Effect of Host Plant Resistance and Seed Treatments on Soybean Aphids (Hemiptera: Aphididae) and Their Natural Enemies[1]

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Abstract The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a serious pest of soybean in the North Central region of the United States. Management tools include resistant varieties and insecticidal seed treatments, used alone or in combination, which may have variable effects on different pest or natural enemy species. In this 1-yr field study we examined the response of soybean aphids and natural enemy abundance to aphid-resistant *Rag1* soybeans, with and without thiamethoxam seed treatment. *Rag1* resistance, thiamethoxam seed treatment, and both together, significantly reduced cumulative aphid days and increased yield compared to the untreated susceptible control, though there was no interaction of these factors. Peak aphid density in the control was 799.4 ± 174.8 aphids/plant. In *Rag1* resistant soybeans, peak aphid density (312.2 ± 121.3 aphids/plant) was above the economic threshold but below the economic injury level, whereas in susceptible soybeans with seed treatment, the peak aphid population (659 ± 164.9 aphids/plant) exceeded the economic threshold and approached the economic injury level. Yield and cumulative aphid days in resistant soybeans and seed-treated soybeans did not differ. The fewest cumulative aphid-days and highest yield were obtained from seed-treated resistant soybeans, where peak aphid density was 39.2 ± 9.2 aphids/plant. Natural enemy abundance was positively correlated with aphid abundance. *Rag1* resistant soybeans and thiamethoxam-treated soybeans were found to have lower natural enemy populations than were present in the control. *Harmonia axyridis* Pallas and *Orius insidiosus* Say were the dominant natural enemies in the study. These results are from one season of study in this system and should be interpreted in that light.

Key Words *Aphis glycines* Matsumura, *Rag1*, thiamethoxam, *Harmonia axyridis* Pallas, *Orius insidiosus* Say

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), has been the principal insect pest of soybean in the North Central region of the United States since its discovery in North America (Gardiner and Landis 2007, Ragsdale et al. 2011, Tilmon et al. 2011). It was first detected in Wisconsin in July 2000 (Alleman et al. 2002) and quickly spread throughout the northern soybean-production areas of North America (Ragsdale et al. 2011, Venette and Ragsdale 2004). The soybean

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aphid can cause severe economic loss and degrade seed quality through its feeding (Beckendorf et al. 2008, Ragsdale et al. 2007).

The soybean aphid has caused many changes in soybean pest management practices in the United States (Ragsdale et al. 2011). Among these changes are increased use of foliar pesticides such as pyrethroids and organophosphates (Johnson et al. 2009), development of aphid-resistant varieties, and increasing use of insecticidal seed treatments at planting (Ragsdale et al. 2011). These tactics are increasingly used in combination, but the impact on different pest or natural enemy species in the system is not always well understood.

Host plant resistance in soybean is an important tool in integrated pest management. In soybean, several resistant genes—*Rag1*, *Rag2*, *Rag3*, and *rag4* —have been identified (Hill et al. 2004; Mian et al. 2008a, b; Zhang et al. 2009, 2010), with eight different resistance genes proposed to date that provide resistance to the soybean aphid (Bhusal et al. 2013, Hesler et al. 2013, Hill et al. 2012). Antibiosis and antixenosis resistance is conferred by the *Rag1* gene (Hill et al. 2006a, b), antibiosis by the *Rag2* gene (Kang et al. 2008, Mian et al. 2008b), antixenosis by the *Rag3* gene (Mensah et al. 2005, Zhang et al. 2010), and antibiosis resistance by the *rag4* gene (Zhang et al. 2010). Hesler et al. (2013) documented the suppression of aphid populations in different parts of the North Central region by lines containing any of the *Rag* genes. Of the 18 commercially available aphid-resistant soybean lines in the Midwest through 2012, 17 of these lines contain the *Rag1* gene (McCarville et al. 2012).

There is a need to determine the relationship between aphid-resistant soybeans and natural enemies that prey upon soybean aphids in those habitats. In North America, a broad complex of natural enemies attack soybean aphids. Although several parasitoid species in the families Braconidae and Aphelinidae and a few entomopathogens are found (Brewer and Noma 2010, Kaiser et al. 2007, Nielsen and Hajek 2005), generalist predators (mainly the coccinellids *Harmonia axyridis* Pallas, *Coccinella septempunctata* L., and the anthocorid *Orius insidiosus* Say) are numerically dominant, and appear to play the greatest role in soybean aphid suppression (Costamagna and Landis 2006; Costamagna et al. 2007; Fox et al. 2004, 2005; Gardiner et al. 2009; Rutledge et al. 2004).

