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Alfalfa Living Mulch Advances Biological Control of Soybean Aphid

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Abstract

Despite evidence for biological control in North America, outbreaks of the invasive soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), continue to occur on soybean (*Glycine max* L. Merr.). Our objectives were to determine whether natural enemies delay aphid establishment and limit subsequent population growth and whether biological control can be improved by altering the within-field habitat. We hypothesized that a living mulch would increase the abundance of the aphidophagous community in soybean and suppress *A. glycines* establishment and population growth. We measured natural enemy and *A. glycines* abundance in soybean grown with and without an alfalfa (*Medicago sativa* L.) living mulch. Soybean grown with an alfalfa living mulch had 45% more natural enemies and experienced a delay in *A. glycines* establishment that resulted in lower peak populations. From our experiments, we concluded that the current natural enemy community in Iowa can delay *A. glycines* establishment, and an increase in aphidophagous predator abundance lowered the rate of *A. glycines* population growth preventing economic populations (i.e., below the current economic threshold) from occurring. Incorporation of a living mulch had an unexpected impact on *A. glycines* population growth, lowering the aphids' intrinsic rate of growth, thus providing a bottom-up suppression of *A. glycines*. We suggest future studies of living mulches or cover crops for *A. glycines* management should address both potential sources of suppression. Furthermore, our experience suggests that more consistent biological control of *A. glycines* may be possible with even partial resistance that slows but does not prevent reproduction.

Keywords

invasive species, habitat management natural enemies, conservation

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ABSTRACT Despite evidence for biological control in North America, outbreaks of the invasive soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), continue to occur on soybean (*Glycine max* L. Merr.). Our objectives were to determine whether natural enemies delay aphid establishment and limit subsequent population growth and whether biological control can be improved by altering the within-field habitat. We hypothesized that a living mulch would increase the abundance of the aphidophagous community in soybean and suppress *A. glycines* establishment and population growth. We measured natural enemy and *A. glycines* abundance in soybean grown with and without an alfalfa (*Medicago sativa* L.) living mulch. Soybean grown with an alfalfa living mulch had 45% more natural enemies and experienced a delay in *A. glycines* establishment that resulted in lower peak populations. From our experiments, we concluded that the current natural enemy community in Iowa can delay *A. glycines* establishment, and an increase in aphidophagous predator abundance lowered the rate of *A. glycines* population growth preventing economic populations (i.e., below the current economic threshold) from occurring. Incorporation of a living mulch had an unexpected impact on *A. glycines* population growth, lowering the aphids' intrinsic rate of growth, thus providing a bottom-up suppression of *A. glycines*. We suggest future studies of living mulches or cover crops for *A. glycines* management should address both potential sources of suppression. Furthermore, our experience suggests that more consistent biological control of *A. glycines* may be possible with even partial resistance that slows but does not prevent reproduction.

KEY WORDS invasive species, habitat management natural enemies, conservation

Disturbance within an agroecosystem frequently results in the loss of habitat and alternative prey for beneficial insects. This can lead to a reduction in natural enemy diversity and abundance, often contributing to pest outbreaks (Thies et al. 2003). Habitat management has been suggested as an effective approach for conserving natural enemy communities, resulting in improved integrated pest management (IPM) within certain agroecosystems (Gurr and Wratten 1999, Landis et al. 2000). The extent to which invasive insect herbivores can be managed by conserving endemic natural enemies is not clear, given the lack of co-evolved natural enemies from the pests' native range.

In North America, soybean (*Glycine max* L. Merr.) had escaped the insect pest and diseases that were associated with it in its native region Asia. As a result, soybean producers in the northcentral region of the United States had a limited need for pest management interventions (Fernandez-Cornejo and Jans 1999). Introduction of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), to North America has resulted in an insect pest of economic importance

for soybean. First discovered in July 2000 in Wisconsin and adjoining states, *A. glycines* is currently distributed in 21 of the United States and parts of Canada (Venette and Ragsdale 2004). *A. glycines* was first reported in Iowa in 2000, and by 2003, it was found in every county within the state. In 2003, >1.6 million ha were treated with insecticides to control *A. glycines* populations that reached several thousand per plant (Pilcher and Rice 2005). Yield reductions have exceeded 50% in grower strip trials, and an average 14% yield loss has been reported in Iowa (Johnson and O'Neal 2005), when populations exceeded an economic threshold of 250 aphids per plant (Rice et al. 2005).

