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Impact of intraguild predation by adult *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Hemiptera: Aphididae) biological control in cage studies

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Abstract

The soybean aphid, *Aphis glycines* Matsumura, has become a principal arthropod pest of soybean in the U.S. since its first detection in 2000. This species threatens soybean production through direct feeding damage and virus transmission. A diverse guild of insect predators feeds on soybean aphid in Michigan including the exotic coccinellid *Harmonia axyridis*, the native gall midge *Aphidoletes aphidimyza* and the native lacewing *Chrysoperla carnea*. In addition to feeding on *A. glycines* some members of this guild may also engage in intraguild predation. These interactions may produce positive, negative, or neutral impacts on *A. glycines* biological control. We explored the impact of intraguild predation on soybean aphid population dynamics by comparing aphid populations in microcosms with either *A. aphidimyza* larvae or *C. carnea* larvae alone, with both a *H. axyridis* adult and either *A. aphidimyza* or *C. carnea* larvae, and without predators. When *H. axyridis* was present with larval *A. aphidimyza* or *C. carnea*, the lady beetle acted as an intraguild predator. However, intraguild feeding did not result in a release of aphid populations compared with microcosms containing only the intraguild and aphid prey. A similar result was found in field cages. Cages allowing large predators had reduced numbers of *A. aphidimyza* and *C. carnea* larvae but also significantly fewer aphids compared with predator exclusion cages. Thus, in both lab and field studies the direct impact of *H. axyridis* on *A. glycines* overcame its negative impact as an intraguild predator. Together, these studies indicate that while the exotic *H. axyridis* does act as an intraguild predator and may contribute to local declines in *A. aphidimyza* and *C. carnea*, it is also currently important in overall biological control of *A. glycines*.

Keywords: Soybean aphid; *Aphis glycines*; Intraguild predation; *Aphidoletes aphidimyza*; *Chrysoperla carnea*; *Harmonia axyridis*

1. Introduction

In agricultural systems, knowledge of food web interactions is often limited due to the complexity of tri-trophic relationships and the number of species involved (Bascompte and Melian, 2005). Invasions of exotic species are likely to profoundly affect the structure of food webs in agroecosystems. The soybean aphid, *Aphis glycines* Matsumura, a native of Asia, was first detected in the U.S. in Wisconsin in July of 2000. Its range now extends throughout the north-central soybean growing states and into Canada. Feeding by *A. glycines* on the leaves, stems, and pods of the soybean plant causes reductions in photosynthetic rate, plant growth, and seed yield of soybean plants (DiFonzo and Hines, 2002; Wang et al., 1996). This pest can also vector several viruses, including alfalfa mosaic, soybean dwarf, soybean stunt, soybean mosaic, tobacco ringspot, and bean yellow mosaic (Van den Berg et al., 1997; Clark and Perry, 2002; Wang and Ghabrial, 2002).

In this system, the invading community extends beyond soybean aphid, involving the introduction, both intentional and unintentional, of an entire exotic food web (Thompson and Townsend, 2003). The primary host plants of soybean aphid are buckthorn species in the genus *Rhamnus*, the

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most abundant and widespread species is *R. cathartica* L. (Rhamnaceae), a native to Asia introduced in the 1880s as a landscape plant (Yoo et al., 2005). The secondary host plant of *A. glycines*, cultivated soybean, *Glycine max* L. (Fabaceae) (also native to Asia), has been cultivated in the US since the early 1800s, and is one of the dominant crops in the north-central region. The guild of predators that attack soybean aphid also contains non-native species, including the following coccinellids: *Harmonia axyridis* Pallas, *Coccinella septempunctata* L. and *Hippodamia variegata* (Goeze) (Gardiner and Parsons, 2005). These organisms interact with native predatory species including the coccinellids, *Coleomegilla maculata* (DeGeer), *Hippodamia convergens* Guérin-Méneville, *Hippodamia parenthesis* (Say), the lacewing *Chrysoperla carnea* (Stephens) and *Aphidoletes aphidimyza* (Rondani) (Cecidomyiidae) that have adapted to utilizing soybean aphid as prey.

1.1. Intraguild interactions

In aphidophagous systems where several predators compete for a single dominant prey, interactions such as intraguild predation are likely to occur (Lucas, 2005). Intraguild predation (IGP hereafter), is defined by Lucas et al. (1998) as predatory interactions between predators within a guild. Generalist predators can be effective biological control agents of native and exotic pests (Symondson et al., 2002); however, interactions such as IGP can also interfere with pest suppression (Snyder and Wise, 1999). Intraguild interactions between predators have resulted in diverse effects on prey population dynamics and plant productivity (Polis et al., 1989; Polis, 1991; Prasad and Snyder, 2004; Rosenheim et al., 1995; Rosenheim, 1998; Snyder et al., 2004a,b). Overall, these studies indicate that the impacts of IGP are community specific. As one of the most abundant predators found in soybean fields (Fox et al., 2004; Rutledge et al., 2004; Costamagna and Landis, 2006), *H. axyridis* has the potential to impact both *A. glycines* population dynamics and populations of native predators that have adapted to utilizing soybean aphid as a food source.

