dynamics has important management consequences, particularly to optimize sampling plans, since it involves considerably more effort for farmers and scouts to estimate field densities of the first group.

2. Material and methods

2.1. Field site

Field experiments were conducted in the Biodiversity Study of the Kellogg Biological Station-Long Term Ecological Research site (KBS-LTER, Hickory Corners, MI), during 2004 and 2005. The KBS-LTER station characterizes typical SW Michigan conditions, with crop yields representative of the US North Central Region (Robertson et al., 2000). The biodiversity study has a series of 21 different agronomic treatments that vary in plant species diversity in time and space, ranging from 0 to >15 species in 3-year rotation cycles (<http://lter.kbs.msu.edu/>). Treatments are replicated in 4 completely randomized blocks in 9.1 × 27.4 m plots, resulting in a large mixture of crops in a relatively small area. This diversity of habitats favors the presence of multiple natural enemy species that may potentially impact *A. glycines*, including several species of generalist parasitoids (Kaiser et al., 2007). Within this site, we utilized the 3 most similar treatments (all typical corn-soybean-wheat rotations) that varied only in the presence of cover crops in the non-soybean portions of the rotations. These included a system with a legume and a small grain cover crop in corn and wheat, a system with only a legume cover crop in corn, and finally a system with no cover crop. Our rationale in selecting these systems was primarily to increase replication (i.e. 4 blocks × 3 agronomic treatments = 12 plots), rather than explore the effects of cover crop systems *per se*, since our previous work had shown little impact of cover crop legacy in *A. glycines* population

growth (Costamagna and Landis, 2006). However, to control for any potential effect of cover crops we included agronomic treatment as a factor in the statistical models (see Section 2.4). Since no significant effects were found for any of the variables analyzed, this factor is not further discussed.

2.2. Natural enemy manipulations

In each plot we manipulated aphid and natural enemies using 3 types of cages. A whole exclusion cage consisted of a cylindrical framework of wire of 0.4×1.0 m (diameter \times height), covered by a fine mesh white no-see-um netting (Kaplan Simon Co., Braintree, MA), buried 25 cm in the soil and tied to the top, enclosing 3 soybean plants. The fine mesh used prevented most insects (even small aphids) from moving in or out. The partial exclusion cage was similar, but was covered with a coarse mesh that had 2-mm openings. This cage allowed free movement of apterous and alate aphids, parasitoids, and small predators (primarily *O. insidiosus*), but prevented large predators (i.e. coccinellids) from entering cages. Finally, we used a sham cage identical to the previous one, except the mesh was held 5 cm above the ground surface, and had 18 rectangular openings (3×20 cm) arranged in 3 circular rings alternating in a manner such that there were openings in all directions. The rings of openings were located from 15 to 45 cm above the soil, encompassing most of the height range of the plant canopy. Thus, the sham cage was designed to allow movement of insects of all sizes, while minimizing microclimate differences with the partial exclusion cage.

Using these cages, we performed 5 insect manipulations in each plot: (1) a no-aphid control, in which we enclosed soybean plants free of insects within whole exclusion cages, (2) an aphid treatment, in which we enclosed a controlled number of aphids at the beginning of the season within whole exclusion cages, (3) a small natural enemy treatment, in which we enclosed aphids in the same manner as in the previous treatment, but within partial (2 mm mesh) exclusion cages, and finally 2 treatments with the whole assemblage of natural enemies, including large predators: (4) a sham treatment, in which we enclosed aphids within sham cages, and (5) an open control, in which we infested soybean plants with aphids but without enclosing them in a cage. In all treatments we visually inspected the soybean plants and removed all resident arthropods at the beginning of the experiment (7/1/04 and 6/24/05). Then, we randomly assigned to each group of plants 1 of the 5 insect manipulations and infested them (except in the no-aphid control) at a rate of 15 aphids per plant on the central plant. This infestation rate ensured aphid establishment in all treatments, and was equivalent to approximately 165 aphids/m² which is slightly higher than the number used in previous studies (i.e. 100–110 aphids/m², Costamagna and Landis, 2006; Costamagna et al., 2007a). Natural aphids were present in the field at very

low levels at the beginning of the experiment in 2004, but were at higher numbers a week prior to the beginning of the experiment in 2005, indicating earlier colonization during that year (0.4 ± 0.3 and 27.6 ± 6.5 aphids/plant, respectively). Plants were infested 24 (2004) and 29 (2005) days after planting, when they were at the V2 and V3–4 phenological stages, respectively (Ritchie et al., 1994). To infest plants we used aphids from a colony maintained on soybeans in growth chambers at Michigan State University (24 °C, 16:8 h photoperiod, 60–80% RH), in order to avoid introducing parasitoids in cages by using field collected aphids (Costamagna and Landis, 2006).

We sampled each cage once a week after initial infestation until populations declined in the aphid treatment (mid August to early September). On each sampling date we quantified all the aphids and natural enemies present in each cage, by carefully inspecting each plant. To minimize plant manipulations and reduce sampling time, when aphids reached high numbers and were evenly distributed on trifoliates, we counted a leaflet or a section of a leaflet and used this number to estimate aphid abundance for the whole leaf. The several orders of magnitude in the differences obtained among treatments make us confident that our results were not affected by a potentially lower accuracy of this method. Parasitoid mummies were identified as Braconidae or Aphelinidae, and they were classified as intact, emerged or damaged by predation from large predators, i.e. bearing large holes with irregular patterns, observed with the aid of hand lenses (Colfer and Rosenheim, 2001). The low numbers of natural enemies that were found in predator exclusion cages were removed, and before closing each cage it was further verified that natural enemies were not enclosed in the treatments designed to exclude them. On each sampling date 1–5 individual mummies were collected for rearing to determine parasitoid species. After the 3rd week of sampling, we removed all but 1 plant in each cage (typically the central one), to allow plants to grow uncrowded within the cage. Before that date, we quantified the number of aphids on the 3 plants and calculated the average per plant in each cage, in order to compare these data with subsequent dates. During 2004, beginning 2 weeks after the start of the experiment, we detected ants tending the aphids in the small natural enemy, sham and open treatments in 7 of the 12 experimental plots. To prevent ants in other plots, we added plastic rings (60 cm diameter) around the cages, buried 15 cm in the soil and rising 20 cm above the soil, coated with Tanglefoot (The Tanglefoot Company, Grand Rapids, MI) on the outside. Ant-exclusion rings were placed around the ant-free plots with no attempt to prevent ant tending in the 7 plots where ants were already present. This allowed us to assess the effects of ant tending on aphid abundance. In 2005, we included ant-exclusion rings to all treatments from the outset. We did not observe ants tending aphids in cages within ant-exclusion rings during either year of the experiment.