In Asia, *A. glycines* is kept below economically important levels by an array of natural enemies (Liu et al. 2004). In North America, Rutledge et al. (2004) found that *A. glycines* is attacked by >30 species of predators, 8 species of parasitoids, and several species of fungal pathogens. In addition, Fox et al. (2004, 2005) found that, by excluding predators, *A. glycines* populations increased, yet when predators had access to *A. glycines*, populations remained relatively low. Despite the evidence for biological control of *A. glycines* in the United States, soybean aphid outbreaks continue to occur across much of the northcentral region, resulting in significant increases in insecticide use (O'Neal 2005). For example, after an outbreak in 2003, most of Iowa experienced subeconomic populations of soy-

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bean aphids in 2004, with a significant reduction in insecticide use in soybean (O'Neal 2006). *A. glycines* populations in 2005 returned to economic levels, and insecticides were applied on nearly one million hectares (O'Neal 2006). Although there is evidence for biological control of *A. glycines* within the United States, it has not shown to be effective in every year. Management of the within-field habitat may improve the effectiveness of biological control and lead to more consistent suppression of *A. glycines.*

In addition to determining if biological control of *A. glycines* occurs in Iowa, our main hypothesis is whether a living mulch, as a form of habitat management, increases the abundance and diversity of the current natural enemy community, which in turn may cascade into greater biological control of the soybean aphid. One form of habitat management used to increase the abundance and diversity of insect natural enemy communities is the use of a cover crop, or a living mulch (Remund et al. 1992, Costello and Altieri 1995, Hartwig and Ammon 2002). Unlike a typical cover crop that is killed early in the growing season, a living mulch grows concurrently with the crop during the entire season. O'Neal et al. (2005) reported increases in activity of ground beetles (Carabidae) when conventionally grown soybean were planted in leguminous living mulches with an accompanying increase in sentinel prey removal. Although O'Neal et al. (2005) focused on the edaphic community of generalist predators, Weiser et al. (2003) showed that conserving natural enemies within alfalfa can contribute to the management of above-ground insect herbivores. To what extent an increase in the current community of aphidophagous natural enemies in Iowa would occur with this practice is not clear. Comprised of mostly generalist predators (Beschinski and Pedigo 1981), the natural enemy community in soybean may not be sufficiently responsive to *A. glycines* to reduce their establishment and population growth.

For this study, our objective was to determine if natural enemies delay *A. glycines* establishment and limit subsequent population growth in Iowa. In addition, we wanted to determine if biological control could be improved through habitat management. Specifically, we hypothesized that an alfalfa living mulch would increase the abundance and diversity of the aphidophagous community in soybean and that such an enhancement would suppress *A. glycines* establishment and population growth.

Materials and Methods

Field Site

We conducted our study at the Iowa State University Agricultural Engineering Research Center in Boone Co., IA. The predominant soil series at the field site is Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic typic endoaquolls). The experimental site was sown to spring triticale (*Triticale hexaploide* Lart.) and alfalfa (*Medicago sativa* L.) on 24 March 2004. Triticale was harvested

(grain and straw) on 15 July 2004. The interseeded alfalfa was cut and removed with the triticale stubble on 16 July 2004. Another alfalfa harvest occurred on 1 September 2004. Postharvest alfalfa stand density was 200 plants/m^2.

In the spring of 2005, we established replicated plots of soybean grown alone (referred throughout as the control treatment) or planted in alfalfa managed as a living mulch (referred throughout as the living mulch treatment). To establish the control treatment, alfalfa was removed with herbicides in the spring using a broadcast spray of 0.9 liters/ha of Roundup WeatherMax (Glyphosate) and 0.9 liters/ha of Dual II Magnum (S-metolachlor) applied on 15 April. Additional spot herbicide applications and hand weeding maintained these plots weed-free during the remainder of the growing season. Other than the herbicides applied to the living mulch treatment (described below), there were no other pesticides applied to the living mulch or control treatments.

Living Mulch Management. Managing the living mulch treatment consisted of planting roundup-ready soybean within rows in which the herbicide was applied (0.25 m wide, centered over the soybean row). On 15 April, herbicide was used to band the future soybean row in alfalfa. Soybeans (Asgrow Brand 'AG2107') were planted at a rate of 445,000 seeds/ha in 0.76-m rows on 9 May. To reduce competition between the remaining alfalfa and soybean, mechanical control occurred on 2 June using a rolling stalk chopper to suppress alfalfa in the interrow. The alfalfa interrows were allowed to grow 25–30 cm in height before chopping occurred. This mechanical control was conducted using a Buffalo rolling stalk chopper (Fleischer Manufacturing, Columbus, NE) configured to affect only the interrow area. Cut alfalfa was 4–8 cm in height. On 20 June, a second herbicide band using glyphosate was applied over the soybean row. The final chopping of the alfalfa interrow occurred on 6 July.

Impact of Habitat Management on *A. glycines* and Natural Enemy Abundance. Using alfalfa as a living mulch, we tested the hypothesis that a form of habitat management (a living mulch) would increase the abundance and diversity of natural enemies and reduce the abundance of *A. glycines* in soybean. Using the methods described above, we established eight plots (30.5 by 27.4 m) in a randomized complete block design with treatments designated as a control or living mulch (Fig. 1). Treatments were randomly assigned in each block.