There is abundant evidence that *H. axyridis* acts as an intraguild predator of other coccinellids and populations of some native species have declined significantly after the establishment of exotics (Colunga-Garcia and Gage, 1998; Elliott et al., 1996; Michaud, 2002). While IGP by *H. axyridis* on other predator groups has received less attention; this study focuses on the intraguild interactions between *H. axyridis*, *C. carnea*, and *A. aphidimyza*. Previous studies have found that IGP by lady beetles on lacewing and *A. aphidimyza* larvae did occur in microcosm studies but not under field conditions. Lucas et al. (1998) established that the lady beetle *C. maculata* was an intraguild predator of both, *Chrysoperla rufilabris* (Burmeister) and *A. aphidimyza* in microcosms. In the field, Brown (2003) compared aphid predator composition before and after the introduction of *H. axyridis* in apple orchards and found no effect of *H. axyridis* on *A. aphidimyza* and chrysopid populations.

1.2. Impact of IGP on early season biological control

A critical time in the biological control of soybean aphid occurs in the early season when aphids begin to colonize soybean plants. We have observed that when aphid populations are low and patchy, adult *H. axyridis* act as "transient predators," spending short feeding bouts within an aphid colony, removing some of the aphid population before moving on to feed elsewhere (Landis unpublished data). Through a review of mark-recapture studies this behavior was also described by Evans (2003) who stated that coccinellids often do not remain long in any one location and will forage in many different habitats throughout their breeding season. When aphid populations are low, adult coccinellids are less likely to lay eggs (Evans, 2003); therefore lady beetle larvae are not yet abundantly present in soybean fields. Our hypothesis was that during this early season time period *H. axyridis* adults may release soybean aphid populations from overall control by acting as intraguild predators of smaller "resident predators", including larvae of the aphid predatory midge *A. aphidimyza* and green lacewing *C. carnea*. As immature stages these species have limited mobility; individuals in soybean fields spend their entire juvenile stage feeding primarily on the soybean aphid on a single plant or foraging among a few plants within a field. If *H. axyridis* acts as an intraguild predator and removes the majority of these small resident predaceous larvae, the remaining aphids could be released from overall control. However, if *H. axyridis* feeds primarily on aphids, these species may have an additive negative effect on *A. glycines* populations. The objectives of this study were to: (1) investigate how the presence of *H. axyridis* impacts populations of the native predators *A. aphidimyza* and *C. carnea* in soybean agroecosystems and (2) determine if IGP among these arthropods impacts soybean aphid population dynamics.

2. Methods

2.1. Rearing

Soybean plants var. 92B16 (Pioneer Hi-Bred International Inc., Johnston, IA) were maintained in a greenhouse at 24 °C on a 16:8 h L:D. Soybean aphid was cultured on soybean in a walk-in growth chamber (Percival Scientific, Perry, IA) at 24:16 °C on a 16:8 h L:D. The predatory midge, *A. aphidimyza*, was obtained from IPM Laboratories (Locke, NY) as pupae. Flies were hatched by placing the pupae into a dark growth chamber for 48 h at 24 °C. Adults were released onto soybean aphid-infested soybean plants and allowed to lay eggs. Larvae hatched within 3 d and reached their second instar within 5 d. The aphid predator *C. carnea* was obtained from Koppert Biological Systems (Ann Arbor, MI) as eggs and first instar larvae. The eggs and larvae were placed in rearing boxes on soybean plants infested with *A. glycines*. A culture of *H. axyridis* was initiated by field-collecting adult beetles from soybean fields at the Michigan State

University Beet and Bean Farm (Saginaw, MI); larvae and adult *H. axyridis* were reared separately on soybean aphid-infested plants. All predator colonies were maintained at 24:16 °C on a 16:8 h L:D.

2.2. Predator consumption rates

Aphid consumption rates of *A. aphidimyza* and *C. carnea* were measured in 60 × 15 mm Petri dish arenas. The bottom of the dish was lined with moist filter paper and contained a soybean leaflet infested with 30 second instar or adult *A. glycines*. One second instar *A. aphidimyza* or second instar *C. carnea* was added to each arena and allowed to forage for 24 h, after which the predator was removed and the number of remaining aphids recorded. Five replications of each treatment were arranged randomly on a cafeteria tray and held in a walk-in growth chamber at 24:16 °C on a 16:8 h L:D.

2.3. Microcosm design

Microcosms consisted of a 3.79 L cylindrical-shaped clear plastic container with three 7 × 13 cm windows of fine mesh netting. The container had a 10 cm opening. The plastic container was inverted over a 10 cm square plastic pot containing three V1 soybean plants (Teare and Hodges, 1994). The opening of the container was held inside the pot; it was buried in the soil and sealed with tape. Microcosms were held on cafeteria trays in a walk-in growth chamber at 24:16 °C on a 16:8 h L:D and watered by filling the tray with water.

2.4. Microcosm experimental procedure

The microcosm experiments were designed to measure the intensity of IGP by the transient predator *H. axyridis* on the resident predators *A. aphidimyza* and *C. carnea* and the resulting impact on *A. glycines* population dynamics. The interaction between *H. axyridis* and the two resident larval predators was evaluated in separate experiments. In both the *A. aphidimyza* and *C. carnea* microcosm experiments four treatments were compared. For *A. aphidimyza* the treatments were: aphids alone (CONTROL), aphids + *A. aphidimyza* (AA), aphids + *H. axyridis* (HA), and aphids + both predators (*H. axyridis* and *A. aphidimyza*) (BOTH). The treatments for the *C. carnea* experiment were: aphids alone (CONTROL), aphids + *C. carnea* (CC), aphids + *H. axyridis* (HA), and aphids + *H. axyridis* and *C. carnea* (BOTH).