2.3. Natural population sampling

In the surrounding field, natural populations of aphids and natural enemies were sampled at the same time cages were observed. During 2004, sampling consisted of total visual counts of aphids and natural enemies on 1 designated plant per plot throughout the season ($n = 12$ plants per week). During 2005, a more comprehensive sampling program consisted of direct visual counts of predators in 3 quadrats of 1 m^2 in each plot, following methods used by Fox et al. (2004) and Costamagna and Landis (2006). Specifically, in each quadrat we recorded large predators during 3 min (weeks 1–4) to 1 min (weeks 5–8) periods. In order to sample smaller predators (i.e. *O. insidiosus*, midge larvae, and syrphid larvae), after the initial non-intrusive observation we conducted a more detailed search in 1 soybean row in 1 of the quadrats per plot. The number of small predators per m^2 (NSP/ m^2) was estimated as: $\text{NSP}/\text{m}^2 = \text{NSP in focal row}/\text{number of plants in focal row} \times \text{total number of plants per quadrat}$ (Costamagna and Landis, 2007). Natural enemy abundance is expressed as individuals/ m^2 , with a plant population of 33.8 ± 7.5 plants/ m^2 (means \pm SD, $n = 229$). Finally, we counted the total number of *A. glycines* on the plant located in the northeast corner of each quadrat sample.

2.4. Statistical analysis

Aphid abundance was analyzed using ANOVA (PROC MIXED, SAS Institute 2001). Treatments were assigned in a split-plot design, with agronomic treatment and year as main factors (3×2 treatments, respectively) at the whole-plot level, and insect manipulation treatment as the subplot level (4 treatments). The statistical model included year, agronomic treatment, insect manipulation treatment and sampling dates, and all the interactions between them as fixed effects, since they do not represent random samples of years, agronomic techniques, and sampling dates. Blocks and their interaction terms were treated as random effects. The error term used for year was the year \times block interaction and sampling dates were nested within year. Thus, a total of 48 experimental units (3 agronomic treatments \times 4 insect manipulation treatments \times 4 blocks) were sampled weekly in each year. Significant interactions were analyzed by slicing main effects (Quinn and Keough, 2002). Our main interest was the relative impacts of the different natural enemy groups in aphid abundance, and therefore we compared treatments until aphid populations reached their peak in the aphid treatment (6 weeks after infestation). Later declines in *A. glycines* populations within exclusion cages indicate bottom-up effects (Costamagna et al., 2007b) that cannot be separated from natural enemy impacts on treatments exposed to ambient levels of predation. To test insect manipulation effects on plant biomass and yield we used a similar ANOVA model and included the aphid free treatment (additional 12 experimental units per week). In all analyses, sampling dates

within each year were treated as repeated measures obtained from each subplot with autoregressive covariance structure selected based on Akaike Information Criteria values (Littell et al., 1996). We also assessed whether our artificial aphid releases were representative of natural populations (open treatment versus field sampling), whether field populations differed between years (2004 versus 2005), and whether the no-aphid treatment resulted in significantly reduced aphids (no-aphid treatment versus open treatment and field sampling), using pre-planned contrasts on separate ANOVA models. To explore potential effects of ants tending the aphids during 2004, we compared treatments with and without ants using a similar ANOVA model, but including ant control treatment at the whole-plot level. The effect of agronomic treatments on the abundance of coccinellids and *O. insidiosus* was assessed with ANOVA for 2005 data, whereas the reduced sampling performed in 2004 prevented statistical comparisons. The cumulative number of mummies (i.e. added across sampling dates) was analyzed using a Split-Plot ANOVA, with agronomic treatment in the whole-plot in a CRBD, and the natural enemy treatments as the subplot factor. Individual means were compared using the Least Square Mean Difference, adjusted for multiple comparisons by the sequential Bonferroni method (Rice, 1989). Data were log- or arcsine-transformed before analyses to meet the assumptions of ANOVA, when necessary. Data that did not fit the assumptions of ANOVA after transformation were analyzed using non-parametric statistical tests. Natural enemy abundance within cages was analyzed using the Kruskal–Wallis tests to compare the effect of insect manipulations independently within years and dates, and the effect of year on the total number of natural enemies sampled in each date (SYSTAT Software Inc., 2004). Similarly, we used the same test to assess the effect of agronomic treatments on un-manipulated populations of large and small predators averaged across all sampling dates for 2005. Agronomic treatments did not affect aphid and natural enemy abundance both within and outside cages, but in some tests they were part of significant interaction terms. Test statistics and graphical exploration of the data indicated consistent effects of the main effects (i.e. insect manipulations and sampling dates) irrespective of blocks and agronomic treatments, and therefore these factors are maintained in the analysis but for simplicity their statistics are not presented. Finally, mummy abundance, percentage parasitism and number and proportion of mummies damaged by IGP were compared between the small natural enemy and sham treatments using separate non-parametric Wilcoxon tests for each sampling date (SYSTAT Software Inc., 2004).