We monitored soybean for *A. glycines* every 3–7 d, beginning on 17 June when plants were at an early vegetative stage (V4, four fully developed trifoliolate leaf nodes; Pederson 2004) and continued through 1 September during leaf senescence (R7; Pederson 2004). On each sampling date, a location within a plot was selected at random, and the total number of apterous (adults and nymphs) and alate *A. glycines* were recorded on a subset of plants. Initially, 20 plants per plot were sampled; however, on 15 July, when 50% of soybean were infested with *A. glycines*, the number of
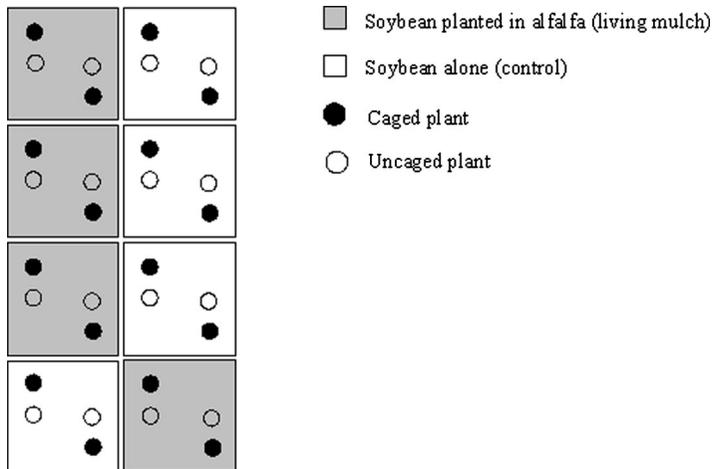
Fig. 1. Schematic diagram of experimental plots used to compare soybean aphid establishment and population growth in soybean grown with (shaded) and without (empty) an alfalfa living mulch. Plots were 30.5 by 27.4 m and separated by a 3-m mowed row.

plants per plot was reduced to 10. Finally on 25 July, when all plants (100%) were infested, sampling decreased to five plants per plot.

We measured the diversity and abundance of aphidophagous natural enemies on a weekly basis using a sweep-net beginning 17 June and continuing through 1 September ($n = 8$). After randomly selecting a row, contents from 20 pendulum sweeps per plot were bagged and stored at $-20°C$ until insect specimens could be sorted. All natural enemies collected were sorted and identified to at least the family level except for members of Coccinellidae, which were identified to species. Both adult and immature stages of natural enemies were counted, and voucher specimens were deposited in the Iowa State Insect Collection at Iowa State University, Ames, IA.

Data Analysis. We used analysis of variance (ANOVA) to determine if the presence of a living mulch affected the abundance of *A. glycines* and their natural enemies from 17 June to 1 September. We calculated an average number of aphids (all stages were conted) per plant. This average was square-root transformed to meet the assumptions of ANOVA (Sokal and Rohlf 1995). To determine if the presence of a living mulch had an effect on the abundance of natural enemies on soybean, we calculated a mean for each aphidophagous natural enemy collected with a sweep-net for each plot. We also calculated a mean to represent the entire aphidophagous natural enemy community that included only the aphid-predaceous life stages of the species collected. This estimate, referred to as the total natural enemy community was square-root transformed to reduce heteroscedascity and meet the assumptions of ANOVA (Sokal and Rohlf 1995). We determined whether the presence of a living mulch affected the abundance of *A. glycines* and their natural enemies (PROC GLM; SAS Institute 2002).

To visualize how the community of aphidophagous natural enemies responded to the control and living mulch treatments, we performed a principal components analysis (PCA) using R statistical software (R Development Core Team 2005). We analyzed the covariance matrix using count data to determine the variance of a species and the degree to which species are correlated. Three analyses were performed; when 0–10, 11–95, and 100% of plants were infested with *A. glycines.* Dates corresponding to these three levels of *A. glycines* infestation were 26 May to 11 July, 15–29 July, and 31 July to 31 August.

Natural Enemy Exclusion Study

Within the plots established for the previous experiment, we tested the hypothesis that the impact of natural enemies on *A. glycines* differs between the control and living mulch treatments. To estimate this impact, we used cages designed to exclude natural enemies from a single soybean plant. In both control and living mulch treatments, four plants per plot were artificially infested with 10 *A. glycines* (two caged and two uncaged). For simplicity, we will refer to the treatments as LMC (living mulch caged), LMU (living mulch uncaged), CC (control caged), and CU (control uncaged). Plants were at least 1 m from the plot edges and from other caged plants. This arrangement was used in all plots for a total of 16 caged and 16 uncaged plants (Fig. 1). To exclude natural enemies, plants were caged using a tomato cage (0.4 m diameter by 1 m tall). A fine-mesh, white no-see-um netting (Balson-Hercules, New York, NY) was sewn to fit the tomato cage, whereas uncaged plants received no cage or netting. To properly fix the cage around the infested plant, four plants on both sides of the infested plant were destroyed. To mimic the caged growing conditions as much as possible and to prevent trivial movement, adjacent soybeans were removed from uncaged plants that were artificially infested. Cages were supported with two metal electric fence posts tied to the tomato cage. At soil line, a trench was dug around the

cage, and the bottom of the no-see-um netting was buried 4–5 cm below the soil surface. Finally, natural enemies were removed within the cage and repeated during each sampling period as needed.