To begin a microcosm experiment, 10 adult and 5 second instar aphids were placed onto clean leaf disks. One disk was then attached to a fully expanded leaf on each of three plants within a pot, for a total of 45 aphids per microcosm. After 48 h, aphids were counted in each microcosm, and in the *A. aphidimyza* experiment one second instar *A. aphidimyza* was introduced onto each of the three soybean plants per microcosm in the AA and BOTH treatments. Similarly, in the *C. carnea* experiment one second instar *C. carnea* was introduced onto each of the three soybean plants per microcosm in the CC and BOTH treatments 48 h after the introduction of *A. glycines*. This was considered the beginning of the experiment, time 0 in all analyses. In the *A. aphidimyza* experiment aphids averaged (±1 SEM) 114.47 ± 6.97 (CONTROL), 113.33 ± 6.85 (AA), 116.2 ± 7.11 (HA), and 107.8 ± 6.51 (BOTH) at time 0 h. In the *C. carnea* experiment *A. glycines* averaged 123.27 ± 10.41 (CONTROL), 111.47 ± 8.24 (CC), 129.4 ± 7.14 (HA), and 119.8 ± 8.57 (BOTH) at 0 h. In both experiments, 24 h after the release of the larval predators aphids were counted again in all treatments and one adult *H. axyridis* was released into the HA and BOTH microcosm treatments. The beetle was allowed to forage for 3 h, after which it was removed to simulate its transient feeding behavior in the field. During this 3 h *H. axyridis* had the opportunity to consume *A. glycines* (HA treatment), or both aphids and the larval predators (BOTH treatment). After the removal of *H. axyridis*, aphids were counted in the HA treatment and aphids and *A. aphidimyza* or *C. carnea* were counted in the BOTH treatment. Aphids were then counted daily in all treatments for the following 2 d and again every 48 h for an additional two sampling periods to track aphid population growth in each treatment.

2.5. Field cage experiment

To determine how the intraguild interactions examined in the microcosm experiment compared with interactions in the field, where a larger complex of generalist predators was present, a field cage experiment was conducted. In the field, *H. axyridis* was one of the two large exotic lady beetle predators, with *C. septempunctata* also abundant. These two coccinellids are both potential intraguild predators of a large community of small resident predators, including syrphid fly larvae, coccinellid larvae, and *Orius insidiosus* adults and larvae in addition to *A. aphidimyza* and *C. carnea* larvae. Three treatments were designed to measure how IGP by large lady beetle adults on small predators impacted *A. glycines* populations. The three treatments were: a predator exclusion cage, preventing all predators from accessing aphid populations, a medium-mesh cage that allowed small predators to gain access to the cage but excluded large lady beetle adults, and a large-mesh cage that allowed access by both large lady beetles and small predators.

2.6. Cage design

All cages consisted of 1 m^3 PVC frames with mesh enclosures that varied in the size of the mesh on the sides of the cage. The predator exclusion cage was made entirely of no-see-um netting (Venture Textiles, Braintree, MA). The medium- and large-mesh cages had no-see-um netting roofs and cage sides made from either 2 mm mesh

(medium-mesh treatment) or 6 mm mesh (large-mesh treatment) (US Netting, Erie, PA). All cages were lined at their base with Velcro attached to a plastic barrier buried to secure the cages at the soil surface and prevent entry of ground-dwelling predators. The cages were arranged in a completely randomized design with three replicates of each treatment in a soybean field planted in 38.1 cm rows with soybean variety 92B16 (Pioneer Hi-Bred International Inc., Johnston, IA) at the Entomology Research Farm at Michigan State University (East Lansing, MI). Each cage contained two rows of plants, with an average of 25.3 plants per cage. When the cages were established aphid density averaged 22.2 per plant. All predators were hand-removed from caged plants prior to beginning the experiment.

2.7. Sampling procedure

All cages were sampled weekly for 4 weeks. A sample consisted of five plants that were randomly selected in each cage. Each plant was examined and the number of apterous and alate aphids and diversity and abundance of predators was recorded.

2.8. Statistics

In the microcosm experiments, we compared the change in the number of *A. aphidimyza* and *C. carnea* before and after exposure to *H. axyridis* using a 2-sample *t*-test. A repeated-measures mixed model analyses of variance (ANOVA) was used to compare differences in aphid abundance between microcosm treatments. Block was included as a random effects factor and treatment, time, and their interaction as fixed effects factors. Differences in aphid and predator abundance between treatments were assessed by comparing least squares means. Aphid counts were square-root transformed prior to analysis to meet the assumptions of the model.

In the field cage experiment, the impact of excluding either large coccinellid predators (*H. axyridis* and *C. septempunctata*) or all predators on aphid populations was assessed using a repeated measures ANOVA random coefficients model with time included as a covariate and random effects for subject and subject-specific slopes on time. Based on residual analysis aphid data were square-root transformed to meet the assumptions of the model. Variation in the abundance of predators was also assessed using a repeated measures ANOVA random coefficients model with time included as a covariate and random effects for subject and subject-specific slopes on time. A square-root transformation was applied to predator means prior to analysis to meet the assumptions the model. Differences in aphid and predator abundance between treatments were assessed by comparing least squares means. Both the microcosm and field cage analyses were conducted using the PROC MIXED procedure of SAS version 9.1 (SAS Institute, 1999).