3. Results

3.1. *A. glycines* field versus caged populations

Un-manipulated field populations of *A. glycines* differed significantly between years ($F = 262.44$; $df = 1, 36$;

$P < 0.001$). Aphid populations were low throughout 2004, but exceeded the economic threshold of 250 aphids/plant at the end of July in 2005, although not the estimated economic injury level of 674 aphids/plant (Ragsdale et al., 2007, Fig. 1). Un-manipulated field populations were significantly lower than those of the open treatment during both years (Figs. 1 and 5, $F = 145.16$; $df = 1, 36$; $P < 0.001$), suggesting that our initial infestation levels resulted in higher aphid establishment than the natural aphid immigration in the field. We also tested whether the aphid treatment inflated aphid abundance due to alate aphid confinement, by contrasting the proportion of alates (number of alates/total number of aphids) between the aphid treatment (with fine mesh), versus the small natural enemy treatment (with coarse mesh that allows alate emigration). The proportion of alates was significantly higher in the aphid treatment than in the small natural enemy treatment only during the 4th week of the experiment, during both years (natural enemy by date [year] interaction: $F = 3.03$; $df = 10, 80$; $P = 0.0014$, LSMD tests on week 4, P values < 0.001). In general alates were at low densities (at week 4: 0.3 versus 2.7% in 2004, 1.8 versus 6.4% in 2005, for the small natural enemy and aphid treatments, respectively), suggesting only a minor effect of alate confinement on aphid abundance. Similar results have recently been reported using different approaches (Donaldson et al., 2007). In addition, these results suggest that to conservatively assess the impacts of large predators, aphid densities on the sham and open treatments should be compared to the small natural enemy treatment. Finally, caging plants successfully reduced aphids in the no-aphid treatment in comparison with the open treatment and aphid field populations (mean aphids/plant \pm SE for the no-aphid treatment, 2004 = 0.4 ± 0.2 ; 2005 = 31.6 ± 12.4 ; contrasts with field, $F = 152.93$; $df = 1, 36$; $P < 0.001$; with open, $F = 596.07$; $df = 1, 36$; $P < 0.001$).

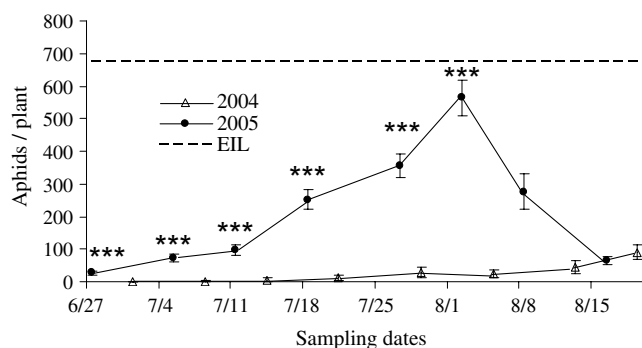


Fig. 1. Open field populations of *A. glycines* at the biodiversity plots of the Kellogg Biological Station-Long Term Ecological Research site in Michigan during 2004 and 2005. Asterisks above the line denote significant differences between years in equivalent sampling weeks (pre-planned contrasts, $P < 0.001$, performed on initial 6 weeks of sampling).

3.2. Natural enemy abundance

In 2004, the single plant sampling yielded only 17 natural enemies, and thus these data were excluded from statistical comparisons. In contrast, in 2005 we observed a total of 1339 natural enemies in the 1 m² quadrat samples, which were dominated by Coccinellidae (73.0% of all predators), with *H. axyridis* the most abundant species (34.6%, Table 1). In addition, we sampled first and second instar coccinellid larvae and pupae (28.4%) that were not possible to identify to species in the field, but most likely were *H. axyridis* (Table 1). Other large-size predators represented only a minor percentage of the predators (9.1%) and included nabids, spiders, mirids, and lacewing larvae (Table 1). Among small-size predators, *O. insidiosus* was the most abundant (14.0%), with midge and syrphid larvae

Table 1
Total number of predators observed in soybeans in 1 m² quadrats throughout the growing season in Michigan, USA, during 2005

Predators	Stage	Total ^a	% ^b
<i>Large-size guild</i>			
Coccinellidae			
<i>Harmonia axyridis</i> Pallas	Adults	291	23.1
	Larvae 3 and 4	145	11.5
<i>Hippodamia variegata</i> (Goeze)	Adults	48	3.8
	Larvae 3 and 4	5	0.4
<i>Coccinella septempunctata</i> L.	Adults	23	1.8
	Larvae 3 and 4	20	1.6
<i>Coleomegilla maculata</i> (De Geer)	Adults	16	1.3
<i>Hippodamia convergens</i> Guérin-Ménéville	Adults	14	1.1
Unidentified to species	Pupae	122	9.7
	Larvae 1 and 2	235	18.7
Nabidae			
<i>Nabis</i> sp.	Adults	37	2.9
	Nymphs	2	0.2
Miridae			
<i>Plagiognathus</i> sp.	Adults	30	2.4
Chrysopidae			
<i>Chrysoperla</i> spp.	Larvae	21	1.7
Araneae			
Unidentified to species		24	1.9
<i>Small-size guild</i>			
Anthocoridae			
<i>Orius insidiosus</i>	Adults	101 [61]	8.0
	Nymphs	75 [51]	6.0
Cecidomyiidae and Chamaemyiidae			
Unidentified to species	Larvae	31 [27]	2.5
Syrphidae			
Unidentified to species	Larvae	19 [14]	1.5

^a Total for small predators are adjusted to compensate for the larger sample size used for large predators, observed values are within brackets (see Section 2 for details).