This experiment was conducted twice during the growing season to reflect the impact of natural enemies before and after populations of *A. glycines* successfully established in soybean. These periods corresponded to mid-June (0% of soybean plants infested) and mid-July (≈30% of soybean plants infested). Before artificially infesting soybean for the first experiment, we scouted 20 plants per plot, and no *A. glycines* were discovered. On 16 June, four plants per plot were infested with 10 *A. glycines.* When this experiment was repeated on 11 July, different plants for both the caged and uncaged plants were randomly selected. For the first experiment, our source of *A. glycines* came from a colony maintained at the Soybean Entomology Laboratory at Iowa State University. This colony was developed from *A. glycines* captured in a soybean field in Story Co., IA, in August 2004 and maintained on vegetative stage soybean in a growth chamber until June 2005. However, *A. glycines* used in the second experiment were from a naturally infested soybean field within 10 mi of the research site.

The number of *A. glycines* (all life stages) on each artificially infested plant were counted every 72–96 h. For the first experiment, cages were removed on 30 June, and sampling continued on the same schedule until 11 July. Sampling continued after cage removal to observe the top down effect of predators. In the second experiment, cages were removed on 26 July, and sampling continued until 17 August. On each sample date, a thorough inspection for natural enemies was conducted, and all were removed from caged plants.

Data Analysis. To compare the impact of each treatment factor to *A. glycines* density over time, we estimated the intrinsic rate of increase (r) of soybean aphid populations for aphids on all caged and uncaged plants from the control and living mulch treatments. We estimated slopes of the natural log (ln) aphid densities per plant over time and calculated averages of these slopes for each treatment. Cages were removed half-way through both natural enemy exclusion studies; therefore, two slopes were calculated for each study: once when plants were caged and a second when cages were removed for a total of four experimental periods. Dates corresponding to the four periods are 17–30 June, 1–11 July, 15–25 July, and 27 July to 17 August. Our data did not meet the assumptions of ANOVA, including a non-normal distribution of slopes; therefore, we used a nonparametric test (the Wilcoxon two-sample tests with exact test for analysis; SAS Institute 2002) to determine whether differences in r occurred across the four treatments.

Soybean Leaf Nutrient Analysis

To determine if soybean varied as a host between the treatments for *A. glycines* we measured total leaf nitrogen (N) as an indicator of host plant quality. On
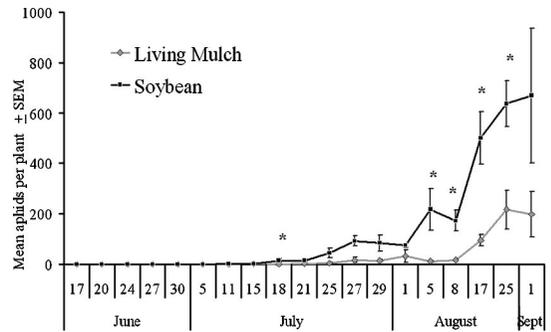

Fig. 2. Mean natural *A. glycines* infestation in soybean growing alone or in an alfalfa living mulch. *Significant differences between the two treatments (1,3 df; $P \leq 0.05$).

13 and 25 of July, 20 leaves per plot were removed and placed in a paper bag. A single leaf from the newest fully developed trifoliolate was randomly collected from 20 separate soybean plants. All leaves were dried in a forced-air oven at 70°C until a constant weight was achieved. All dried shoot material was ground to pass through a 1-mm sieve and analyzed for total N using the Dumas combustion method (AOAC Method 990.03).

Data Analysis. A Student's *t*-test was used to determine if the N concentration differed between the two treatment groups (PROC TTEST; SAS Institute 2002). This analysis was performed separately for both dates.

Results

Impact of Habitat Management on Soybean Aphid and Natural Enemy Abundance

We observed significant treatment differences in the natural infestation of *A. glycines* between the living mulch and control treatments ($F = 20.85$; df = 1,3; $P < 0.0001$). During the growing season *A. glycines* were found sooner and in greater abundance in the control treatment (Fig. 2). *A. glycines* were first observed in the control on 24 June at an average of 0.05 aphids per plant (1% of plants infested), whereas, in the living mulch treatment, *A. glycines* were not detected until 11 July (16 d later) at an average of 0.15 aphids per plant (9% of plants infested). On specific dates (18 July and 5, 8, 17, and 25 August) significant treatment effects were reported. Overall, there were always more *A. glycines* in the control than in the living mulch treatment (Fig. 2).

