Inventory and Assessment of Foliar Natural Enemies of the Soybean Aphid (Hemiptera: Aphididae) in South Dakota

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ABSTRACT   Soybean aphid (*Aphis glycines* Matsumura) (Hemiptera: Aphididae) is a major pest of soybean in northern production regions of North America, and insecticides have been the primary management approach while alternative methods are developed. Knowledge of arthropod natural enemies and their impact on soybean aphid is critical for developing biological control as a management tool. Soybean is a major field crop in South Dakota, but information about its natural enemies and their impact on soybean aphid is lacking. Thus, this study was conducted in field plots in eastern South Dakota during July and August of 2004 and 2005 to characterize foliar-dwelling, arthropod natural enemies of soybean aphid, and it used exclusion techniques to determine impact of natural enemies and ants (Hymenoptera: Formicidae) on soybean aphid densities. In open field plots, weekly soybean aphid densities reached a plateau of several hundred aphids per plant in 2004, and peaked at roughly 400 aphids per plant in 2005. Despite these densities, a relatively high frequency of aphid-infested plants lacked arthropod natural enemies. Lady beetles (Coleoptera: Coccinellidae) were most abundant, peaking at 90 and 52% of all natural enemies sampled in respective years, and *Harmonia axyridis* Pallas was the most abundant lady beetle. Green lacewings (Neuroptera: Chrysopidae) were abundant in 2005, due mainly to large numbers of their eggs. Abundances of arachnids and coccinellid larvae correlated with soybean aphid densities each year, and chrysopid egg abundance was correlated with aphid density in 2005. Three-week cage treatments of artificially infested soybean plants in 2004 showed that noncaged plants had fewer soybean aphids than caged plants, but abundance of soybean aphid did not differ among open cages and ones that provided partial or total exclusion of natural enemies. In 2005, plants within open cages had fewer soybean aphids than those within cages that excluded natural enemies, and aphid density on open-cage plants did not differ from that on noncaged plants and those accessible by small predators. In a separate 3-yr experiment, exclusion of ants from soybean plants did not lead to differences in soybean aphid density compared with ant-accessible plants. Overall, these results suggest that the soybean aphid natural enemy guild is unsaturated and could be enhanced to improve biological control of soybean aphid in South Dakota.

KEY WORDS   *Aphis glycines*, Coccinellidae, exclusion cage, ant–aphid association

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is native to Asia, but only a sporadic pest of soybean there (Wang et al. 1996). However, since 2000, northern soybean production areas of North America have suffered outbreaks of soybean aphid, which may cause up to 40% yield loss (Myers et al. 2005, Ragsdale et al. 2007, Beckendorf et al. 2008). Soybean aphid vectors several persistently and nonpersistently transmitted viruses of soybean, and these viruses may cause further yield loss (Clark and Perry 2002, Burrows et al. 2005, Wang et al. 2006). Insecticides have been used widely to manage soybean aphid in North America until other tactics, such as biological control and host plant resistance, can be evaluated and developed (Rutledge et al. 2004, Ragsdale et al. 2007).

Soybean is a major field crop in South Dakota (National Agricultural Statistics Service [NASS] 2004, 2012). Historically, soybeans in South Dakota lacked significant arthropod pests (Hesler et al. 2005, Ragsdale et al. 2011), and this crop was generally not included in surveys of natural enemies (Kieckhefer et al. 1992, Elliott et al. 1996, Hesler et al. 2005). Thus, relatively little information has been available on its composition of arthropod natural enemies in South Dakota.

Evaluation of biological control of soybean aphid includes an inventory and assessment of incumbent natural enemies (Luck et al. 1988, van den Berg et al. 1997, Rutledge et al. 2004). The composition and effectiveness of natural enemies in soybean agroecosys-

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tems may differ regionally owing to such factors as climate, crop phenology, crop management practices, and the composition and severity of various pests (Rutledge et al. 2004, Brosius et al. 2007, Rhainds et al. 2007, Liu et al. 2012). One tool that is used to evaluate natural enemy efficacy involves exclusion techniques to partition impact of particular classes of natural enemies on pest populations. The use of cages with various-sized openings selectively excludes particular natural enemies and, thereby, allows inference about which class(es) (e.g., small predators and parasitoids versus large predators) may effectively suppress the target pest (Luck et al. 1988).

In addition, ants (Hymenoptera: Formicidae) interact with aphids and arthropod natural enemies in crop fields, and the interactions can reduce effectiveness of natural enemies against aphids. For example, ants may reduce attack of particular aphids from parasitoids and predators (Stary 1966, Buckley 1987, Kaplan and Eubanks 2002). Moreover, ants may act as higher-order predators of natural enemies and indirectly release pest species from regulation through intraguild interactions (Banks and McCauley 1967, Jiggins et al. 1993, Eubanks et al. 2002). Thus, it is important to determine how ants, as well as natural enemies, mediate population growth of soybean aphid in soybean.

In this article, foliar-dwelling, arthropod natural enemies of soybean aphid were characterized over two seasons in soybean plots in eastern South Dakota, and exclusion techniques were used to determine impacts of foliar natural enemies and ants on population levels of soybean aphid in soybean.

Materials and Methods

Experiments were conducted at the Eastern South Dakota Soil and Water Research Farm near Brookings, SD, in 1.2-ha plots of soybean (nontreated seed of '91B91,' Pioneer Hi-Bred International, Inc., Johnston, IA) that followed successive annual plantings of wheat and maize. Soybean was planted on 15 May 2004 and 27 May 2005 at a seeding rate to achieve 39 plants per m-row, with rows spaced 76 cm apart. The herbicides glyphosate and alachlor were applied before soybean emergence, and plots were hoed and hand-weeded for postemergent weed management. No insecticides or fungicides were used in the plots. Weed densities and soybean disease were negligible each year within plots.