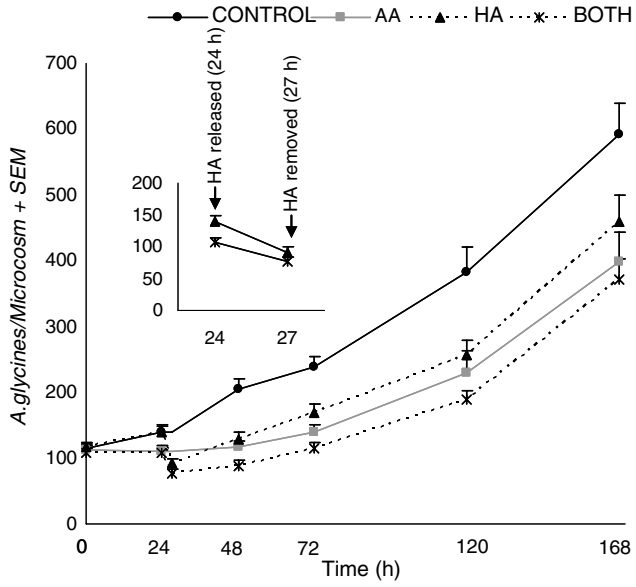
3. Results

3.1. Predator consumption rates

Second instar *A. aphidimyza* consumed on average 6.2 h ± 1.71 second instar and 9.4 ± 0.4 adult *A. glycines* per 24 h, while second instar *C. carnea* consumed 17 ± 4.06 second instar and 3.4 ± 0.98 adult aphids per 24 h.

3.2. Aphidoletes aphidimyza and H. axyridis

In the *A. aphidimyza* and *H. axyridis* microcosm experiment, aphid populations in the CONTROL and HA treatments, both of which initially contained no predators, rose in the first 24 h while AA and BOTH treatments which contained *A. aphidimyza* remained relatively constant during this time period (Fig. 1). At 27 h, i.e., after introduction and 3 h of feeding by *H. axyridis*, aphid populations in both the HA and BOTH treatments declined. During its 3 h presence, *H. axyridis* acted as an intraguild predator, significantly reducing numbers of *A. aphidimyza* larvae ($t = 6.87$, $df = 14$, $P < 0.0001$) in the BOTH treatment. At time 24 h an average of 2.87 ± 0.09 midges were found in the AA and BOTH treatments. An average of 1.80 ± 0.26 midge larvae

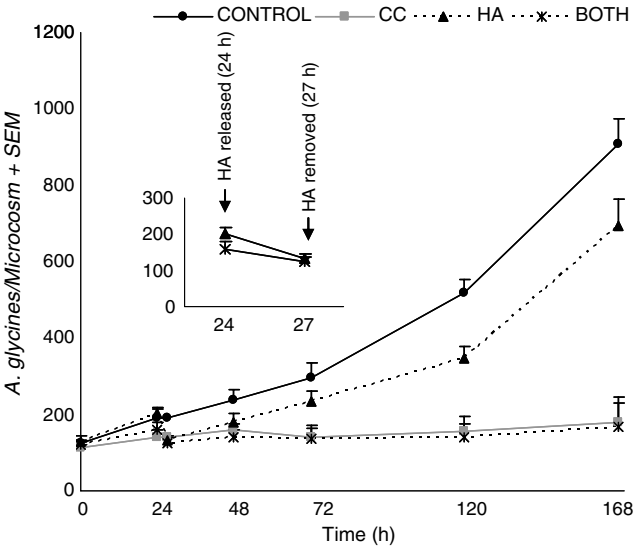


TREATMENT	0	24	27	48	72	120	168
CONTROL	a	a	-	a	a	a	a
AA	a	a	-	bc	bc	bc	c
HA	a	a	a	b	b	b	b
BOTH	a	a	a	c	c	c	c

Fig. 1. Mean number of *A. glycines* per microcosm in the CONTROL (*A. glycines* only), AA (*A. glycines* + *A. aphidimyza*), HA (*A. glycines* + *H. axyridis*) and BOTH (*A. glycines* + *A. aphidimyza* + *H. axyridis*) treatments. Larval *A. aphidimyza* were released at 0 h, *H. axyridis* were released at 24 h and removed at 27 h. Only the BOTH and HA treatments (which included *H. axyridis*) were counted at 27 h. The figure inset shows the change in aphid populations after 3 h of feeding by *H. axyridis* in the HA and BOTH treatments. Mean comparisons are based on square-root transformed data ($P < 0.05$).

per microcosm were removed by *H. axyridis* during 3 h of foraging, leaving only 1.06 ± 0.23 midges per microcosm at 27 h in the BOTH treatment.

From 27 to 168 h overall aphid populations increased but were not consistent in all treatments as indicated by a significant interaction between treatment and time ($F_{16,308} = 3.00$, $P = 0.0001$). In the AA treatment, *A. aphidimyza* reduced aphid populations relative to the control beginning at 48 h and continuing until the end of the experiment (Fig. 1). In this treatment *A. aphidimyza* reduced aphid populations in the first 48 h of the experiment (107.8 ± 6.51 aphids per microcosm at 0 h to 87.93 ± 7.84 aphids per microcosm at 48 h) but after 48 h aphid populations increased, reaching 372.33 ± 30.0 aphids per microcosm by 168 h. Three hours of *H. axyridis* feeding in the HA treatment also significantly reduced aphid abundance, from 140.53 ± 9.31 aphids per microcosm at 24 h to 91.47 ± 7.97 per microcosm at 27 h. Following the removal of *H. axyridis*, aphid populations in the HA treatment increased and were not significantly different from the AA treatment from 48 to 120 h. At 168 h the HA treatment had significantly higher aphid populations compared with the AA and BOTH treatments. In the BOTH treatment aphid population remained constant over the first 24 h in the presence of *A. aphidimyza*. Three hours of feeding by *H. axyridis* reduced *A. glycines* populations in the BOTH treatment from 107.47 ± 6.06 to 75.86 ± 7.6. The BOTH treatment was similar to the AA treatment throughout the experiment and significantly lower than the HA treatment beginning at 48 h and continuing through 168 h (Fig. 1).