A total of 989 natural enemies were collected with sweep-nets, indicating a significant treatment effect between the control (353 total natural enemies) and living mulch treatment (636 total natural enemies). The living mulch treatment had greater species richness with 16 species and a total of 21 different categories of natural enemies when species were separated between predacious life stages (i.e., adult and immature), whereas only 15 species and a total of 19 different categories of natural enemies were found when species were separated between life stages in

Table 1. Natural enemy community[a] in soybean grown with and without an alfalfa living mulch

Species	Seasonal totals[bc]	
	Soybean with living mulch	Soybean alone
Nabis spp.	275	104
Araneae	115[e]	22
Opiliones	70[d]	21
Syrphidae	38	37
Harmonia axyridis	21	50
Podisus maculiventris nymphs	21	13
Orius insidious	20	13
Parasitic wasps	20	12
Podisus maculiventris	12	6
Chrysoperla spp. larvae	10	8
Chrysoperla spp.	9	40[d]
Coccinella septempunctata	8	2
Hippodamia parenthesis	4	1
Coleomegilla maculata	3	2
Hippodamia convergens larvae	2	2
Orius insidious nymphs	2	1
Coccinella septempunctata larvae	2	—
Cycloneda munda	1	6
Anatis quindecimpunctata	1	—
Hippodamia convergens	1	—
Hemerobius spp. larvae	1	—
Harmonia axyridis larvae	0	12
Hemerobius spp.	0	1
Grand total	636[d]	353
Species richness	16	15

[a] Natural enemies collected with a sweep-net.

[b] Seasonal totals are samples taken from four replicates of the two treatments.

[c] Significant treatment differences are represented by ([d] $P \leq 0.05$ and [e] $P \leq 0.01$; df = 1,3).
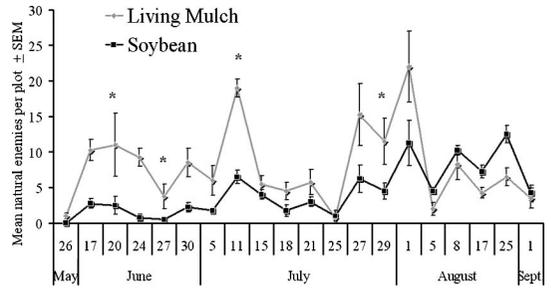

Fig. 3. Mean total natural enemies collected with sweep-net in soybean growing alone or in an alfalfa living mulch. *Significant differences between the two treatments (1,3 df; $P \leq 0.05$).

PCAs explained a cumulative 87% (PC1–76%, PC2–6%, and PC3–5%) of the variance in the data. Axis 1 was the most informative in explaining the variability of *Nabis* spp. abundance (Fig. 4). Axes 1 and 2 explained a slight positive correlation between Araneae and Opiliones, with Araneae being more variable than Opiliones (Fig. 4). Hulls based on site scores indicated that there was greater variability in the species composition of natural enemies within the living mulch treatments (Fig. 4).

When 11–95% of plants were infested with *A. glycines* in control and living mulch treatments, the first three PCAs explained a cumulative 82% (PC1– 49%, PC2–20%, and PC3–13%) of the variance in the data. Axes 1 and 2 were the most informative for explaining the variability of *Nabis* spp. and Araneae, in addition to explaining a positive correlation between Araneae, Opiliones, syrphids, and adult *O. insidiosus* (Fig. 5). Again, hulls indicated that the living mulch treatment had a more variable species composition of natural enemies (Fig. 5).

When 100% of plants were infested with *A. glycines* in control and living mulch treatments, the first three PCAs explained a cumulative 81% (PC1–63%,

the control treatment (Table 1). *Nabis* spp. (Hemiptera: Nabidae) were the most abundant predator in both control and living mulch treatments, and along with spiders (Araneae) and Opiliones, these were the only taxa to respond positively to the presence of a living mulch. These three taxa comprised 72% of the natural enemies collected in the living mulch treatment. In contrast, we found a more evenly composed natural enemy community in the control treatment. Although *Nabis* spp. were the most commonly collected natural enemy in the control treatment, they comprised only 29% of the total community. In the control treatment, we collected more (both larva and adult) *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Chrysoperla* spp. (Neuroptera: Chrysopidae) than in the living mulch treatment; however statistically significant differences were only detected for *Chrysoperla* spp. (Table 1). Throughout the growing season, more natural enemies were found in the living mulch, with significant treatment differences occurring on four dates (20 and 27 June and 11 and 29 July; Fig. 3). Although not statistically significant, on 5 August, more natural enemies were found in the control treatment. This transition occurred at the same time that the abundance of *A. glycines* was nearly 16 times as great in the control (218.4 ± 83.4) than in the living mulch (13.6 ± 1.6) treatments (Fig. 2).