Three studies were run each year with respective goals of 1) inventorying natural enemies and associating them with soybean aphid, 2) determining effect of exclosure on soybean aphid-population growth, and 3) determining the effect of ants on soybean aphid-population growth. The ant experiment was also repeated in 2006.

Inventory of Natural Enemies Associated With Soybean Aphid. Soybean aphids and their natural enemies were monitored on soybean plants at 300 sampling stations among 12 rows of soybean within the interior of the plots. Twenty-five sampling stations were es-

tablished per row, and rows with sampling stations were separated by two nonsampled soybean rows. Individual sampling stations were roughly 1.2 m long, 7.5 row-m apart, and ≥15 m from plot margins. Soybean plant density was modified within stations during V2 to V3 plant-development stages (Pedersen 2004) by thinning to eight plants per station (roughly 0.2 m between plants) so that sampling could be performed and quantified on a per-plant basis. Sampling within each station commenced around onset of soybean aphid infestation in plots during late V4 to early V5 stages on 26 July 2004 and during early V4 stage on 28 June 2005 and continued until R7 stage (early bean maturity, initial leaf drop) on 31 August 2004 and R6 stage (full seed stage, initial leaf senescence) on 9 August 2005. Soybean development was notably faster in 2005 than in 2004. Soybean plants were sampled weekly for 6 wk in 2004 and 7 wk in 2005. One plant per station was sampled in the first week, and successively adjacent plants were sampled individually in subsequent weeks.

Individual plants were sampled as one person approached slowly from the north side of each row to preclude sudden movement and to avoid casting a shadow on sample plants, either of which might disturb mobile natural enemies and cause them to flee before they could be identified (Hesler et al. 2004, Schmidt et al. 2008). Sampling time was increased as crop canopy enlarged (i.e., 30 s per plant, each of first 2 wk; 45 s, third week; and 60 s in later weeks). Sampling time was partitioned by spending roughly the first 5–10 s reconnoitering arthropod natural enemies at ≈0.2 m from the sample plant, and remaining sample time was spent scouring the canopy by carefully moving stems and turning leaves to locate obscured natural enemies. The number and type of natural enemies per plant were recorded at each sampling station.

The number of soybean aphids on each sample plant was estimated by two different methods depending on year. In 2004, aphid infestation per plant was categorized as "0" = no aphids; "1," 1–10 aphids per plant; "2," 11–100 aphids per plant; "3," 101–1000 per plant; and "4," >1000 per plant (modified after survey protocol in Rutledge et al. [2004]). In 2005, soybean aphids were counted on each plant when aphid numbers were 50 or fewer, but estimated to the nearest 50-aphid interval (i.e., 100, 150, 200, 250, etc. soybean aphids per plant) at higher aphid densities as a practical necessity because of sampling time constraints (Elliott et al. 2002). Samplers had been trained in recognizing natural enemies and in estimating and counting soybean aphid on soybean plants. Associations between aphid density and numbers of natural enemies per plant were tested separately for each year (aphid ratings, 2004; actual numbers, 2005) by using Pearson's correlation technique (PROC CORR; SAS Institute 2010, Zar 2010). Across years, a logit model was used to test whether the proportion of soybean plants with natural enemies varied by aphid-infestation level and year (PROC GLIMMIX). Post hoc comparisons of proportions among aphid ratings were made using the LSMEANS statement. Finally, counts of 16 individual

Fig. 1. Cage with open windows to permit ingress and egress of arthropod natural enemies.

groups of natural enemies were summed across sampling dates each year, and rank correlation in counts per natural enemy group between 2004 and 2005 was tested using Spearman's correlation procedure (PROC CORR; SAS Institute 2010).

Cage Treatments. In this experiment, exclusion cages were used to evaluate the impact of different sized natural enemies against soybean aphid. Four treatments were applied: three with cages (57 cm in height, 36 cm diameter bottom and 29 cm diameter top) and a fourth no-cage control. The three cage treatments respectively provided total, partial, or no exclusion of arthropod natural enemies from artificially aphid-infested soybean plants (Rice and Wilde 1988). "Total exclusion" cages were covered with fine mesh screen (0.27 by 0.82 mm), "partial exclusion" cages had eight openings covered by small mesh screen (1.1 by 1.1 mm) to allow entry by parasitoids and "micropredators" [Rice and Wilde 1988; early immature instars of spiders (Araneae), anthocorids (Hemiptera), etc.], and "open" cages had the eight openings without screen to allow entry of all sizes of arthropod natural enemies (Fig. 1). Each cage had four 12.5- by 12.5-cm and four 15.2- by 15.2-cm windows to which screen was applied and secured tightly with Velcro around window perimeters. Cages each had a 12.5- by 12.5-cm window on top that was covered by the fine mesh screen. Each of the four treatments appeared within a block row of soybean, and cages within a row were ≥5 m apart from each other. Cage treatments were arranged in a randomized block, and each cage-row represented one of five (2004) or seven (2005) replicate blocks. Blocks were six rows (or 6 m) apart. Each cage covered two soybean plants (majority V3 to V4 stages), which were each infested with 30 soybean aphids by attaching an infested soybean leaf

via paper clip to the newest unfurled leaf of each plant and then covering the two plants with fine-mesh cages for 4 d. Aphid-infested leaves were obtained from plants that supported a multiclonal laboratory colony of soybean aphids collected from nearby fields of soybean in 2003. Before artificial infestation, treatment plants were searched for insects. No aphids were found, and other insects were removed. After 4 d of infestation, establishment cages were removed and cage treatments were applied and maintained for the next 23 d. Temperature was recorded within individual cage treatments during this time by placing a HOBO Pro Series (Onset Computer Corp., Bourne, MA) temperature logger between paired cage-treatment plants (2004, $n = 3$; 2005, $n = 4$). After 23 d, treatment plants (V6 to R1 stages) were cut at soil level and carefully placed into large plastic bags that were stored in a laboratory freezer. Plants were later thawed, and aphids were counted on each plant in the laboratory. Counts for each two-plant sample were square-root transformed to homogenize variances among treatments and then subjected to analysis of variance (PROC ANOVA) to test for effect of cage treatment, with significant outcomes followed by a Tukey HSD test to separate means (Zar 2010). Mean temperatures over the experimental period were also subjected to analysis of variance to test for cage effect.