TREATMENT	0	24	27	48	72	120	168
CONTROL	a	ab	-	a	a	a	a
AA	a	a	-	b	b	c	c
HA	a	b	a	ab	a	b	b
BOTH	a	ab	a	b	b	c	c

Fig. 2. Mean number of aphids per microcosm in the CONTROL (*A. glycines* only), CC (*A. glycines* + *C. carnea*), HA (*A. glycines* + *H. axyridis*) and BOTH (*A. glycines* + *C. carnea* + *H. axyridis*) treatments. Larval *C. carnea* were released at 0 h, *H. axyridis* were released at 24 h and removed at 27 h. Only the BOTH and HA treatments (which included *H. axyridis*) were counted at 27 h. The figure insert shows the change in aphid populations after 3 h of feeding by *H. axyridis* in the HA and BOTH treatments. Mean comparisons are based on square-root transformed data ($P < 0.05$).

3.3. Chrysoperla carnea and H. axyridis

In the *C. carnea* and *H. axyridis* microcosm experiment, aphid populations increased in the CONTROL and HA treatments and remained relatively constant in the CC and BOTH treatments (Fig. 2). As in the *A. aphidimyza* microcosm experiment, there was a significant treatment by experimental time interaction ($F_{16,308} = 16.67$, $P < 0.0001$). Initially, aphid populations increased in all treatments, both in the CONTROL and HA treatments which did not contain any predators at the beginning of the experiment and in the CC and BOTH treatments which contained *C. carnea* larvae. At time 24 h *H. axyridis* was added to the HA and BOTH treatments, resulting in a decline in aphid numbers in these treatments by time 27 h. During its 3 h presence, *H. axyridis* acted as an intraguild predator significantly reducing numbers of *C. carnea* larvae ($t = 2.82$ df = 14, $P = 0.014$) in the BOTH treatment. At time 24 h an average of 2.26 ± 0.12 and 2.6 ± 0.13 lacewings per microcosm were found in the RP and BOTH treatments respectively. The intraguild predator *H. axyridis* removed an average of 1.07 ± 0.28 lacewing larvae within 3 h of foraging, leaving 1.67 ± 0.29 lacewing larvae per microcosm in the BOTH treatment at 27 h. In the CC and BOTH treatments aphid populations were significantly lower than the CONTROL beginning at 48 h and continuing throughout the experiment (Fig. 2). In the CC treatment aphid populations decreased between 48 and 120 h and then began to increase ending the experiment at 179.13 ± 64.90 aphids per microcosm at 168 h (Fig. 2). Despite the IGP, aphid populations in the BOTH treatment were relatively constant from 48 to 120 h (139.0 ± 18.89 aphids per microcosm at 48 h to 141.33 ± 34.13 aphids per microcosm at 120 h) at which time populations began to increase reaching 168.53 ± 61.96 aphids per microcosm at 168 h.

3.4. Field cage experiment

Aphid populations increased in all three field cage treatments (Fig. 3), however the impact of these treatments on aphid populations was not consistent across the 4 weeks of the experiment, as indicated by a significant treatment-by-time interaction ($F_{6,9} = 3.79$, $P = 0.036$). By week four, aphid populations were significantly lower in the large-mesh treatment compared with the predator exclusion treatment (Fig. 3). Allowing access to *C. septempunctata* and *H. axyridis* delayed aphid populations from reaching the economic threshold of 250 aphids per plant by two weeks in the large-mesh treatment compared with populations in the medium-mesh and predator exclusion treatments. Adult *H. axyridis* and *C. septempunctata* were found only in the large-mesh treatment, indicating that

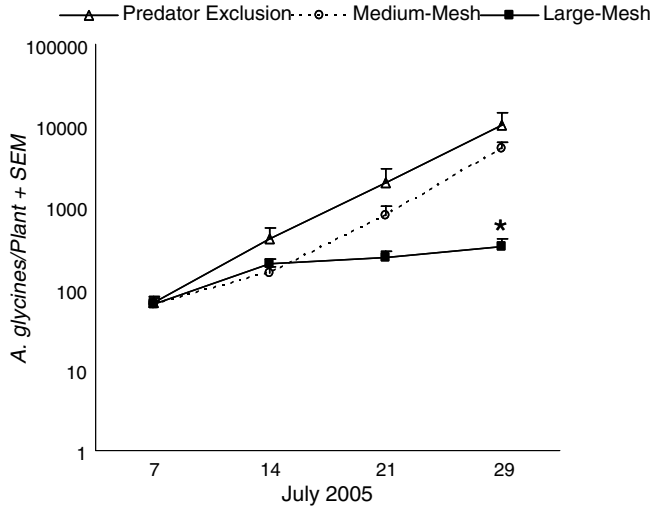


Fig. 3. Mean number of *A. glycines* per plant in predator exclusion, medium-mesh (small predators only) and large-mesh (small and large predators) cage treatments. Asterisk indicates a significant difference between square-root transformed mean *A. glycines* in the predator exclusion and large-mesh treatments ($P < 0.05$).

