

Density-Dependent Responses of Natural Enemies to Soybean Aphid (Hemiptera: Aphididae) Populations¹

D.R. Kandel², K.J. Tilmon, and T.L. Shuster

Plant Science Department, South Dakota State University, SAG 345, Box 2207A, Brookings, South Dakota 57007 USA

J. Entomol. Sci. 51(2): 168–177 (April 2016)

Key Words soybean aphid, Coccinellidae, natural enemy, biological control, density dependence

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was introduced into the United States in 2000 and quickly became an important pest of soybean throughout much of the North Central United States (Ragsdale et al. 2011, Annu. Rev. Entomol. 56: 375–399; Venette and Ragsdale 2004, Ann. Entomol. Soc. Am. 97: 219–226). Studies on its interactions with host plants, natural enemies, and pesticides including seed-applied insecticides have been conducted (Hill et al. 2004, Crop Sci. 44: 98–106; McCarville and O’Neal 2013, J. Econ. Entomol. 103: 1302–1309; Ragsdale et al. 2011; Rutledge et al. 2004, Ann. Entomol. Soc. Am. 97: 240–248).

Surveys of natural enemies in the United States have shown a complex community of predators, parasitoids, and pathogens attacking soybean aphid (Ragsdale et al. 2011). Among them, generalist predators have demonstrated a significant impact (Costamagna and Landis 2006, Ecol. Appl. 16: 1619–1628; Fox et al. 2004, Environ. Entomol. 33: 608–618; Ragsdale et al. 2011) and, in many experiments, show strong top-down control of the soybean aphid (Costamagna and Landis 2006; Costamagna et al. 2007, Ecol. Appl. 17: 441–451; Costamagna et al. 2013, PLoS One 8: 1–10). Influential predators attacking soybean aphids include the coccinellids *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae), *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), *Orius insidiosus* Say (Hemiptera: Anthracoridae), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), *Leucopis* spp. (Diptera: Chamaemyiidae), and various species of Syrphidae

¹Received 13 July 2015; accepted for publication 30 November 2015.

²Corresponding author (email: devi.kandel@sdstate.edu).

(Costamagna et al. 2007; Desneux et al. 2006, *Environ. Entomol.* 35: 1342–1349; Fox et al. 2004; Gardiner et al. 2009a, *Ecol. Appl.* 19: 143–154; Gardiner et al. 2009b, *Div. Distrib.* 15: 554–564; Rutledge et al. 2004). McCarville and O'Neal (2012, *J. Econ. Entomol.* 105: 1835–1843) reported a 64.4% reduction in aphid populations when aphid-infested microplots were exposed to natural enemies in the field as compared to microplots without natural enemies.

Soybean aphid population suppression in soybean lines containing *Rag* resistance genes, which confer antitibiosis, antixenosis, or both, has been reported (Bhusal et al. 2013, *Crop Sci.* 53: 491–499; Bhusal et al. 2014, *Crop Sci.* 54: 2093–2098; Hill et al. 2004; Mian et al. 2008a, *Crop Sci.* 48: 1055–1061, Mian et al. 2008b, *Theor. Appl. Genet.* 117: 955–962; Zhang et al. 2010, *Theor. Appl. Genet.* 120: 1183–1191). Other studies show early-season aphid suppression from plant systemic insecticide applied as a seed treatment (Kandel et al. 2015, *J. Entomol. Sci.* 50: 186–205; McCarville and O'Neal 2013).

Several studies report that the coccinellid abundance in agricultural crops is affected by habitat composition, quality, and patchiness (Gardiner 2009a; Schmidt and Tschamtko 2005, *J. Biogeog.* 32: 467–473; Thies et al. 2003, *Popul. Ecol.* 42: 81–90). Schellhorn and Andow (2005, *Popul. Ecol.* 47: 71–76) documented the species-specific aggregation of coccinellid predators on aphids at different spatial scales. Colfer and Rosenheim (2001, *Oecologia* 126: 292–304) noted density-dependent mortality of cotton aphid (Hemiptera: Aphididae) by *H. convergens*. Similar results were obtained by Donaldson et al. (2007, *Biol. Control* 43: 111–118) with predators showing strong, density-dependent responses to isolated patches of soybean aphid at a small scale.