Ants and Soybean-Aphid Population Growth. Ants were excluded from or allowed access to aphid-infested soybean plants to test for their effect on population growth of soybean aphids. Ants were excluded by applying a roughly 1-cm-thick coat of adhesive (Stikem Special, Michel and Pelton, Emeryville, CA) to the basal 10 cm of stem of soybean plants. Nontreated plants allowed ants to access soybean plants. A completely randomized design was applied to a row of

soybean plants within an experimental plot to intersperse adhesive-treated and nontreated plants. Experimental plants were ≈7.5 m apart. Their unifoliolate leaves were removed, and plants within 0.5 row-m were removed to prevent bridging with experimental plants. Experimental plants were perused to ensure they had no natural infestation of soybean aphid. Single aphid-infested leaves of colony plants were cut, thinned to 35 soybean aphids per leaf, and attached to individual field plants by paper clip to establish aphids. Plants (V4 to V5 stages) were immediately covered for 48 h with exclusion cages used in the experiment above. After this the cages were removed, and plants were checked once per week to ensure that ants were excluded during each experiment (8–23 July 2004, 8–21 July 2005, 3–17 July 2006). In 2005, a storm with strong winds lodged experimental plants ≈40 h before their removal, and thus allowed brief (<15 h) access by ants to experimental plants. Each year, plants (V6 to R1 stages) were removed at the end of the experimental period by cutting them at soil level, and placing into large plastic bags for storage in a laboratory freezer. Plants were later thawed, and aphids were counted on each plant. Because of similarity in experimental procedures among years, aphid counts were compared between adhesive-treated and nontreated plants across years using a mixed model analysis of variance (PROC GLIMMIX; Littell et al. 2006, SAS Institute 2010), with treatment (ant manipulation) as the main, fixed factor and year and replicate within year and treatment as random factors. Accordingly, interactions of ant treatment and year were also random and unpredictable, and thus excluded from the model. To obtain supplementary information on ants associated with soybean aphid, soybean plants sampled in the natural enemy inventory in 2004 were also monitored for the presence of ants, and the frequency of ant-occupied plants was tested for association with aphid-density rating (PROC CORR). In addition, a sample of the predominant ant associated with soybean aphid on soybean plants was collected for identification in the laboratory.

Results

Population Levels of Soybean Aphid in Field Plots. Soybean aphids steadily increased in abundance each year, but in 2005 their numbers decreased appreciably on the last sampling date as senescent plants became prevalent (Fig. 2). Densities of soybean aphids were rated mainly in categories 3 and 4 (100–1,000 aphids per plant and >1,000 aphids per plant, respectively) on the last four sampling dates in 2004, and peaked at roughly 400 aphids per plant on 4 August in 2005.

Inventory of Natural Enemies and Their Association With Soybean Aphid. Seven major groups of foliar-dwelling, arthropod natural enemies were found on sample plants each year (Table 1). These included arachnids (spiders, Araneae; harvestmen, Opiolones), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae, adults and nymphs), damsel bugs (Hemiptera: Nabidae, *Nabis americoferus* Carayon,
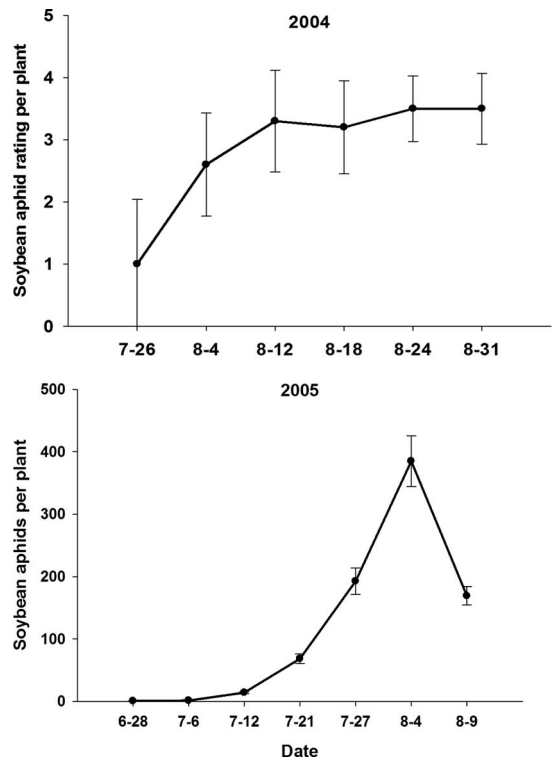

Fig. 2. Number of soybean aphids per plant ($x \pm$ SD) in field plots near Brookings, SD, during sampling periods for natural enemies in 2004 and 2005. Aphid ratings in 2004: "0" = no aphids; "1," 1–10 aphids per plant; "2," 11–100 aphids per plant; "3," 101–1000 per plant; and "4," >1000 per plant.

adults and nymphs), lady beetles (Coleoptera: Coccinellidae; all stages), green lacewings (Neuroptera: mainly Chrysopidae and some Hemerobiidae; all stages), predaceous fly larvae (Diptera: Cecidomyiidae and Syrphidae), and wasp parasitoids (Hymenoptera: Aphidiidae) in the form of tan mummified aphids. Parasitoids were identified based on adults that emerged from mummies that were collected, placed into small gel vials, and held in the laboratory. A subsample ($n = 8$) of emerged parasitoid wasps from 2004 contained four *Lysiphlebus testaceipes* (Cresson) and four *Aphidius* sp. More natural enemies were found in 2004 than in 2005, corresponding to respectively higher levels of soybean aphids, but yearly rank abundance of 16 individual natural enemy groups (Table 1) was similar between 2004 and 2005 ($r = 0.76$; $n = 16$; $P < 0.001$). Active feeding on soybean aphid by all of these natural enemies was observed in the plots. Evidence of nonarthropod natural enemies associated with *A. glycines* was rarely seen, e.g., only occasionally were aphids observed with symptoms of infection by entomopathogens, mainly on later sampling dates.