When 0–10% of plants were infested with *A. glycines* in control and living mulch treatments, the first three
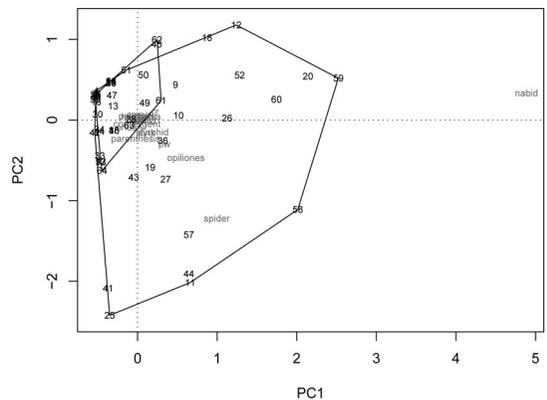

Fig. 4. PCA of natural enemy community in control and living mulch treatments when 0–10% of plants were infested with *A. glycines* (26 May to 11 July). Hulls represent the natural enemy community collected in control and living mulch treatments.
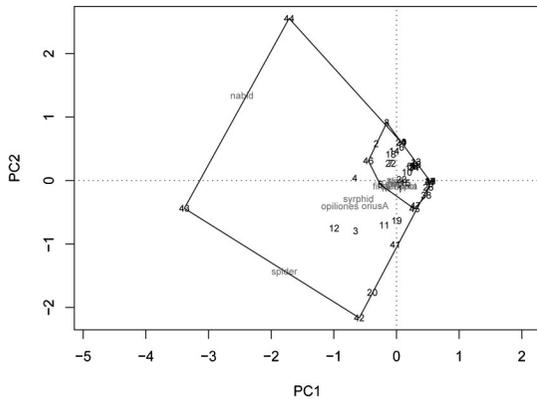
Fig. 5. PCA of natural enemy community in control and living mulch treatments when 11–95% of plants were infested with *A. glycines* (15–29 July). Hulls represent the natural enemy community collected in control and living mulch treatments.

PC2–12%, and PC3–6%) of the variance in the data. Axes 1 and 2 were the most informative in explaining the distribution of the natural enemy community composition among sites (Fig. 6). Unlike in Figs. 4 and 5, where the control treatment was nested within the living mulch treatments, in Fig. 6, a separation occured in the natural enemy community composition between the control and living mulch treatments. Axis 1 was the most informative in explaining the variability of *Nabis* spp. abundance, again indicating a positive correlation with Araneae and Opiliones (Fig. 6). Axis 2 indicated that *Chrysoperla* spp. were the second-most variable, in addition to being somewhat positively correlated with *Harmonia axyridis,* and syrphids (Fig. 6). It also seems that *Chrysoperla* spp. and *Harmonia axyridis* were positively correlated with *A. glycines* abundance. Hulls indicated that the living mulch and control treatments both have variable species composition of natural enemies (Fig. 6).
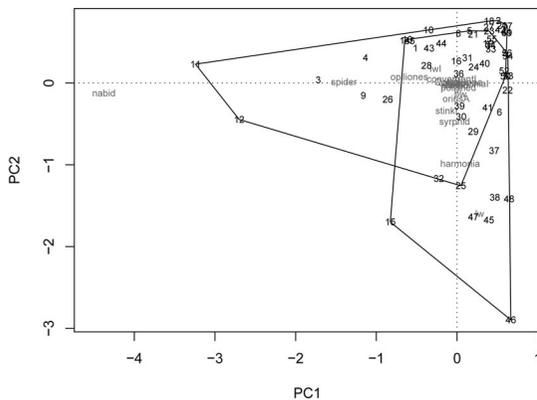

Fig. 6. PCA of natural enemy community in control and living mulch treatments when 100% of plants were infested with *A. glycines* (31 July to 31 August). Hulls represent the natural enemy community collected in control and living mulch treatments.

Natural Enemy Exclusion Study

Experiment 1. In plots established for the previous experiment, we observed fewer *A. glycines* and more natural enemies in the living mulch than in the control treatment. To determine whether natural enemies were responsible for the difference in *A. glycines* between these treatments, we used cages designed to exclude natural enemies from artificially infested plants. Our first natural enemy exclusion experiment was conducted before the natural *A. glycines* infestation occurred. The growth rate of *A. glycines* was significantly greater on caged plants (CC > CU, LMC > LMU); in addition, aphid populations grew faster in the control plots than in living mulch treatments (CC > LMC). Although aphid populations decreased in the caged treatments (CC and LMC) once cages were removed, only aphid populations in the CC decreased significantly from the other treatments (Fig. 7).