Regardless of how natural enemies locate prey patches, it is desirable to have a positive numerical response of natural enemies to increasing densities of soybean aphids (Kandel et al. 2015; Noma and Brewer 2008, *J. Econ. Entomol.* 101: 278–287). Understanding the response of natural enemy communities in different host plant environments, and to different soybean aphid population densities, will help us understand how different pest management strategies, such as biological control and host plant resistance, may work together in soybean integrated pest management. The purpose of this study was to examine the relationship between aphid density and natural enemy response to them by experimentally manipulating aphid densities in the field in different host plant environments (with different ambient insect communities).

The study was conducted at the South Dakota State University (SDSU) research farm in Volga, SD, in 2011. There were four types of plots: an aphid-resistant *Rag1* soybean (LD [05] 16137) or a susceptible variety (SD 76 R), with or without insecticidal seed treatment (thiamethoxam [Cruiser 5 FS, Syngenta Crop Protection, Greensboro, NC] at a rate of 0.0756 mg ai per soybean seed). The two soybean lines used in the study are near-isogenic lines and differed primarily by the presence or absence of the aphid-resistant *Rag1*, a source of antitibiosis.

A randomized complete block design was used with four replications. Plots were 30.5 × 30.5 m with 3.05-m bare borders maintained around each plot to minimize edge effects from other treatments. Soybeans were planted on 19 May 2011 in 75-cm rows at a rate of 296,400 seeds/ha. There were 40 rows in each plot. Glyphosate (Roundup®, Monsanto, St. Louis, MO) was applied on 29 June for weed control. During the peak period of natural aphid infestation on 15 August, aphid-free

and aphid-dense sentinel plants were placed in each plot to monitor the responses of natural enemies.

Sentinel plants were prepared by growing potted, aphid-susceptible plants (SD 76R) in field cages and either inoculating them with aphids or keeping them aphid-free. On 13 June, 128 round plastic pots (17-cm height, 15.2-cm diameter) were each filled with approximately 2 kg of a soil mixture which contained equal proportions of field soil, sand, and potting mix. Three seeds were planted per pot and thinned to one plant per pot after germination. Plants were grown in four large, fine-meshed, walk-in field cages (3.65×1.82 m) erected on a grassy area of the SDSU Plant Pathology farm adjacent to campus. Each cage contained two plastic wading pools with 16 potted soybean plants per pool (i.e., 32 plants per cage). High aphid density was created on 64 potted sentinel plants in two of the cages by infesting them with aphids reared in outdoor field insectary cages erected over soybeans that had been stocked with aphids collected from the field earlier in the season. Sentinel plants were inoculated on 21 July when plants were at V3/V4 growth stage. The other 64 potted soybeans in the remaining two field cages were kept free from infestations to serve as aphid-free sentinel plants.

On 15 August, these potted sentinel plants were placed in each field plot (aphid-resistant and susceptible soybeans, with and without insecticidal seed treatment) at arbitrary locations. Each plot received four aphid-dense and four aphid-free sentinel plants. Potted plants were well-watered before being placed in the field. Pots were buried halfway to the rim to conserve moisture and provide stability. The initial number of aphids on each infested sentinel plant was determined based on visual counts in units of 500, and the aphid-free status of each clean plant was verified. Initially, there were 0 aphids per plant in all aphid-free sentinel plants and $>2,900$ aphids per plant in aphid-dense sentinel plants (Fig. 1).