Lady beetles were the most frequent natural enemies sampled each year, although their abundance was >3× greater in 2004 than in 2005 (Table 1). Correspondingly, they represented a greater proportion of natural enemies in 2004 than in 2005, with their

Table 1. Number of arthropod natural enemies sampled on soybean plants in eastern South Dakota

Type of natural enemy	2004 (n = 1800 plants)	2005 (n = 2097 plants)
Arachnida: Araneae and Opiolones	155	257
Hemiptera: Anthocoridae, *O. insidiosus*	144	174
Hemiptera: Nabidae, *N. americoferus*	13	31
Neuroptera: mainly Chrysopidae, *Chrysoperla* spp.		
Eggs	195	567
Larvae	12	25
Adults	33	19
Coleoptera: Coccinellidae		
Egg clusters	56	50
Larvae	1482	445
Pupae	691	116
Adults	238	163
C. maculata	12	12
Hi. tredecimpunctata tibialis	36	7
Hi. convergens	17	47
Co. septempunctata	64	30
H. axyridis	104	66
Hymenoptera, mummified aphids (parasitoids)	31	21
Diptera, mainly Ceccidiomyiidae (larvae)	40	15
Other natural enemies	19	1
Total	3109	1883

proportions peaking at 90 and 52% of all natural enemies in respective years (Fig. 3). Adult lady beetles consisted primarily of five species, including three native species (*Coleomegilla maculata lengi* Timberlake, *Hippodamia tredecimpunctata tibialis* (Say), and *Hippodamia convergens* Guerin-Meneville) and two naturalized species (*Coccinella septempunctata* L. and *Harmonia axyridis* Pallas). *H. axyridis* was the most abundant adult coccinellid each year. Although it was impractical to identify larvae to species in the field or via collected specimens in the laboratory, nevertheless it was obvious that larval *H. axyridis*, which are distinct from the other coccinellids found in soybean (Schellhorn 2013), accounted for the majority of larvae sampled each year, and this corresponded to proportionally greater abundance of adult *H. axyridis.* Lacewings rivaled lady beetles in abundance in 2005. However, whereas various stages of lady beetles were represented in samples over time, lacewing abundance was largely owing to high numbers of their eggs found throughout the season. Arachnids, *O. insidiosus*, and eggs of green lacewings were generally present throughout the sampling period each year, whereas immature lady beetles were proportionally more abundant in the middle of the sampling period and predaceous fly larvae were most abundant on the last few sampling dates (data not shown).
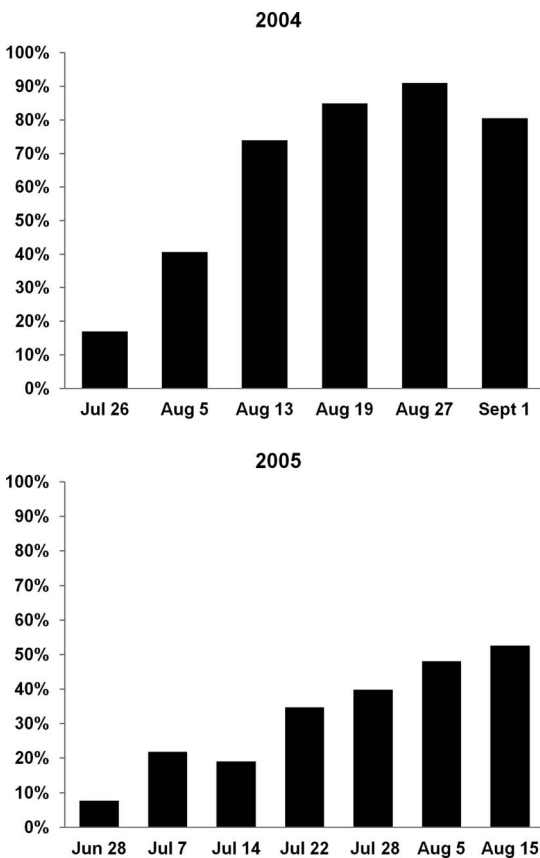
For the four most abundant natural enemy groups (arachnids, *O. insidiosus*, chrysopids, and lady beetles), numbers of arachnids and coccinellid larvae were positively correlated with soybean aphid levels each year, and the number of chrysopid eggs was positively correlated with aphid density in 2005 (Table 2). Numbers of *O. insidiosus* were not significantly correlated with aphid density. The overall number of natural enemies was also positively correlated with aphid levels in each year (Table 2; Fig. 4; aphid counts in 2005 converted to ratings for consistency). The proportion of plants with natural enemies varied with aphid-infestation rating ($F = 172.39$; df = 4, 4; $P < 0.001$) but not year ($F = 0.37$; df = 1, 4; $P = 0.574$), and for both years combined, the proportion increased significantly (0.09, 0.32, 0.51, 0.74, and 0.86) with respective aphid ratings (Fig. 4).

Fig. 3. Proportion of lady beetles (all stages) among all foliar, arthropod natural enemies in aphid-infested soybean plots in 2004 and 2005.