they were effectively excluded from the medium-mesh and predator exclusion treatments. Populations of large lady beetles were low throughout the experiment, peaking at 0.4 ± 0.24 per plant on July 21. The population composition of large coccinellids in the large-mesh treatment was 55% *C. septempunctata* and 44% *H. axyridis*. Coccinellid larvae were found in both the medium- and large-mesh treatments and the difference in their abundance between the two treatments varied across time, indicated by a marginally significant interaction ($F_{3,12} = 3.33$, $P = 0.056$). Coccinellid larvae were significantly more abundant in the large-mesh treatment on July 21 reaching 1.47 ± 0.48 per plant compared with 0.07 ± 0.07 per plant in the medium-mesh treatment. Their populations were similar in the two treatments on the other three sampling dates (Fig. 4). *Harmonia axyridis* was the most abundant species of coccinellid larvae found, averaging 0.05 ± 0.28 per plant in the medium-mesh treatment and 0.32 ± 0.09 per plant in the large-mesh treatment. Larvae of *C. septempunctata* were the second most abundant species, averaging 0.02 ± 0.02

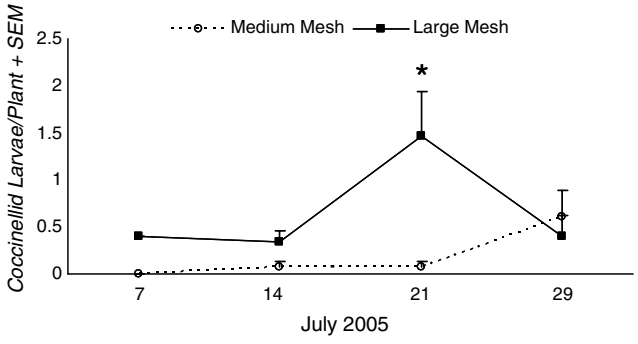


Fig. 4. Mean number of lady beetle larvae per plant in the medium-mesh and large-mesh cage treatments. Asterisk indicates a significant difference between treatments based on square-root transformed data ($P < 0.05$).

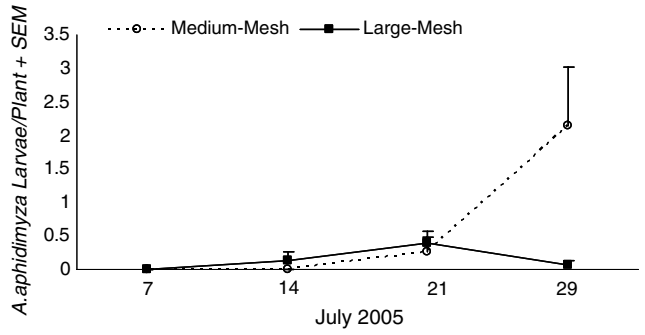


Fig. 5. Mean number of *A. aphidimyza* per plant in medium-mesh and large-mesh cage treatments.

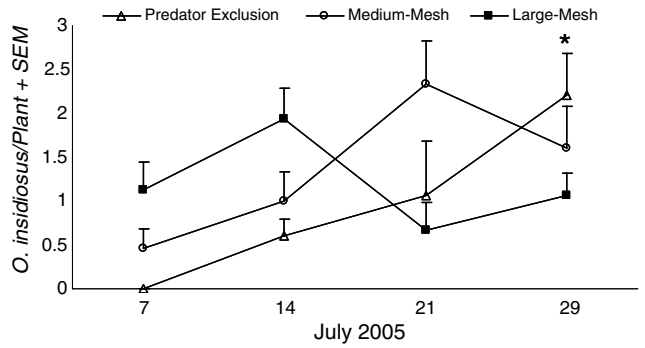


Fig. 6. Mean number of *O. insidiosus* per plant in the predator exclusion, medium-mesh and large-mesh treatments. Asterisk indicates a significant difference between the predator exclusion and large-mesh treatments based on square-root transformed data ($P < 0.05$).

per plant in the medium-mesh treatment and 0.06 ± 0.05 per plant in the large-mesh treatment. Lacewing larvae were only detected in the medium-mesh treatment, at 0.4 ± 0.16 larvae per plant on July 21. There was no difference in the number of *A. aphidimyza* found in the medium- and large-mesh treatments on any dates ($F_{1,4.5} = 0.19$, $P = 0.679$) (Fig. 5). We were unable to restrict *O. insidiosus* from entering the predator exclusion treatment. There was a significant interaction in the abundance of *O. insidiosus* between the three treatments and time ($F_{6,28} = 2.75$, $P = 0.05$). Despite removing individuals while sampling the predator exclusion treatment, *O. insidiosus* increased throughout the experiment and was significantly higher in this treatment compared with the large-mesh treatment ($P = 0.027$) by July 29 (Fig. 6).

4. Discussion

Generalist predators can exert strong top-down population regulation of *A. glycines* in soybean fields (Costamagna and Landis, 2006). *Harmonia axyridis* is one of the most abundant predators found in soybean across the invasive range of *A. glycines* (Fox et al., 2004; Rutledge et al., 2004; Costamagna and Landis, 2006), thus understanding its role in shaping the *A. glycines* predator guild is of critical importance in the overall suppression of this invasive species.