Starting 1 d after sentinel plants were placed in the field, natural enemies and aphids were counted on each plant on each day for 5 subsequent days from 16–20 August. Four field plants from each plot were also selected arbitrarily each day for counting. Thus, each day in each plot, four aphid-infested sentinel plants, four aphid-free sentinel plants, and four field plants (a different set on each date) were surveyed for aphids and natural enemies. Aphid counts were made with high precision up to 100, after which estimates were made based on visual units of approximately 100 (up to 500 per plant), and then visual units of approximately 500 thereafter. Each plant was carefully inspected for natural enemies, and all species were recorded to the lowest taxonomic level that could be determined in the field.

Besides visual sampling of aphids and natural enemies, we also sampled natural enemies in each field plot using sticky cards (Scentry® Multigard® Unbaited AM Trap, Gempler's, Janesville, WI). The sticky cards were fluorescent yellow and measured 27.9×22.9 cm. One sticky card was placed in the center of each plot, mounted on a wooden post, and secured directly above the plant canopy with a zip tie. Cards were mounted on 15 August and removed on 22 August. Cards were stored in a freezer and natural enemies trapped on the cards were identified.

Aphid population changes in aphid-free sentinel plants and aphid-dense sentinel plants were calculated by subtracting the initial aphid count for a given plant from the count taken on the last sampling day. Changes in aphids per plant and natural enemy counts were log transformed to satisfy the assumptions of normality and analyzed using the split plot analysis of variance design in PROC MIXED (SAS

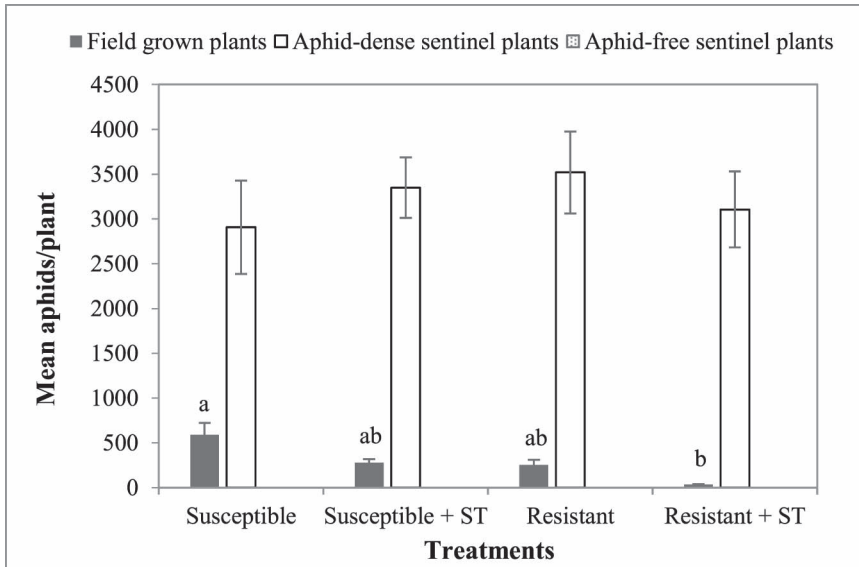


Fig. 1. Mean aphid densities on aphid-free and aphid-dense sentinel plants and field-grown plants at the beginning of the study (aphid-free sentinel plants had means of 0 aphid per plant and do not appear on the figure). Sentinel plants were all of the aphid-susceptible soybean variety, without seed treatment, whereas the field-grown plants were whatever variety or seed treatment (ST) (or both) was in the plot. Error bars are standard errors of means. Bars with different letters in field grown plants are significantly different between treatments ($P < 0.05$, least significant difference).

Institute 2008, Cary, NC). Treatment means were compared by a least significant difference test at 0.05% level of significance.

At the beginning of this study, aphid populations were about the same in the different treatments except in resistant soybeans with seed treatment, where aphids were fewest (Fig. 1). Initially, aphid densities on aphid-dense plants ($>2,900$) did not vary nor did aphid densities on aphid-free plants (0).