Table 2. Correlation coefficients (r) for soybean aphid density per soybean and corresponding density of natural enemies

Type of natural enemy	2004	2005
Arachnida: Araneae and Opiolones	0.074*	0.132*
Hemiptera: Anthocoridae, *O. insidiosus*	0.167	−0.003
Neuroptera: Chrysoperlid eggs	0.366	0.088*
Coleoptera: Coccinellid larvae	0.605*	0.245*
All arthropod natural enemies	0.528*	0.086*

Asterisk indicates significant correlation ($P < 0.05$).

Cage Treatments. The number of soybean aphids differed among cage treatments in 2004 (cage: $F = 9.26$; df = 3, 18; $P = 0.005$; block: $F = 1.70$; df = 6, 18; $P = 0.18$) and 2005 ($F = 11.93$; df = 3, 26; $P < 0.0001$; block: $F =$ 1.63; df = 9, 26; $P = 0.16$). In 2004, noncaged plants had fewer soybean aphids than caged plants, and the number of soybean aphids did not differ among cage treatments (Fig. 5). In 2005, numbers of soybean aphids were greater in total-exclusion cages than open or no-cage treatments, and aphid numbers in open cage treatments did not differ from those on noncaged plants and ones with partial-exclusion cages. Ambient temperature did not differ among cage treatments in 2004 (= 22.6 ± 0.3°C; $F = 1.24$; df = 3, 4; $P = 0.40$; block: $F = 0.73$; df = 2, 9; $P = 0.54$) and 2005 (= 23.7 ± 0.1°C; $F = 3.54$; df = 3, 9; $P = 0.06$; block: $F = 1.89$; df = 3, 9; $P = 0.20$).

Ants and Soybean-Aphid Population Growth. The mean number of soybean aphids per plant (±SE) did
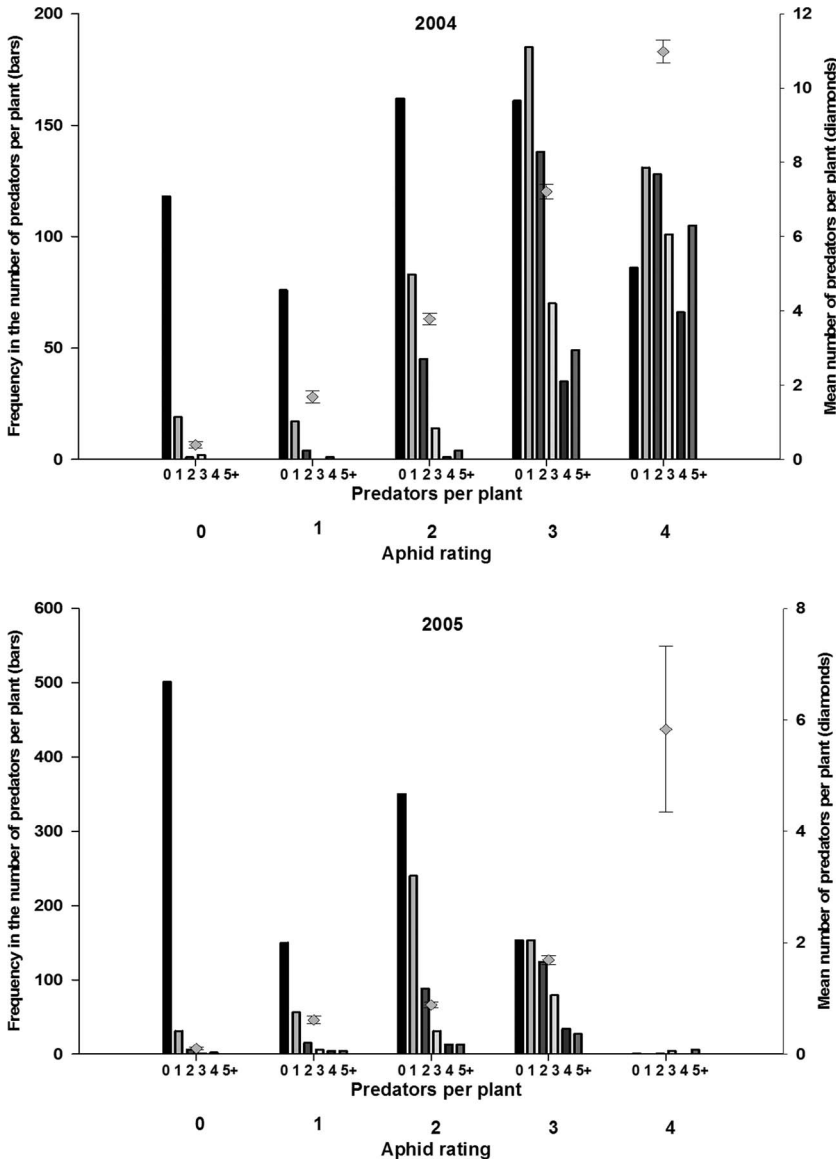