4.1. Microcosm studies

In microcosm experiments we found that *H. axyridis* acting as both an intraguild and a direct predator had differential impacts on both the survival of the intraguild prey and *A. glycines* population dynamics. In tests with *A. aphidimyza*, the presence of this predator alone slowed, but did not prevent, the eventual increase of *A. glycines* to high levels. Based on this result and the aphid consumption rate data we conclude that under the conditions tested, *A. aphidimyza* was not efficient enough in preventing the aphid from reaching outbreak levels. In contrast, only 3 h of feeding by *H. axyridis* provided suppression of *A. glycines* essentially equal to the continuous presence of *A. aphidimyza* over the full 168 h. During its 3 h tenure, *H. axyridis* also acted as a significant intraguild predator of *A. aphidimyza*. This IGP did not result in a release of aphid populations from overall control; aphid populations exposed to both predators were not significantly different from aphids exposed only to *A. aphidimyza*. The overall picture that emerges from this experiment is that while *H. axyridis* is an intraguild predator of *A. aphidimyza*, IGP did not release *A. glycines* from control as *A. aphidimyza* is simply not efficient enough at the densities tested to suppress the aphid on its own.

A somewhat different picture emerges for the *C. carnea* and *H. axyridis* interaction. As in the previous experiment, *H. axyridis* alone slowed but did not prevent the eventual increase of *A. glycines* to high levels. However, in contrast to *A. aphidimyza*, *C. carnea* larvae alone were able to maintain *A. glycines* populations at low levels throughout the experiment. IGP of an effective predator could potentially release aphids from control. While *H. axyridis* did significantly reduce *C. carnea*, it did not reduce the levels of this intraguild prey to the same degree as it did *A. aphidimyza*. This may be because *C. carnea* are larger, more mobile, or less preferred intraguild prey than *A. aphidimyza*. The overall result was that either *C. carnea* alone or the combination of *C. carnea* and *H. axyridis* together resulted in experiment-long aphid control.

Taken together, the microcosm experiments illustrate a complex set of interactions between *H. axyridis*, *A. aphidimyza*, *C. carnea* and *A. glycines*. First, even a single visit by a *H. axyridis* may produce long term impacts on aphid suppression. Three hours of *H. axyridis* feeding significantly reduced overall *A. glycines* population growth in both experiments. In the field, a similar result could mean a difference of several days in when a threshold population is reached or could conceivably even maintain aphid populations below thresholds, particularly if repeated visits by transient coccinellid predators occur. Second, intraguild predators can have strong or weak impacts on intraguild prey depending on their ability to exploit particular guild members and the intraguild prey's ability to escape predation. Third, the ability of *A. aphidimyza* and *C. carnea* to suppress aphid populations after IGP depends on both the number which survive and their inherent ability to consume prey. Finally, it is likely that relatively stochastic events such as the exact timing of intraguild interactions and the numbers of all predators and prey will shift the outcome of these interactions.

4.2. Field cages

In the field cage experiment aphid populations were reduced in cages, allowing colonization by *H. axyridis* and *C. septempunctata*. Predation by coccinellids delayed *A. glycines* from reaching the 250 per plant threshold for 2 weeks compared with the medium-mesh and predator exclusion treatments. Throughout this investigation numbers of all predators remained low in all treatments, indicating that small numbers of predators can have a significant impact on *A. glycines* populations in the field. We were unable to exclude *O. insidiosus* from our predator exclusion treatment; its abundance was highest in this treatment by the end of the experiment. These data indicate that *O. insidiosus* may have benefited from the exclusion of other predators; however, it appears to have little impact on aphid biological control when acting alone at the population levels found in this experiment. While *A. aphidimyza* larvae were more abundant and lacewing larvae were only found in the medium-mesh treatment, there was not a significant difference in the number of these intraguild prey between the medium- and large-mesh treatments. Since IGP did occur in our microcosm experiments, it is likely to also occur at some level in the field. It is possible that the confined arena of a microcosm elevated the extent of IGP relative to what occurs in soybean fields. Alternatively, the relatively low and variable number of *A. aphidimyza* and *C. carnea* found in our field study may have reduced our ability to detect differences. Coccinellid larvae were more abundant in the large-mesh treatment and *H. axyridis* was the most common species. Since *H. axyridis* and *C. septempunctata* were able to deposit eggs inside the large-mesh cage treatment, larvae would not have to colonize these cages. This could have led to higher populations in this treatment compared to the medium-mesh where coccinellid larvae would have to disperse into the cages on their own. Coccinellid larvae are likely to greatly impact both IGP and aphid population dynamics. Aphid consumption by *H. axyridis* can vary from 90 to 370 aphids (depending on the species of aphid) during its larval stage (Hukusima and Kamei, 1970 in Koch, 2003). Coccinellid larvae are also known to act as intraguild predators (Lucas et al., 1998; Kajita et al., 2000; Snyder et al., 2004a,b). It is likely that in this study coccinellid larvae contributed to decreased aphid populations in the large-mesh treatment and may have engaged in intraguild interactions with other predators.