We found a diverse community of natural enemies in soybean in this study. A total of 1,007 natural enemies was observed on plants (Table 1) and 412 natural enemies were trapped on sticky cards (Table 2) deployed in each plot (1/plot) during the study period. Among the plant-counted natural enemies, active predators (i.e., the larvae and adults of coccinellids, syrphids, and lacewings; aphid midge larvae; spiders; *Orius* spp. nymphs and adults) comprised 52.8% of observations whereas 23.5% of observations were aphid parasitoid mummies. Among the active predators observed, coccinellids were the most abundant (49.4% of the active predators) followed by anthocorid *Orius* spp. (16.9% of the active predators) and cecidomyiid *Aphidoletes* spp. (15.7% of the active predators), respectively. In sticky trap counts, *O. insidiosus* was the most-common observed natural enemy (38.1%).

Table 1. Diversity and abundance of natural enemies observed on plants for 5 d in aphid-free sentinel plants, aphid-dense sentinel plants, and in field-grown plants across four treatments of soybean. A total of 960 plants were visually inspected for natural enemies throughout the study period.

Order	Family	Identity	Number observed by treatment*			
			Sus	Sus + ST	Res	Res + ST
Coleoptera	Coccinellidae	<i>Harmonia axyridis</i> adults	29	3	7	2
		<i>H. axyridis</i> larvae	43	18	30	10
		<i>Coccinella septempunctata</i> adults	8	4	11	2
		<i>Hippodamia convergens</i> adults	10	2	9	3
		Other adult lady beetles	5	2	2	4
		Other lady larvae†	31	20	11	1
		Lady beetle pupae	54	16	7	0
		Lady beetle eggs	38	0	78	7
Diptera	Syrphidae	Syrphid adults	8	4	4	6
		Predatory fly larvae	8	8	9	5
	Cecidomyiidae	<i>Aphidoletes</i> spp. larvae	22	21	9	32
Hemiptera	Anthocoridae	<i>Orius</i> spp. adults	24	25	23	6
		<i>Orius</i> spp. nymphs	4	2	3	3
Neuroptera	Chrysopidae	<i>Chrysoperla carnea</i> adult	6	2	5	4
		<i>Chrysoperla carnea</i> larvae	7	4	5	3
		<i>Chrysoperla carnea</i> eggs	6	0	11	21
Araneae		Spider	1	1	1	0
Hymenoptera		Parasitized aphid mummies	49	89	78	21
Total natural enemies			353	221	303	130

* Sus = susceptible; ST = seed treatment, Res = resistant.

† Other lady larvae = lady beetle larvae excluding *H. axyridis*.

Table 2. Diversity and abundance of adult natural enemies on sticky-card traps. A total of 16 sticky card traps deployed were inspected for natural enemies (one per plot).

Order	Family	Identity	Number observed by treatment*			
			Sus	Sus + ST	Res	Res + ST
Coleoptera	Coccinellidae	<i>Harmonia axyridis</i>	1	0	0	0
		<i>Hippodamia convergens</i>	7	3	3	3
		<i>Hippodamia tredecimpunctata</i>	1	0	0	1
Diptera	Syrphidae	Syrphid fly	2	2	2	2
Hemiptera	Anthocoridae	<i>Orius insidiosus</i>	50	60	32	15
		<i>Orius tristicolor</i>	3	0	1	0
		<i>Orius</i> spp.	3	5	2	0
Neuroptera	Chrysopidae	<i>Chrysoperla carnea</i>	15	10	12	6
	Hemerobidae	Brown lace wing	7	6	9	3
Araneae		Spider	7	4	0	2
Hymenoptera	Braconidae		1	0	0	1
	Aphelinidae		40	36	25	30
Total natural enemies			137	126	86	63

* Sus = Susceptible, ST = Seed treatment, Res = Resistant.