^b Percentages for small predators are based on adjusted abundance values.

accounting for 4.0% of the predators. Overall, coccinellid abundance was more or less constant, fluctuating around 3 predators/m², whereas other large predators and small predators were initially at densities of 1 predator/m² and then decreased (Fig. 2). *Orius insidiosus* reached 3 individuals/m² in early to mid July, and then peaked again at similar density at early August (Fig. 2). These relatively low predator densities (i.e. approximately 1 predator/5 plants) were sufficient to maintain *A. glycines* field populations under the economic injury level (Fig. 1) and substantially below the densities reached in the predator exclusion treatments (see Section 3.3).

Cage manipulations resulted in the expected reduction of large predator densities in the complete and partial exclusion cages, and of *O. insidiosus* in the complete exclusions during the initial weeks of manipulation (Fig. 3). During 2004, coccinellids showed a trend of very low densities in all treatments, except in the sham treatment that reached marginally significantly higher densities in week 3, and significantly higher densities in week 4 (Fig. 3a). Similarly, during 2005, coccinellids were detected at very low densities on all treatments initially, reaching significantly higher densities in the sham treatment only during week 4. In weeks 5 and 6 of 2005, similar levels of coccinellids in small natural enemy, open and sham treatments were obtained due to the presence of small coccinellid larvae in the small natural enemy treatment, in response to increased aphid densities in that treatment (Fig. 3b). The abundance of *O. insidiosus* showed the expected trend of lower numbers in the aphid treatment during initial weeks, being significantly lower in weeks 2, 3, and 4 of 2004, and 2 and 6 in 2005 (Fig. 3c and d). In addition it showed a trend of higher numbers in the small natural enemy treatment during most of the season, probably in response to increasing densities of *A. glycines* (see Section 3.3). Large predators other than coccinellids were significantly more abundant in sham cages only during the 4th week of 2004, although at very low levels (Fig. 3e and f). Small predators (i.e. midge and syrphid larvae) were initially higher

in the sham and open treatments at low levels, and then reached high but variable abundances in the small natural enemy and aphid treatments, probably as a response to the increase densities of aphids on those treatments (Fig. 3g and h). In summary, our results indicate that the initial weeks of manipulation resulted in reduced coccinellid density in the aphid and small natural enemy treatments, and reduced *O. insidiosus* density in the aphid treatment. Successful manipulation of natural enemies during these initial weeks of the experiment resulted in highly contrasting aphid trajectories of population growth.

To test for a potential difference in the abundance of natural enemies between 2004 and 2005, we compared the total number of natural enemies observed, grouped for all 4 natural enemy treatments, for each sampling date. Overall, predators were more abundant in 2005 (898 versus 231 total predators observed in 2004, notice the different scales in Fig. 3), probably responding to increased aphid densities in the field at large (Fig. 1). In 2005, significantly more *O. insidiosus* and coccinellids were observed in weeks 2, 5, and 6 after the beginning of the experiment, and more large predators in weeks 2 and 5 (Fig. 3a–f). The abundance of small predators did not differ between years (Fig. 3g and h).

During 2004, we observed 1934 parasitoids in our cage manipulations, most of which were Braconids (85%) and the rest Aphelinidae (15%). The subsamples collected included 3 parasitoid species: the braconids *L. testaceipes* and a new species, *Binodoxys kelloggensis* Pike et Starý (Pike et al., 2007), and the aphelinid *Aphelinus asychis* (Walker). We did not observe parasitoids in the aphid treatment and only 1 mummy in the open treatment, and therefore these treatments were excluded from statistical comparisons. Sampling of natural populations did not yield any parasitoids outside our cage manipulations during 2004. Despite the aphid outbreak and the more intensive sampling performed in 2005, only 5 braconid mummies were detected outside of cages, and 42 mummies in our cage manipulations (95% Braconidae, 5% Aphelinidae). Therefore, we only considered data from 2004 in the following analyses of parasitism.

Overall, parasitoids were more abundant in the small natural enemy treatment than in the sham treatment, as indicated by the significantly higher cumulative number of mummies obtained ($F = 8.30$; $df = 1, 9$; $P = 0.0182$). Parasitism levels were low and very variable, with some plots that did not have parasitism through most of the season (5 out of 12 plots). Thus, although there was a trend of higher parasitism in the small natural enemy treatment throughout the experiment, it was only marginally significant in weeks 4 and 6 (Fig. 4a). Percentage parasitism was lower than 1% throughout the experiment and did not differ between treatments (Wilcoxon tests, all $P > 0.176$, Fig. 4b), indicating that the higher number of mummies observed in the small natural enemy treatment were more likely due to a numerical response of the parasitoids to the higher aphid densities of that treatment

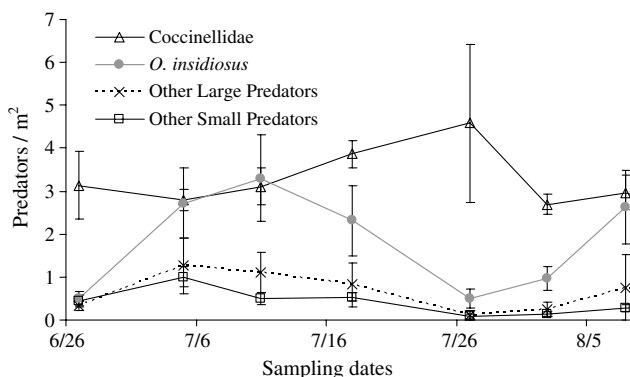


Fig. 2. Open field predator populations sampled in the biodiversity plots of the KBS-LTER site during 2005. Other large-size predators include nabids, spiders, small carabids, mirids, and lacewing larvae; other small-size predators include midge and syrphid larvae.