Fig. 4. Frequency in the number of predators observed on soybean plants (bars) and the mean number of predators per plant (diamonds, ± SEM) across aphid-infestation ratings in 2004 and 2005.
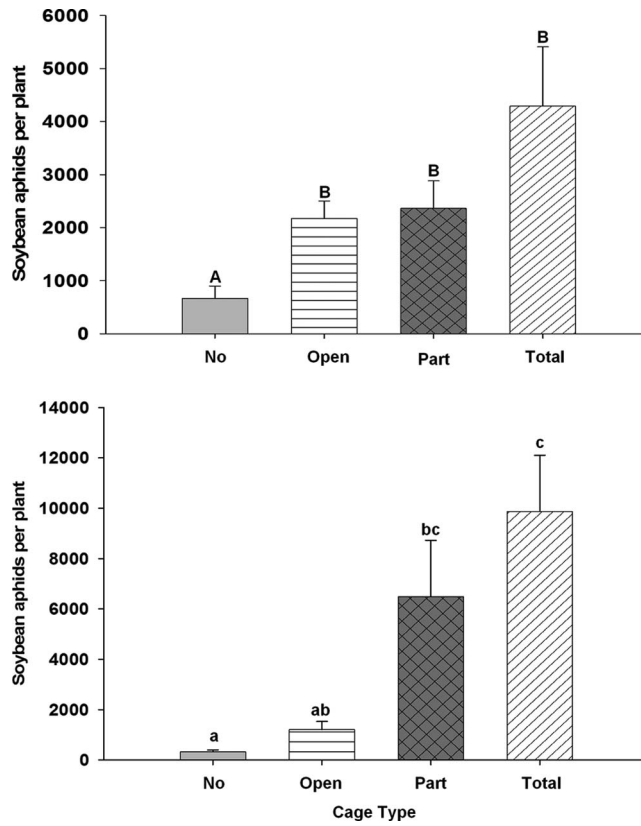
Fig. 5. The number of *A. glycines* per plant among treatments of no cage ("No"), open cage with unscreened windows ("Open"), cage with screened windows to exclude larger arthropod natural enemies ("Part"), and cage that excludes all arthropod natural enemies ("Total"). For each year, bars accompanied by same letter do not differ statistically ($\alpha = 0.05$, Tukey HSD test).

not differ between adhesive-treated and nontreated plants across years (908.6 ± 271.6 and 729.3 ± 219.7, respectively; $F = 3.56$; df = 1,24.78; $P = 0.310$). Variance in counts among years was >0 ($\chi^2 = 28.93$; $P < 0.001$). Thus, although respective means (±SE) between ant-accessible and ant-excluded treatments were comparable each year, they were nearly an order of magnitude higher in 2006 (2109.2 ± 315.6 vs. 1624.6 ± 350.5) than in 2004 (264.3 ± 65.8 vs. 296.8 ± 28.6) and 2005 (223.4 ± 92.4 vs. 180.0 ± 49.5). Ants were not found on ant-excluded plants, but were observed, though not quantified, in low numbers on ant-accessible plants (≪20 per plant).

During sampling of soybean plants for natural enemies, various undetermined species of ants were occasionally seen on soybean plants associating with soybean aphids, often grooming them or actively foraging for honeydew. However, the frequency was low, as one or more ants were recorded on only 188 of 1800 plants (10.4%) sampled in 2004, although the frequency of ant occupancy on the 188 plants was linearly correlated with aphid-infestation rating ($r = 0.90$; $P = 0.038$). *Lasius neoniger* Emery was by far the predominant ant observed in association with soybean aphids in both years, although identity and proportions of other ant species were not determined. Nonetheless,

ants were generally sparse (<20 ants per plant), even when aphid densities reached several hundred per plant or greater. Furthermore, relatively few direct encounters were observed between ants and predatory arthropods during the extensive periods of sampling natural enemies. These encounters seldom appeared antagonistic to natural enemies, and were mainly brief, nonaggressive encounters when paths of ants and predators coincided, except for a few instances in which ants were found entangled in spider webs.

Discussion

Natural Enemies of Soybean Aphid. A variety of methods is available to sample natural enemies in soybeans (Schmidt et al. 2008), and each has its own set of tradeoffs. Of the various methods, this study used direct field counts of aphid natural enemies on individual soybean plants. This method samples foliar natural enemies that are typically in closest proximity to and in many cases even actively feeding on soybean aphids. A main disadvantage is missing agile natural enemies that flee before they can be sampled. Alternative sampling methods include sweepnetting and yellow sticky cards. Each is more efficient at capturing

agile natural enemies, but sweepnetting biases samples to insects in the upper plant canopy and sticky traps typically do not capture less mobile or inactive stages that are on plants (Schmidt et al. 2008).

The types of arthropod natural enemies found in this study were generally comparable with that reported for other South Dakota field crops, although their proportions were not necessarily similar. For instance, composition of arthropod natural enemies in soybean was comparable with that found in sunflower fields of eastern South Dakota (Royer and Walgenbach 1991), and five coccinellid species found in my study (*C. maculata lengi, Hi. tredecimpunctata tibialis, Hi. convergens, Co. septempunctata* L., and *H. axyridis* Pallas) had been found previously in South Dakota soybean fields and are common in other major field crops there (Elliott et al. 1996, Hesler and Kieckhefer 2008). However, composition of aphidophagous natural enemies found in soybean contrasts with that of alfalfa, another major legume crop in South Dakota (NASS 2004). In a 13-yr survey of aphidophagous insects of alfalfa in eastern South Dakota, *N. americoferus* composed >40% of predators, and lady beetles and lacewings made up the remainder (Elliott and Kieckhefer 1990). Given this, a greater proportion of *N. americoferus* was expected in soybean, but it represented a small percentage of natural enemies in my study. Similarly, few nabids were found in Nebraska soybean fields (Brosius et al. 2007). However, *N. americoferus* is not strictly aphidophagous, but also preys on weevil larvae, caterpillars, and leafhoppers (Lattin 1989), which all readily occur in alfalfa. Lack of significant alternative prey may explain low proportions of *N. americoferus* in soybean relative to alfalfa. Regardless of differences in relative abundance among crops, soybean and other field crops in eastern South Dakota share several natural enemies, and this suggests that management practices within an agroecological landscape could potentially affect the pool of natural enemies available to colonize various crops, such as soybean. Indeed, Landis et al. (2008) found that biological control services to soybean decline significantly with increasing proportion of corn in the landscape, and Noma et al. (2010) found that habitats dominated by corn and soybean were associated with higher populations of soybean aphid.