When evaluating our field cage experiment, it is critical to assess the role of cage design in influencing the results. Our primary concern was that our predator exclusion cages prevented dispersal of alate aphids, which could have been responsible for the elevated aphid populations in this treatment. While a lack of alate dispersal may have contributed

to higher *A. glycines* populations, there is evidence that this is not the only factor resulting in lower populations in the large-mesh cage treatment. If dispersal was driving the difference in aphid abundance, the medium-mesh and large-mesh treatments should have both had significantly fewer aphids, as alates could disperse from these treatments. Instead, we found that the number of aphids in the medium-mesh which allowed alate dispersal did not differ from populations in the exclusion treatment where dispersal was prevented. This indicates that it was the difference between the large-mesh and medium-mesh treatments that resulted in better aphid suppression. The only difference between these treatments was the presence or absence of *H. axyridis* and *C. septempunctata*. A second concern was that our medium-mesh was small enough to exclude large lady beetles but still provided equal colonization of small predators compared with the large-mesh treatment. We evaluated this in a preliminary greenhouse experiment prior to conducting our field experiment. We established two portable hoop-houses on top of a greenhouse bench and inside each placed one medium-mesh cage and one large-mesh cage. Inside each cage were 12 heavily infested soybean plants. The cages was surrounded on all sides with 2 rows of uninfested soybean plants onto which we released *O. insidiosus* adults, *A. aphidimyza* adults and *C. carnea* larvae (100 individuals of each species). The cages were sampled at 48, 96, and 144 h after the release of the predators. For both *O. insidiosus* ($F_{1,2}=1.84$, $P=0.779$) and *C. carnea* ($F_{1,2}=0.07$, $P=0.817$) there was no difference in colonization across the three sampling periods. As adult *A. aphidimyza* were released we did not begin to detect larvae until 96 h, when one larva was found in a large-mesh cage. At 144 h there was not a significant difference in colonization by *A. aphidimyza* in the medium-mesh and large-mesh treatments ($F_{1,2}=2.13$, $P=0.281$). Our third concern was that abiotic conditions were equal in all cages. To prevent greater shading in our predator exclusion treatment we used the exclusion mesh for the roofs of all three cage treatments, so that all treatments were equally shaded.

4.3. Impacts of H. axyridis on biological control

The impact of guild member displacement by an intraguild predator in overall biological control of aphids is likely to depend on several factors, including the contribution of each natural enemy to aphid suppression and the severity of the IGP. The benefit of an intraguild predator such as a lady beetle to overall biological control may be tied to its foraging strategy. Rosenheim and Corbett (2003) modeled how foraging strategy can impact the outcome of biological control of a sedentary pest. They found that when the top predator exhibited a sit and wait foraging strategy, actively foraging intermediate predators were suppressed, releasing sedentary herbivore populations from overall control. This outcome changed when the top predator was an active forager, as is the case with *H. axyridis*. Here the sedentary herbivore was not released from overall control because both the intermediate and top predators were likely to encounter and consume it, so any losses of intermediate predators due to IGP had less of a direct effect on pest suppression (Rosenheim and Corbett, 2003). This is what we find in the soybean system, where biological control of the sedentary *A. glycines* was not impacted by the removal of *A. aphidimyza* and *C. carnea* by the active top predator *H. axyridis*.

While adult coccinellids are often significant intraguild predators (Losey and Denno, 1998; Lucas et al., 1998; Colfer and Rosenheim, 2001; Snyder et al., 2004a,b; Colunga-Garcia and Gage, 1998; Phoofolo and Obrycki, 1998; Cottrell, 2005), inclusion of these predators often results in increased biological control (Colfer and Rosenheim, 2001; Cardinale et al., 2003; Snyder et al., 2004a; Aquilino et al., 2005; Costamagna, 2006). The presence of *H. axyridis* has been found to increase biological control of aphid pests in communities containing other predators as well as predators and parasitoids. Aquilino et al. (2005) manipulated the diversity of host plant species and predators in microcosms to determine how top-down and bottom-up diversity affected consumption of pea aphid, *Acyrthosiphon pisum* Harris. They found that increasing the diversity of predators increased aphid consumption. This was attributed to poor performance of *Nabis* sp. and *C. maculata* when each predator was present alone. In contrast, consumption by *H. axyridis* alone in the majority of treatments was typically equal to or exceeded consumption in microcosms containing all three predators (Aquilino et al., 2005). Cardinale et al. (2003) found that when *H. axyridis*, *Nabis* sp., and the parasitic wasp *Aphidius ervi* Haliday were combined in field cages greater pea aphid suppression was achieved than was predicted by the summed impact of each species alone (Cardinale et al., 2003). Snyder et al. (2004a) found that on caged rose plants infested with *Macrosiphum euphorbiae* Thomas, the presence of *H. axyridis* dampened aphid populations without impacting the density of *Aphelinus asychis* (Walker) pupae. Similarly, in soybean Costamagna (2006) found that although high levels of IGP of parasitoids by predators were detected, no evidence for disruption in the level of parasitism was found. In contrast, the presence of coccinellids, including *H. axyridis*, was responsible for strong suppression of *A. glycines* and restored soybean biomass and yield to levels similar to control treatments lacking aphids (Costamagna, 2006).

4.4. Conclusions

Our hypothesis was that early in the season when lady beetles are highly mobile, IGP events occurring between the exotic coccinellid *H. axyridis* and larvae of the native *C. carnea* and *A. aphidimyza* could impact the success of soybean aphid biological control. While the transient top predator *H. axyridis* did act as an intraguild predator of both intermediate predators *A. aphidimyza* and *C. carnea*, we did not find evidence of a release of soybean aphid due to IGP in either our microcosm or field cage experiments.

Based on the results of this study, the presence of *H. axyridis* may contribute to local declines in *A. aphidimyza* and *C. carnea*; however, biological control of soybean aphid would not likely be improved by removing *H. axyridis* from the system. When *H. axyridis* is excluded from aphid colonies in the field, populations grow to levels not significantly different from cages excluding all predators. This suggests that intraguild prey in the soybean system, including *A. aphidimyza*, *C. carnea*, and *O. insidiosus*, while important to native biological diversity, are not currently major contributors to overall biological control of this invasive pest.

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