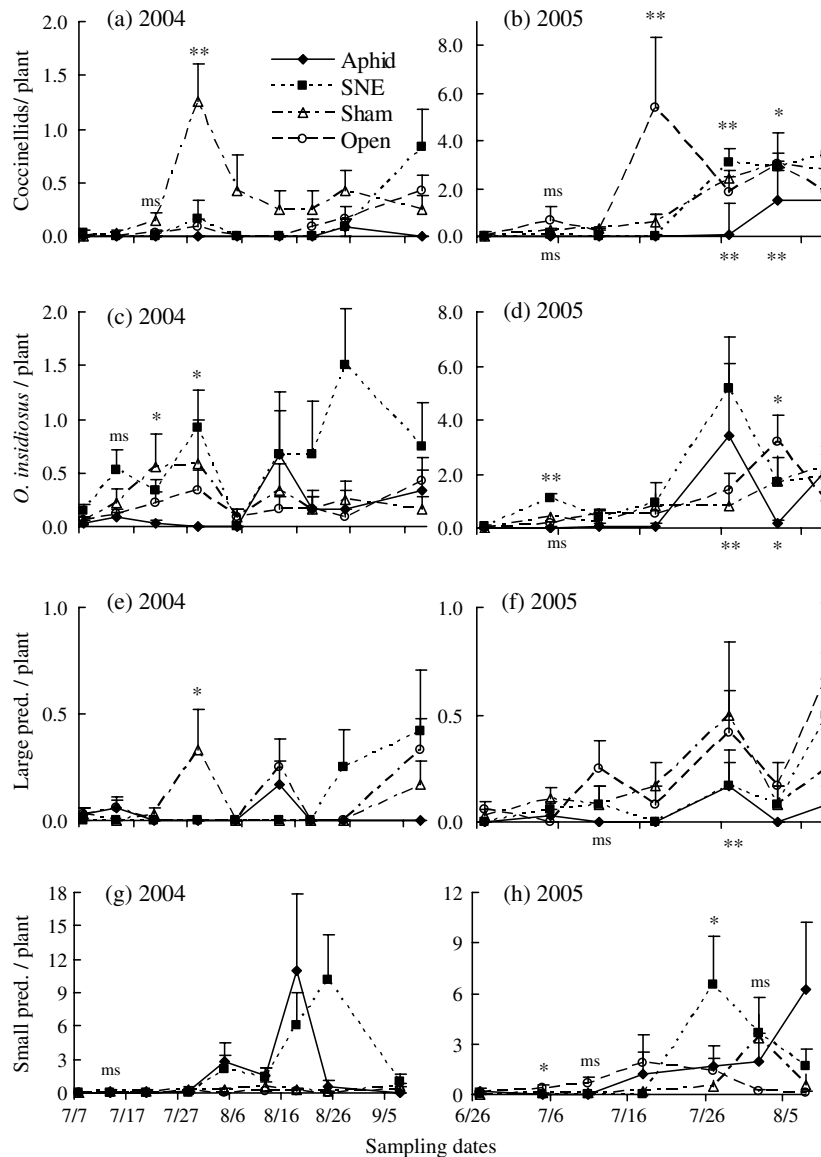


Fig. 3. Effect of natural enemy manipulations on the abundance (mean + SE) of the 4 major groups of predators sampled in the biodiversity plots of the KBS-LTER during 2004 and 2005. Asterisks above treatment lines indicate significant differences among natural enemy treatments, asterisks below sampling dates indicate significantly higher abundance for all treatments combined in that year (Kruskal–Wallis tests; ms, marginally significant, $P < 0.10$; * $P < 0.05$, ** $P < 0.01$). Statistical comparisons were performed for the 6 initial weeks. SNE, small natural enemy treatment. Please notice the independent scales for each graph.

than due to a refuge from IGP by large predators. Finally, we directly assessed IGP by comparing the number and percentage of mummies damaged by predation. Although we observed a trend of more mummies damaged in the sham treatment, the highly variable incidence of IGP in this treatment resulted in an absence of significant differences (Wilcoxon tests, all $P > 0.180$, Fig. 4c). A more refined test was performed by contrasting IGP on all the possible pairs in which parasitism was obtained in both treatments within the same plot ($n = 16$). This test revealed that a significantly greater proportion of mummies was damaged in the sham ($18.1 \pm 7.6\%$, range 0–94.4%) than in the small natural enemy treatment ($3.7 \pm 2.4\%$, range 0–36.4%, Wilcoxon test $z = 2.1$, $P = 0.036$). Together,

these results indicate that low levels of IGP by large predators did occur at the very low levels of parasitism observed in our study, but did not result in parasitoid disruption.

3.3. Aphid suppression

Despite an expected positive role of ant tending on aphids (e.g. Völkl, 1992; Stadler et al., 2002), we did not find significant effects of ants on aphid abundance. The presence of ants in 2004 did not result in significant differences in aphid abundance either as a main effect ($F = 3.49$; $df = 1, 2$, $P = 0.027$) or as an interaction with agronomic or natural enemy treatments (all $P > 0.20$), therefore this factor was removed from subsequent analysis. However,