The taxa and proportion of natural enemies in my study share similarities and dissimilarities to that reported in mid- to late season soybean for other regions of North America where soybean aphid is a pest. In several studies, coccinellids are a dominant natural enemy group (Fox et al. 2004, Rutledge et al. 2004, Nielsen and Hajek 2005, Mignault et al. 2006, Donaldson et al. 2007, Rhainds et al. 2007, Meihls et al. 2010), and Meihls et al. (2010) also found *O. insidiosus* to be codominant with coccinellids in Missouri soybean fields. In contrast, Brosius et al. (2007) found relatively low numbers of coccinellids and relatively high numbers of *O. insidiosus* in Nebraska soybean, and Desneux et al. (2006) found that *O. insidiosus* composed from 85 to 90% of predators found in Indiana soybean. Likewise, Noma et al. (2010) found that

predatory bugs (principally *O. insidiosus*) were the most abundant type of aphid predators in soybean among four north central states, and they were >4 times more abundant than lady beetles, the second most abundant natural enemy group. Costamagna and Landis (2007) also found *O. insidiosus* was the most abundant predator and had the highest consumption rate of soybean aphid, but found coccinellids, which were the second most abundant predator group, had greater per capita aphid consumption than *O. insidiosus.* It is unclear why coccinellids were particularly abundant in several studies, including my own (Fig. 3), whereas *O. insidiosus* was more abundant in other studies. *O. insidiosus* preys preferentially on soybean thrips (*Seriothrips variabilis* Beach; Thysanoptera: Thripidae; Butler and O'Neil 2008, Harwood et al. 2009). Thus, levels of this alternative prey may influence abundance of *O. insidiosus* and its likelihood to suppress soybean aphid populations (Butler and O'Neil 2008, Desneux and O'Neil 2008).

In my study, arachnids and chrysopids were relatively abundant, especially in 2005 for chrysopid eggs. Similarly, spiders and chrysopids were two of five dominant aphidophagous taxa in aphid-infested soybean fields in Iowa (Schmidt et al. 2008) and South Dakota (Seagraves and Lundgren 2012, Lundgren et al. 2013), and a diverse assemblage of arachnids was collected from pitfall traps throughout soybean fields in the upper Midwest (Gardiner et al. 2010). Despite abundance of chrysopid eggs, few larvae were found in my samples. This might have been because chrysopids are subject to intraguild predation in soybean fields (Gardiner et al. 2007), and this may have decreased their relative larval abundance in my samples.

Foliar, arthropod natural enemies in my study were generally analogous to those found in China, where soybean aphid is native (Liu et al. 2004, 2012; Miao et al. 2007). As Miao et al. (2007) noted, the same families and usually same genera of predators are associated with soybean aphid in both China and North America, but parasitoids of the aphid are rare in North America compared with China. Parasitoids were uncommon in my study, and they have generally been rare in other inventories of aphid natural enemies in North American soybean fields (Nielsen and Hajek 2005, Desneux et al. 2006, Donaldson et al. 2007, Kaiser et al. 2007, Costamagna et al. 2008, Noma and Brewer 2008).

Signs of soybean aphids infected by entomopathogens were uncommon in my study. Most other North American studies have typically found very low levels of entomopathogenic infection in soybean aphid populations (Fox et al. 2004, Desneux et al. 2006), although Nielsen and Hajek (2005) found mycoses from entomopathogenic fungi that were strongly associated with aphid density, including epizootic levels of infection that were associated with aphid declines in New York state.

Arachnids, chrysoperlid eggs, coccinellid larvae, and the overall numbers of natural enemies were positively correlated with soybean aphid levels in my study (Table 2). Although this indicated that these

natural enemies tracked soybean aphids, no natural enemies were sampled on many aphid-infested plants, even some with >1000 aphids (Fig. 4). This suggests that soybean aphid was underexploited in my field plots, and that the natural enemy guild could be enhanced to improve biological control of soybean aphid.

Cage Treatments. Cage treatments were used in one of my experiments to exclude arthropod natural enemies from aphid-infested soybean plants to various degrees. Caged plants generally had more soybean aphids than noncaged plants, except in 2005 when aphid densities did not differ between open cage and noncaged plants. The lack of differences in aphid densities between partial and total exclusion cages suggests that parasitoids and very small predators did not impact soybean aphid populations in either year, and it is consistent with the very low level of mummified aphids observed in my study. In a 2-yr study, Brosius et al. (2007) found more soybean aphids per plant in total exclusion cages than other cage treatments, and more soybean aphids per plant in partial exclusion cages than in no-cage treatments.

Three hypotheses (Liu et al. 2004) may possibly explain greater aphid numbers on caged versus noncaged plants observed in my study: 1) differences in microclimate affected aphid growth rates between caged and noncaged plants; 2) caged plants reduced aphid emigration; and 3) natural enemies had differential access to caged and noncaged plants, and this resulted in differential mortality among treatments. In my study, temperature may be ruled out as a microclimate factor because it did not differ among cage (and noncage) treatments. Similarly, some other studies have found no differences in mean temperature of soybean canopy among cage treatments (Fox et al. 2004, Brosius et al. 2007, Liu et al. 2012). Meihls et al. (2010) found relatively small differences in temperatures between cage treatments, and concluded that cage environment had minimal effect on soybean aphid populations. Cages may have also protected aphids from microclimatic factors such as rain and consequent splashing of soil onto leaf surfaces (Hand and Keaster 1967, Mann et al. 1995). Indeed, a 4.5-cm rain occurred 13 d after initial caging in 2004, and rains of 2.1 and 2.9 cm fell 3 and 8 d after caging in 2005. Nonetheless, the effects of the rain may have been minimal, as I have observed splashed soil does not reach younger leaves colonized by the majority of soybean aphids on V3 and older plants as used in my study.