Although we observed a greater density of natural enemies in the living mulch treatment (Fig. 3), we did not see a difference in *A. glycines* abundance between the control and living mulch when natural enemies had access to the aphids (LMU = CU; Fig. 7). These results suggest that the natural enemy community in control plots was sufficient to suppress *A. glycines* population growth. What differences there were between the control and living mulch treatments were only revealed when aphids were caged, suggesting that aphid growth on soybean grown with alfalfa was reduced compared with soybean grown alone.

Experiment 2. Our second natural enemy exclusion experiment was conducted after *A. glycines* had infested our study site. In this second experiment, as in the first experiment, *A. glycines* growth rates were greater when natural enemies were excluded (CC > CU, LMC > LMU; Fig. 7). However, *A. glycines* growth rates did not significantly differ among the LMC, CU, and LMU treatments. Once cages were removed, there was a significant decline in population growth within the CC treatment. Although numerically similar to the CC treatment, the amount of variation within the LMC treatment prevented us from declaring it significantly different from the other treatments. Interestingly, only during the final period of the caged studies (Fig. 7, 2b) did we see a significant difference in aphid population growth in the uncaged treatments (CU > LMU). Only within this second experiment did we see evidence that the increased abundance of natural enemies caused by the presence of the living mulch (our conservation technique) produced a decrease in aphid population growth.

Soybean Leaf Nutrient Analysis

There was evidence of competition between soybean and the living mulch compared with soybean in the control treatment. Soybean planted in alfalfa were shorter compared with soybean planted alone (N.P.S., unpublished data). In addition, *A. glycines* populations grown in the control treatment had higher populations
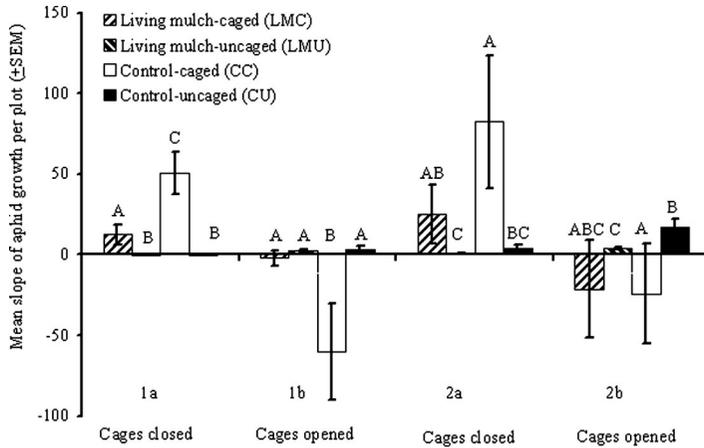
Fig. 7. Mean slope of *A. glycines* growth during two natural enemy exclusion studies: (1a) both caged and uncaged plants 17–30 June, (1b) cages removed 1–11 July, (2a) new caged and uncaged plants 15–25 July, and (2b) cages removed 27 July to 17 August. Significant treatment differences from Wilcoxon two-sample test for each experimental period are represented with letters.

than the living mulch treatment. This led us to consider the quality of soybean as a host for *A. glycines,* specifically N content. We found significant differences on 13 July ($t = 4.27$; df = 6; $P = 0.005$) and on 25 July ($t = 2.67$; df = 6; $P = 0.037$) in total N concentration between control and living mulch treatments. At both sampling periods, we measured a greater concentration of N in soybean leaves taken from plants grown without a living mulch.

Discussion

Our results suggest that the natural enemy community in Iowa is capable of delaying *A. glycines* establishment and limiting subsequent *A. glycines* population growth. The results supported our hypothesis that a living mulch would increase natural enemies (Fig. 3), and in turn lower *A. glycines* abundance compared with conventional soybean plots (Fig. 2). However, the impact of this natural enemy community in the control plots was sufficient to prevent significant *A. glycines* growth until August. Comparison of *A. glycines* on caged and uncaged plants within the larger experiment supports the inference that natural enemies were responsible for this difference. We consistently observed greater *A. glycines* abundance on caged versus uncaged soybeans. The lower abundance of naturally occurring populations of *A. glycines* in living mulch plots and those on artificially infested uncaged plants both suggest that natural enemies contributed to the suppression of *A. glycines* establishment and population growth in Iowa.

Despite the impact of insect predators on *A. glycines,* additional factors may have contributed to this top-down suppression of *A. glycines* populations. The comparison between caged plants in control and living mulch treatments revealed that *A. glycines* did not reproduce as well on soybean grown with an alfalfa living mulch. Given the smaller size and lower N concentration of the soybean grown with an alfalfa

living mulch, it is likely that these plants were a poor host for *A. glycines.* The availability of N within a plant has been shown to be responsible for many cases of aphid reproductive success on host plants (Dixon 1998). This bottom-up regulation of *A. glycines* likely influenced the difference in *A. glycines* abundance observed in our study. Based on this interaction between these two potential population suppressive factors, we suggest that soybean varieties that merely retard *A. glycines* reproduction may contribute to lowering the carrying capacity of aphids below the economic injury level (EIL). It is likely that biological control of *A. glycines* may become more consistent when *A. glycines*–resistant soybeans are made available to growers. Our experience suggests that even partial resistance that slows but does not prevent *A. glycines* reproduction could be significant for limiting economic outbreaks. Such a multitrophic interaction between host plant resistance and natural enemies has been suggested for managing *Schizaphis graminum* (Hemiptera: Aphididae) on sorghum (Dogramaci et al. 2005).