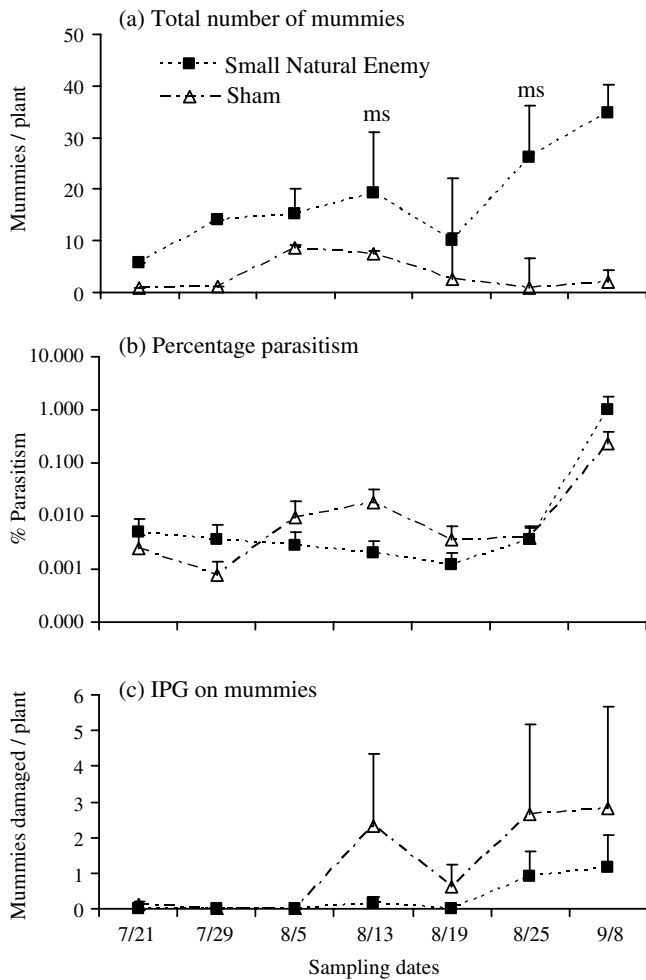


Fig. 4. Effect of predator manipulations on (a) number of mummies/plant, (b) percentage parasitism (proportion of mummies to aphids $\times 100$), and (c), number of mummies damaged by predators; averaged across agronomic treatments. Only 1 mummy was obtained in the open treatment and none in the aphid treatment, and therefore these treatments were excluded from statistical comparisons. Treatments were compared independently for each date using Wilcoxon tests (ms = marginally significant, $P < 0.10$).

we can not make general statements regarding the importance of ant tending in the *A. glycines* system, since our study was not explicitly designed to test this factor, and we did not manipulate the ants during 2005.

Natural enemy manipulations had significant effects on aphid abundance that changed in intensity with year and sampling date (significant natural enemy by date [year] interaction, Table 2). Large predators dramatically reduced aphid populations during 2004 (207-fold reduction comparing aphid and open treatments, and 86-fold reduction comparing small natural enemy and open treatments at peak aphid populations during 8/13/04) and although this effect was lower in 2005 (63- and 36-fold reduction during 7/27/05, respectively) in both cases it was highly significant (slicing tests: $F = 49.38$ [2004], and $F = 24.24$ [2005]; $df = 3, 54$, $P < 0.0001$; Fig. 5).

Table 2

Results of the ANOVA for effects of year, agronomic treatment, and natural enemy treatment on *A. glycines* abundance

Source	df	F	P
Year	1, 3	40.86	0.0078
Agronomic treatment	2, 6	3.23	0.1116
Agronomic treatment \times year	2, 6	0.26	0.7804
Natural enemies	3, 54	65.67	<.0001
Agronomic treatment \times natural enemies	6, 54	1.38	0.241
Natural enemies \times year	3, 54	7.96	0.0002
Agron. treat \times natural enemies \times year	6, 54	1.12	0.3624
Date (year)	10, 360	155.02	<.0001
Date (year) \times agronomic treatment	20, 360	1.62	0.0457
Date (year) \times natural enemies	30, 360	7.85	<.0001
Date (year) \times agronomic treatment \times natural enemies	60, 360	1.39	0.0365
Block	3, 12	6.66	0.2429
Block \times year	3, 6	0.57	0.6567
Block \times agronomic treatment	6, 6	1.2	0.4142
Block \times year \times agronomic treatment	6, 54	1.32	0.2659
Block \times year \times agronomic treatment \times natural enemies	54, 360	4.45	<.0001

Aphid populations increased rapidly in the aphid and small natural enemy treatments and exceeded economic injury levels 4 (2004) and 3 (2005) weeks after initial infes-

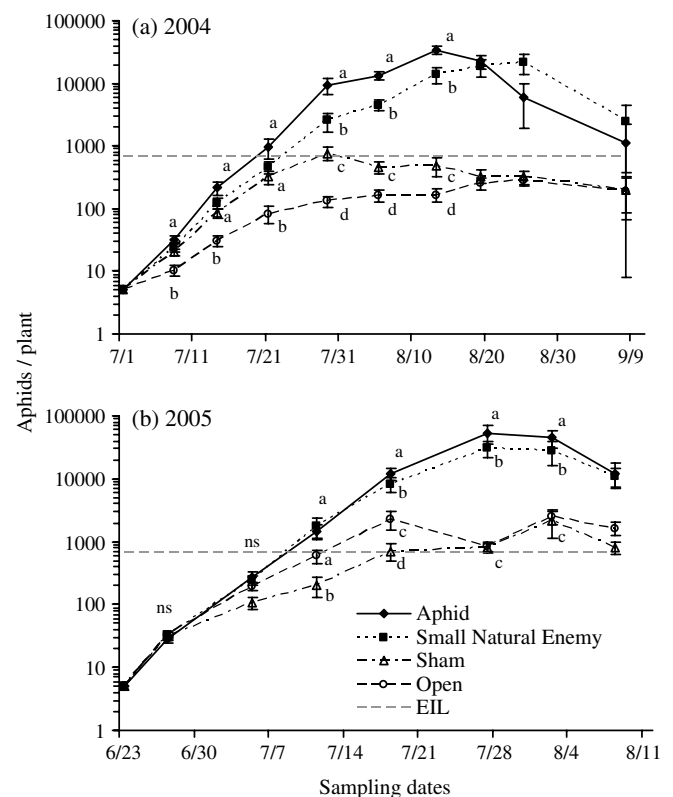


Fig. 5. Effect of natural enemy treatments on the number of *A. glycines* (mean \pm SE) during (a) 2004 and (b) 2005. Different letters on each sampling date indicate significant differences ($P < 0.05$, Least Mean Square Differences adjusted by the sequential Bonferroni method for multiple comparisons). Statistical comparisons were restricted to the 6 initial sampling dates, when aphids reached peak populations.

tation. In contrast, large predators maintained aphids below threshold for the entire season in 2004 (Fig. 5a). In 2005, an outbreak of *A. glycines* occurred throughout the US Midwest, and aphids reached threshold populations in our experiment 5 weeks after infestation, with a maximum level of 2500 aphids/plant at week 6 in the sham and open treatments. However, these population levels were reached 3 weeks earlier in the aphid and small natural enemy treatments, from which large predators were excluded (Fig. 5b).