The cage study results suggest natural enemies were not a significant factor in 2004, but that in 2005 predators and perhaps emigration played a role in reducing densities, and that either or both of these factors in combination with small natural enemies had the greatest reduction in aphid densities, as seen with no-caging. Aphid emigration, if operative, would have been expected to produce differences in residual aphid levels between total exclusion cages and the open and partial cages, and higher rates of emigration might have been expected from open compared with partial

exclusion cages. However, aphid numbers did not differ among cage types in 2004. Aphid levels were roughly twofold greater on plants in the partial- and total-exclusion cages in 2005 than in 2004. Aphid density on open-cage plants in 2005 was lower than that on plants totally excluded from natural enemies but did not differ from that on noncaged plants. Thus, emigration may have been operative under higher mean densities in 2005 that exceeded 6,000 aphids per plant. Indeed, Donaldson et al. (2007) found that significant emigration occurred only when densities of soybean aphid were >4,000 aphids per plant. Other studies have suggested that lack of emigration has a minimal influence on differences in densities of soybean aphid among cage treatments (Rhainds et al. 2007, Costamagna et al. 2008).

It is unclear why the open cage treatment lowered aphid densities in 2005 and not 2004, but one or more of the following factors peculiar to 2005 may have been responsible for the effect. These factors include suppression by a relatively larger proportion of arachnids, reduced interference of predation due to proportionally lower numbers of lady beetles (Gardiner et al. 2007), and a density-dependent response of natural enemies to the higher levels of aphids in cages in 2005. Spiders and harvestmen collectively made up a greater proportion and lady beetles composed a relatively smaller proportion of foliar natural enemies in 2005 than in 2004, whereas proportions of the active feeding stages of other arthropod natural enemies were either insignificant or similar between years. Arachnid abundance was weakly, but significantly and positively associated with soybean aphid density in both years of my study, but with slightly stronger association in 2005 (Table 2).

My exclusion cage results agree in part with those of other studies. As in my study, other cage studies have shown that total exclusion of predators increases soybean aphid levels compared with no-cage treatments (Brosius et al. 2007, Miao et al. 2007, Costamagna et al. 2008, Meihls et al. 2010). Other studies have shown marked reductions in aphid levels within cages that allow access by aphid predators (Brosius et al. 2007, Miao et al. 2007, Costamagna et al. 2008, Meihls et al. 2010), whereas this was true only for open cage treatments in 2005 in my study. Some other studies have also shown no to modest reductions in aphid levels within cages that allow access only by small predators (Brosius et al. 2007, Miao et al. 2007, Costamagna et al. 2008, Meihls et al. 2010), and my study showed no effect in cages accessible only to small natural enemies. For experiments in which aphid levels declined in cages accessible only to small predators, complementary noncage experiments in the same study often found that smaller natural enemies such as anthocorids and parasitoids were associated with decreased numbers of soybean aphids (Brosius et al. 2007, Miao et al. 2007), whereas my study showed no comparable association involving small natural enemies (Table 2).

Ants and Soybean-Aphid Population Growth. Ants were occasionally seen on soybean plants grooming

and collecting honeydew excreted by soybean aphids. Although a varied but undetermined number of ant species was observed in my study, *L. neoniger* was predominant. Even though ant occupancy of soybean plants correlated with aphid density ratings, only a few individual ants were typically observed per soybean plant in 2004, even when soybean aphid densities reached or exceeded several hundred per plant. Likewise, Wyckhus et al. (2009) found only one ant-tended colony of soybean aphid, and that by *L. neoniger*, over multiple dates in soybean fields from four regions of Minnesota, whereas Herbert and Horn (2008) found ants (principally *Monomorium minimum* Buckley) commonly tending soybean aphids in Ohio.

I found no differences in population levels of soybean aphids between ant-excluded and ant-accessible plants in my soybean plots, and observed almost no antagonistic interactions between ants and natural enemies of soybean aphid. These results were largely owing to the low numbers and frequency of ants on plants. These results contrast with laboratory results in which the ant, *M. minimum*, attending soybean aphid populations was observed harassing or killing *O. insidiosus* and *H. axyridis*, and ant attendance reduced predation and led to 10-fold increase in soybean aphid numbers (Herbert and Horn 2008). The lack of effect by ants on soybean aphid populations in my 2-wk exclusion experiment and the low frequency of ant occupancy on soybean plants may suggest that the *L. neoniger*-dominated ant fauna in this study had no measurable effect on population levels of soybean aphids. Given the relatively short time period in which resident ants and *A. glycines* have coexisted in North America, some ant species, such as *L. neoniger*, may not yet have adapted protective behavior toward soybean aphids that would be detected in a short-term study like mine. Alternatively, ant–aphid mutualisms alter other aphid demographic measures besides population numbers (Flatt and Weisser 2000), and thus ants might have had other effects on *A. glycines* that I did not measure. For instance, Schwartzberg et al. (2010) found no differences in numbers of soybean aphids between *L. niger*-accessible and *L. niger*-excluded soybean plants in open cages over 16 d, but they found a twofold increase in aphid biomass when ants tended aphids.

Summary. A diverse assemblage of arthropod natural enemies was associated with soybean aphid in eastern South Dakota. Excluding these natural enemies from soybean plants through caging caused >4-fold increases in soybean aphid densities, and large insect predators were important in suppressing aphid populations in one of two years. Nevertheless, no natural enemies were found on many aphid-infested soybean plants. Furthermore, the assemblage had low numbers of some aphidophagous taxa, such as damsel bugs and parasitoids, and no meaningful levels of entomopathogens. These latter results suggest that the soybean-aphid natural enemy guild is unsaturated and that it could be enhanced to boost biological control of soybean aphid.

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