Greater densities of coccinellids were found in plots of soybeans with more aphids compared with the fewest coccinellids in plots with the fewest aphids. There were 4.7 times more coccinellid larvae and 9.2 times more coccinellid adults in untreated susceptible soybeans than in seed-treated resistant soybeans (Table 1); this points to coccinellids as the natural enemies most responsive to aphid density. Such behavioral responses of coccinellids to aphids have also been illustrated in other studies (Conway and Kring 2010, J. Entomol. Sci. 45: 129–139; Costamagna et al. 2008; Elliott and Kieckhefer 2000, Popul. Ecol. 42: 81–90; Gardiner 2009a; Schellhorn and Andow 2005, Popul. Ecol. 47: 71–76). However, coccinellids were under-represented on sticky traps compared to vegetation counts. Stephens and Losey (2004, Environ. Entomol. 33: 535–539) suggested that coccinellids are better at escaping sticky cards than are other natural enemies because they are relatively large, strong, and have spheroidal bodies.

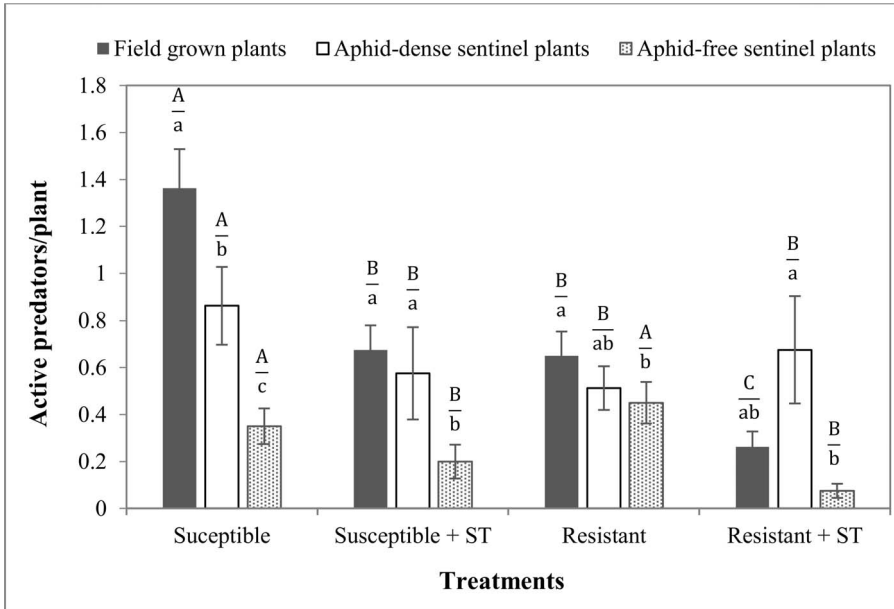


Fig. 2. Mean abundance of active predators (adults and larvae) in aphid-free sentinel plants, aphid-dense sentinel plants, and field-grown plants in the four plant habitats. Sentinel plants were all susceptible without seed treatment whereas the field-grown plants were whatever variety or seed treatment (ST) (or both) was in the plot. Error bars are standard errors of means. Bars with different uppercase letters are significantly different than corresponding bars in different treatments. Bars with different lowercase are significantly different than other bars within a given treatment ($P < 0.05$, least significant difference).

The average of the active predators (larvae and adult of coccinellids, syrphid larvae and adults, *Aphidoletes* spp. larvae, *Orius* spp. nymphs and adults, larvae and adults of chrysopids, and spiders) observed on all types of plants (i.e., field-grown plants, aphid-free sentinel plants, and aphid-dense sentinel plants) in each treatment are presented in Figure 2. We focus here on predators capable of movement, as the purpose of this study was to assess predator immigration within a short time frame. Among the tested factors, active predator abundance varied significantly by plant variety (resistant or susceptible) ($F = 17.45$; $df = 1, 12$; $P = 0.0001$), seed treatment ($F = 42.44$; $df = 1, 12$; $P < 0.0001$), and the type of plant (field plant, aphid-free sentinel plant, aphid-dense sentinel plant) ($F = 41.04$; $df = 2, 24$; $P < 0.0001$), and also showed a variety by plant-type interaction ($F = 7.24$; $df = 2, 24$; $P = 0.003$).