During 2004, we observed a significant delay (approximately 2 weeks) in the timing of peak aphid density in the small natural enemy versus the aphid treatments (Fig. 5a). We observed a shorter delay in 2005, although in general aphid populations were slightly lower in the small natural enemy treatment (Fig. 5b). Since we observed much more parasitism in 2004 than in 2005, we tested whether the delay in aphid peak populations may have been a result of parasitism. To assess this, we contrasted aphid abundance for the first 6 weeks of the experiment in the small natural enemy treatment between plots in which we found parasitoids ($n = 5$) versus plots in which we did not find parasitoids ($n = 7$), irrespective of block and agronomic treatment. We obtained no significant differences related to the presence of parasitoids ($F = 2.70$, $df = 1, 10$; $P = 0.1311$) and a trend opposite to that expected, with more aphids in plots with parasitoids. Thus, the lower numbers of aphids in the small natural enemy treatment in comparison with the aphid treatment were not attributable to parasitism.

3.4. Soybean biomass and yield

We observed significant differences in biomass ($F = 42.69$; $df = 4, 80$; $P < 0.0001$) and yield ($F = 70.11$; $df = 4, 80$; $P < 0.0001$) due to our natural enemy manipulation treatments (Fig. 6). The presence of soybean aphids decreased biomass and yield between 72% and 90% in comparison with the controls with no aphids, but exposure of aphids to predators in the sham and open treatments resulted in a trophic cascade that restored both plant yield and biomass (Fig. 6). The presence of small natural enemies resulted in intermediate soybean biomass and yield that differed significantly from the aphid and aphid free treatments during both years, and from the open and sham treatments during 2005. Although we can not discard that the higher levels of plant manipulation required to sample high aphid numbers affected plant biomass and yield, we are confident that most of the reductions observed were due to increased number of aphids for two reasons. First, our sampling method was specifically designed to minimize plant manipulations. Second, two recent studies using larger cages in which only a random subset of plants was sampled every week (and therefore considerable less overall manipulations on the plants were exerted) showed the same results as in our study (Costamagna et al., 2007a; Rhainds et al., 2007). Biomass was significantly lower in 2004 than

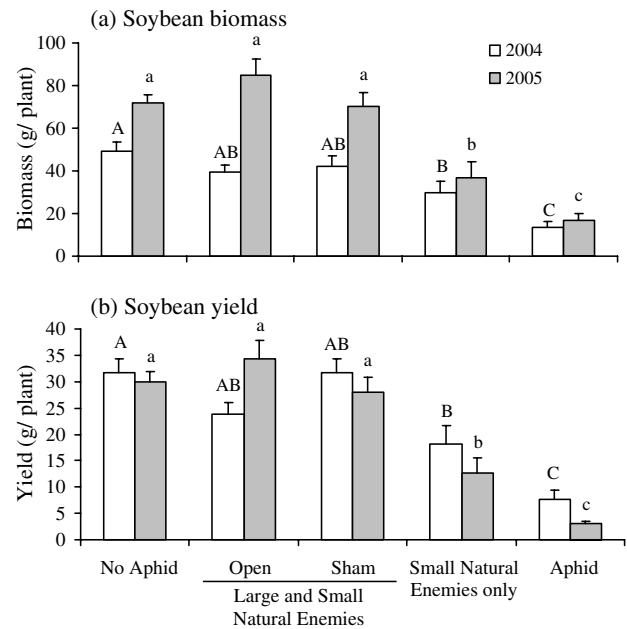


Fig. 6. Effects of natural enemy manipulations on soybean (a) above ground biomass and (b) yield (g of seed/plant), in the biodiversity plots of the KBS-LTER site, during 2004 and 2005. Different letters indicate significant differences ($P < 0.05$, LSMD, adjusted by the sequential Bonferroni method) among treatments within the same year (capital letters for 2004, lower case letters for 2005).

in 2005 ($F = 10.77$; $df = 1, 15$; $P = 0.0050$). We observed a non-significant trend of lower yield in 2005 in all except the open treatment (year by treatment interaction: $F = 5.65$; $df = 4, 80$; $P = 0.0005$). These results confirm independent findings of the potential for extant predator assemblages to reduce *A. glycines* populations to levels that prevent significant plant damage (Costamagna et al., 2007a; Rhainds et al., 2007), and suggest that the delayed in aphid peak populations due to exposure to small natural enemies resulted in a partial recovery of soybean yield.

4. Discussion

During the 2 years of study, we observed strong suppression of *A. glycines* due to large generalist predators, and only small suppression due to small natural enemies. Our findings thus support an increasing number of studies showing an important role of generalist predators controlling *A. glycines* in North America (Brown et al., 2003; Fox et al., 2004, 2005; Rutledge et al., 2004; Nielsen and Hajek, 2005; Rutledge and O'Neil, 2005; Costamagna and Landis, 2006; Desneux et al., 2006; Costamagna et al., 2007a; Gardiner and Landis, 2007). Moreover, our results showed that even during outbreak years such as 2005, large predators dominated by coccinellids were able to exert strong suppression of soybean aphid, confirming earlier studies (Fox et al., 2004; Costamagna and Landis, 2006; Costamagna et al., 2007a). In addition, we showed that soybean aphid has the potential to cause a 3.5- to 10-fold reduction on soybean yield and biomass, and that cascading effects of suppression by large

predators restored them to normal levels during both years of study. Similar results were obtained in different experiments using larger field cages (Costamagna et al., 2007a; Rhainds et al., 2007).