It should be noted that throughout the duration of our study we only collected two parasitized *A. glycines;* therefore, the contribution of natural enemies to *A. glycines* suppression was solely caused by insect predators. Overall, we collected more predators in the living mulch treatment on every sampling date until 5 August, excluding 25 July, when we collected very few in either treatment, presumably because of wet conditions from a heavy rain the night before (N.P.S., unpublished data). 5 August correlates with an increase in *A. glycines* populations in both treatments. Once *A. glycines* populations reached >100 per plant, we collected significantly more *H. axyridis* than collected before 5 August. Furthermore, the density of *H. axyridis* at our research site was highest in August when *A. glycines* populations were also at their peak, with >70% more *H. axyridis* collected in the control than living mulch treatment. *H. axyridis* is considered

a significant *A. glycines* predator (Van Den Berg et al. 1997). Apparently, *H. axyridis* was not necessary for *A. glycines* suppression in the living mulch treatment (Table 1). The majority of the predators collected in the living mulch treatments before August have a fairly broad host range (*Nabis* spp., Araneae, and Opiliones) that may include *A. glycines.* In no-choice feeding trials, *Nabis* spp. adults reduced *A. glycines* numbers by 77% (Rutledge et al. 2004). In addition, Opiliones have been identified as preying on *A. glycines* (Allard and Yeargan 2005). Our results are consistent with Fox et al. (2005) and Costamagna and Landis (2006) and suggest that the community of generalist predators present before the arrival of *A. glycines* are important to delay their establishment.

An economic threshold for *A. glycines* has been set at 250 aphids per plant (Hodgson et al. 2004, Rice et al. 2005). In our case, increasing the abundance of natural enemies through the addition of a living mulch resulted in subeconomic *A. glycines* populations. Living mulches have been used in annual crop production with limited competition to the main crop (Affeldt et al. 2004). We found evidence for significant competition between soybean and alfalfa, resulting in a yield reduction of ≈26% (N.P.S., unpublished data). Within this limited test of a living mulch, the yield protection that would come from suppressing *A. glycines* outbreaks would not have been cost effective. However, additional benefits of a living mulch should be considered. For example, we observed fewer bean leaf beetles (*Ceratoma trifurcate*) within the living mulch than control plots (N.P.S., unpublished data). Both *C. trifurcate* and *A. glycines* are vectors for soybean diseases that reduce yield and seed quality. Whether the use of a living mulch could improve seed quality is not clear. In summary, we did not address the question of whether a living mulch could be used successfully for soybean production. It is likely that the production techniques that we used would have varied had this been our goal. We selected a management plan for suppression of the living mulch that would maintain as much of an alfalfa stand for as long as possible to result in an impact on the insect community. To optimize soybean production, the type of living mulch used and the method and timing of mulch suppression would be altered. How such changes would affect the pest management benefits of a living mulch is not clear. We have studied other living mulch options (Prasifka et al. 2006); however, further research into the optimal use of a living mulch for a corn–soybean rotation is required.

Although one of our objectives was to determine if a living mulch could increase natural enemy abundance, we also made observations related to mechanisms that may have contributed to this increase. In addition to taking *A. glycines* counts on soybean, we also monitored the alfalfa for potential alternative prey for predators. We only observed potato leafhoppers, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae), in June; however, the alfalfa living mulch was chopped on 6 July, which may have limited the development of a resident leafhopper population. By mid-July, after the alfalfa had regrown, we observed green peach aphids, *Myzus persicae* (Sulzer), in low numbers (two aphids per plant) and cowpea aphids, *Aphis craccivora* Koch, in moderately high numbers (55 aphids per plant).

Since the introduction of *A. glycines* to the United States, it seems that aphid densities greatly vary between years. In 2004, test plots reported very low densities of *A. glycines* in Iowa (<65 aphids per plant; N.P.S. unpublished data) compared with 2005, where test plots reported relatively high densities of aphids (71–740 aphids per plant; N.P.S. unpublished data). In addition, it seems that Iowa has a relatively static natural enemy community, when comparing 2004 and 2005 (N.P.S. unpublished data). In this context, our results indicate that, in two separate trials in 2005, excluding natural enemies created an order of magnitude difference in *A. glycines* populations from a common initial density in <2 wk. This clearly shows that natural enemies play an important role in suppressing *A. glycines* under the conditions tested.

Acknowledgments

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