In general, natural enemies were found in greater numbers on plants with the most aphids compared to plants with the fewest aphids, showing their response to prey availability. The plants with the greatest number of aphids (aphid-dense sentinel plants) had significantly higher predator densities than did the plants with

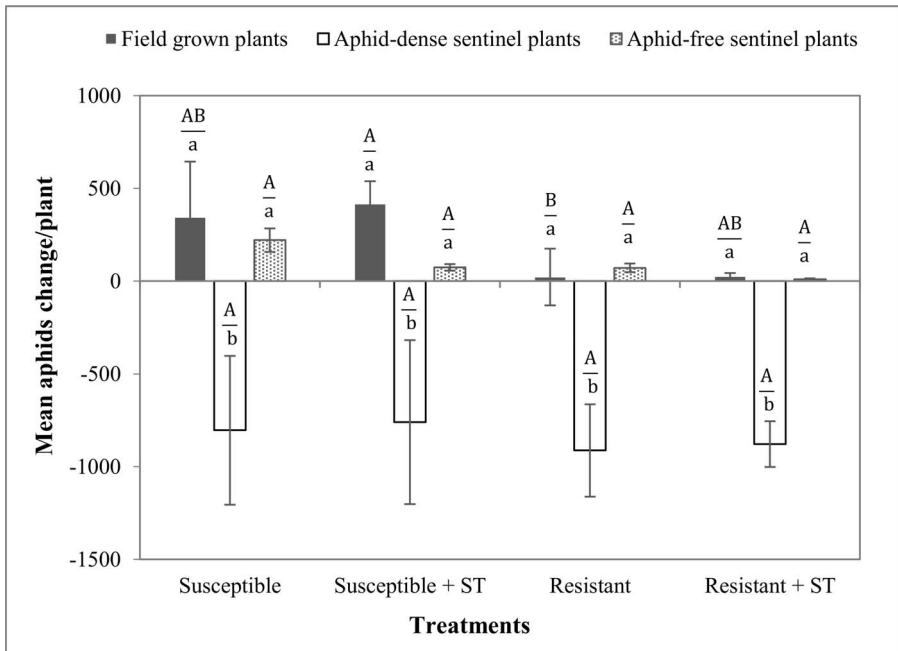


Fig. 3. Aphid population change in aphid-free sentinel plants, aphid-dense sentinel plants, and field-grown plants during the study period. Sentinel plants were all susceptible without seed treatment whereas the field-grown plants were whatever variety or seed treatment (ST) (or both) was in the plot. Error bars are standard errors of means. Bars with different uppercase letters are significantly different than corresponding bars in different treatments. Bars with different lowercase letters are significantly different than other bars within a given treatment ($P < 0.05$, least significant difference).

the fewest aphids (aphid-free sentinel plants), regardless of field plot treatments, with the exception of non-seed-treated resistant soybeans (Fig. 2). The field plot treatment with the most active natural enemies on field-grown plants (susceptible soybeans without seed treatment) also had the most natural enemies on the aphid-dense sentinel plants (Fig. 2), suggesting at least some influence of the surrounding field plot soybeans on natural enemy recruitment, even though there were fewer natural enemies on the sentinel plants than on the field-grown plants. In the other treatments, the average predator abundance on aphid-dense sentinel plants was comparable with the predator abundance on field-grown plants and was also comparable among treatments. Taken all together this indicates that, at this spatial scale, natural enemies are mobile and effective at finding dense aphid patches.