We observed 3 species of parasitoids attacking *A. glycines*, but did not detect significant effects of parasitism in reducing aphid populations, even when IGP by large predators was reduced in the small natural enemy treatment. Since we only excluded large predators, we can not discard IGP on parasitoids by small predators. However, laboratory studies demonstrated that the dominant small predator, *O. insidiosus* would not feed on mummies of *L. testaceipes* or *A. asychis* (Costamagna and Landis, unpublished data). By contrast, the same experiments showed that *H. axyridis* will readily feed on mummies of both parasitoids (Costamagna and Landis, unpublished data). Thus, although we can not discard coincidental IGP by *O. insidiosus* on parasitoid larvae before they reach the mummy stage, these laboratory experiments in conjunction with other field studies (Colfer and Rosenheim, 2001), suggest that by excluding large predators we reduced a major source of IGP.

We did observe direct evidence of IGP by large predators by quantifying damaged mummies, and this measure is likely to be conservative, since predators may completely dislodge some of the parasitoid mummies during consumption (Brodeur and Rosenheim, 2000). Colfer and Rosenheim (2001) studied IGP of *L. testaceipes* by the coccinellid *Hippodamia convergens* in cotton and found that despite levels of IGP of up to 98–100%, the addition of the coccinellids significantly increased suppression of cotton aphid over the presence of the parasitoid alone. In contrast, in the *A. glycines* system we observed very low parasitism levels, typically lower than 1%, no evidence of reduction in aphid population growth due to parasitism, and less than 20% IGP on mummies by large predators. Failure to run the experiment long enough to develop more than 1 parasitoid generation can result in underestimation of IGP effects on parasitoid populations (Snyder and Ives, 2003). However, the biology of the 2 most common species we observed suggests that the time frame used in our experiment was adequate to detect IGP effects on 2–3 parasitoid generations. Development from egg to emergence for *L. testaceipes* is not known on *A. glycines*, however, studies conducted using the aphid *Schizaphis graminum* (Rondani) as host required between 10 and 15 days to emergence in the range of temperatures of our experiments (Kring and Kring, 1988; Elliott et al., 1999). Similarly, generation times of *A. asychis* on the aphid *Diuraphis noxia* (Modvilko) range between 12 and 24.8 days (at 26–18 °C, Lee and Elliott, 1998). Overall, our results suggest that the low levels of parasitism currently observed in the *A. glycines* system may be more likely due to a lack of adaptation of the extant community of generalist parasitoids to search in soybeans rather than limitation through IGP by large predators. A similar hypothesis has been proposed to explain the initial low

levels of parasitism of the exotic Russian wheat aphid, *D. noxia*, by indigenous parasitoids that now are dominating the natural enemy assemblage of this pest in wheat (Brewer et al., 2005). To our knowledge, our study is the first one to explicitly test the effect of IGP on the impact extant parasitoids on a new invasive agricultural pest.

Previous studies have assessed the role of the entire assemblage of natural enemies suppressing soybean aphid by contrasting complete predator exclusions with open and sham controls (Fox et al., 2004; Costamagna and Landis, 2006; Desneux et al., 2006). The different cages used in our experiments allow us to separate the effect of *O. insidiosus*, other small predators, and parasitoids, from the impact of Coccinellids and other large predators. Small natural enemies, largely dominated by *O. insidiosus*, caused a delay in aphid peak populations, but did not prevent aphids from reaching economic injury levels in either year. We observed a 2-week delay in peak aphid populations in the small natural enemy treatment with respect to the aphid treatment in 2004, and slightly reduced overall aphid densities during 2005 (Fig. 5). Parasitism was low during 2004 and almost non-existent in 2005. However, plots with parasitoids in 2004 had a trend for higher aphid populations than plots without parasitism, suggesting that reduction of *A. glycines* in that treatment was mainly due to *O. insidiosus*. Several studies have suggested that *O. insidiosus*, due to its abundance on soybeans early in the season, potential for aphid consumption in laboratory conditions, and negative correlation with aphid growth, can play an important role reducing *A. glycines* population growth (Rutledge et al., 2004; Rutledge and O'Neil, 2005; Desneux et al., 2006). Moreover, a recent study conducted in Nebraska using a similar methodology as in our study, showed significant reductions in *A. glycines* populations primarily associated with *O. insidiosus* (Brosius et al., 2007). In contrast, our results suggest that similar densities of *O. insidiosus*, in addition to other small natural enemies, have a low to moderate impact on aphids, but do not prevent exponential increase of *A. glycines*. The stronger impact of *O. insidiosus* in the Nebraska study may be related with the lower *A. glycines* population growth potential observed in their study. Our peak aphid populations reached over 20,000 aphids per plant in predator exclusion conditions, whereas in the Nebraska study aphid peak populations reached only 3000 aphids per plant. One potential explanation for the lower aphid peak populations observed in Nebraska could be plant phenological limitations for *A. glycines* growth, since plants were infested around 60 days after planting, whereas in our experiment plants were infested less than 30 days after planting. Previous studies suggest a decrease in host plant suitability with plant age for several aphid species (Williams, 1995; Kift et al., 1998), including *A. glycines* (Van den Berg et al., 1997; Rhainds et al., 2007), although other studies did not corroborate

that effect (Rutledge and O'Neil, 2006). In summary, the weak aphid suppression by *O. insidiosus* obtained in our study could be associated with higher potential for aphid growth on the younger plants infested, mimicking early field colonization typical of the US Midwest.

In conclusion, we observed strong reduction in aphid numbers only in the treatments that were exposed to coccinellids and large predators (sham and open treatments), indicating that these were the predators that had the greater impact on aphid populations in the field. In contrast, the guild of small natural enemies, dominated by *O. insidiosus*, delayed but did not prevent *A. glycines* from reaching the EIL during the 2 years of study. In general, our findings suggest that generalist predators, particularly coccinellids, are a key factor in *A. glycines* control and management efforts should focus on determining their impact on action thresholds for this pest.

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