The aphid population change (growth or decline) on the sentinel and field-grown plants in the four treatments are presented in Fig. 3. On field-grown plants, there was a significant variety effect, with the least aphid population growth observed on the aphid-resistant variety ($F = 6.06$; $df = 1, 9$; $P = 0.03$). The suppression of

soybean aphid growth on plants with *Rag* genes has been well-documented (Hill et al. 2004; Mian et al. 2008a, 2008b; Zhang et al. 2010). However, aphid population growth did not vary with the presence or absence of insecticidal seed treatment. By this point in the season (mid-August), there was no longer an insecticidal effect. Because the experiment was started 88 days after soybean planting, the thiamethoxam may have been absent from the majority of plant tissue by that time. A loss of seed-treated insecticide activity over time was observed by McCornack and Ragsdale (2006, Crop Manag. doi: 10.1094/CM-2006-0915-01-RS), Seagraves and Lundgren (2012, J. Pest Sci. 85: 125–132), and McCarville and O'Neal (2013) at 49, 46, and 42 d after planting, respectively.

The most interesting significant variation in aphid population change was by plant type ($F = 46.93$; $df = 2, 24$; $P < 0.0001$). In all aphid-dense plants (which initially had >2,900 aphids per plant), aphid populations significantly decreased compared to the aphid population change on sentinel plants that began with no aphids (and where aphids increased slightly) or compared to field-grown plants that began with moderate aphid density (Fig. 3). Aphid population decrease on aphid-dense sentinel plants was four times greater than the overall increase observed in field-grown plants. Also, the magnitude of the decline of aphid populations on the aphid-dense sentinel plants was universal across habitats. Even in plots with the lowest natural enemy and aphid abundance in general (seed-treated resistant soybean), aphid-dense plants placed in those plots received similar attention from natural enemies and experienced the same dramatic reduction in aphid population over only a 5-d period. This again demonstrates that natural enemies were able to successfully locate and respond to these high-density aphid pockets quite rapidly, perhaps from further away than the plot itself. This is a case of strong, density-dependent responses of natural enemies to prey availability. A similar result of density-dependent response was documented by Donaldson et al. (2007) in which generalist predators responded to soybean aphids at a small spatial scale. Fox et al. (2004) also reported that *H. axyridis* and *O. insidiosus* lowered dense populations of aphid after the removal of cages. In addition, Desneux et al. (2006) documented the effectiveness of *O. insidiosus* for slowing the growth of aggregated aphids, though this effect was not observed for randomly distributed aphids.

Intraspecific competition due to limited host-plant nutrients, lack of space (Colfer and Rosenheim 2001, Oecologia 126: 292–304; Noma et al. 2010, Environ. Entomol. 39: 31–41; Williams et al. 1999, For. Entomol. 1: 119–125), and emigration (Donaldson et al. 2007; Hodgson et al. 2005, Environ. Entomol. 34: 1456–1463) are other factors besides natural enemies which may lead to aphid decline. The design of our experiment did not allow us to measure those impacts. However, the time period of this study was short (5 d), and the rapid decline of aphids on aphid-dense plants was most likely due to the immediate influence of natural enemies.

In conclusion, our results show a trend toward higher natural enemy abundance in treatments, or on plants which have more aphids, with a particularly strong response by coccinellids. Natural enemies showed a strong, density-dependent response to aphids in high aphid density pockets (the aphid-dense sentinel plants), which resulted in the rapid decline of aphids on these plants within 5 d. This was the case even in environments where the ambient density of natural enemies was

lower, showing that the natural enemies have the ability to locate high-density aphid patches from at least some distance in this system. This demonstrates the potential for natural enemies to regulate soybean aphid as part of a sustainable, integrated pest management program.

Acknowledgments. We appreciate the help of Mathew Sachen, Rachel Boone, Larissa Giddings, Ben Pflueger, Ethan Wiese, Jeff Augspurger, and Earl LaMaack for assistance with field work. We thank Dr. Cuirong Ren for assistance with statistical analysis, Syngenta for providing custom seed treatment, and the Severin-McDaniel Insect Research Collection, SDSU, for access to insect specimens. Research was funded by the North Central Soybean Research Program, the South Dakota Soybean Research and Promotion Council, and the South Dakota Agricultural Experiment Station.