Host plant resistance can facilitate or interfere with the action of natural enemies (Boethel and Eikenbary 1986). If the resistance keeps pest densities lower than in susceptible varieties, then natural enemies may be more likely to suppress the pests further (Starks et al. 1972, van Emden and Wearing 1965). In one microplot study conducted by McCarville and O'Neal (2012), a combination of host plant resistance and biological control reduced the soybean aphid population by 89.1% compared to the susceptible control free from natural enemies. Cai et al. (2009) found that host plant resistance enhanced parasitism by the parasitoid *Aphidius* spp. on *Sitobian avenae* (F.) in wheat in China. Conversely, morphological and physiological traits of the plant hosts, such as trichome structure or density, leaf-surface texture or waxiness, and allelochemicals could directly interfere with predators and parasitoids (Kauffman and Kennedy 1989a, b; Kennedy 2003; Lundgren et al. 2009a). Also, reduced quality and availability of aphids due to resistant plants could have an adverse impact on natural enemies (Chacon et al. 2012, Kauffman and Flanders 1985, Lundgren et al. 2009a, van Emden 1995).

Treating seed with insecticides designed to function systemically in the plant is another pest management approach. Neonicotinoids are popular insecticides having systemic action and can be highly effective against piercing–sucking insects (Tomizawa and Casida 2003, 2005). Thiamethoxam is a neonicotinoid insecticide commonly used for soybean seed treatment, which may affect different insect species in different ways. Thiamethoxam is absorbed by roots and translocated throughout the plant by the xylem, encountering insects wherever they feed (Tomizawa and Casida 2005, O'Neal and Johnson 2010). Though it is frequently marketed for management of soybean aphid, its efficacy for this purpose remains under investigation. The fact that insecticidal seed treatments tend to decrease in concentration at a certain period after planting and germination could be problematic for managing a pest such as soybean aphid, which often does not reach a population peak until late July or August (i.e., 2 or 3 mo after planting). In a study in Iowa, McCarville and O'Neal (2013) observed thiamethoxam seed treatment affected aphids until 42 d after planting, after which thiamethoxam toxicity no longer suppressed aphid population growth. However, in a study in Nebraska, Megalhaes et al. (2009) found that thiamethoxam seed treatment maintained aphid population below the economic threshold throughout the season and that treated soybeans had higher yield than untreated soybeans.

Thus, inconsistent results are obtained in studies on insecticidal seed treatment used for soybean aphid suppression. It may be necessary to evaluate seed treatments in relation to the particular environment, seasonality, and the crop and insect phenology of a given region. In addition, seed treatments are sometimes sold in combination with aphid-resistance traits. Little independent information exists on whether this combination provides good value for producers. Furthermore, there is a need to assess the impact of insecticidal seed treatments, alone or in combination with resistance traits, on natural enemies, which are important in the soybean system. Koch and Ragsdale (2011) did not find an adverse impact of thiamethoxam seed treatment on a fungal pathogen (*Pandora neophidis* Remaudiere and Hennebert) of soybean aphid. Moser and Obrycki (2009) found higher mortality of *H. axyridis* larvae on corn seedlings germinated from seeds treated with neonicotinoid insecticides compared to non–seed-treated corn seedlings.

The objective of this study was to study how an aphid-resistant *Rag1* soybean line, either alone or in combination with thiamethoxam seed treatment, affect changes in soybean aphid populations. The second objective of the study was to survey and determine the impact of aphid-resistant soybean and thiamethoxam seed treatment on natural enemies. Understanding how factors such as host plant resistance and seed treatments affect soybean aphid and also natural enemy populations is important to develop sustainable integrated pest management strategies in the soybean system.

Materials and Methods

The experiment was conducted during the summer of 2011 on the South Dakota State University Plant Science Research Farm located in Volga, SD. This farm is located 11.2 km west of the main campus of South Dakota State University in

Brookings at 44.30022°N latitude and 96.92592°W longitude, near Caspian Road (465th Avenue).

This study had four treatments in a two by two factorial design. The factors were soybean genotype (an aphid-resistant or a susceptible variety) and insecticidal seed treatment (present or absent). The aphid-susceptible variety was 'SD 76R', which is the breeding parent of the aphid-resistant soybean variety used in this study. The aphid-resistant soybean variety was the breeding line 'LD (05) 16137′ (a near-isoline of SD 76R), containing the aphid resistance gene *Rag1*. Thus, resistant and susceptible varieties had very similar genetic backgrounds. Both soybean varieties were Roundup Ready and from maturity group I.

The insecticidal seed treatment used was thiamethoxam (Cruiser 5 FS, Syngenta Crop Protection, Greensboro, NC), applied at the rate of 0.0756 mg active ingredient (thiamethoxam) per soybean seed, the standard commercial rate for this product. This insecticidal seed treatment is often sold commercially in combination with fungicidal seed treatments; however, for this study seed was custom-treated with thiamethoxam only.

Each treatment was replicated four times and planted in a randomized complete block design. Plots were 30.5 m by 30.5 m with 3.05-m bare borders maintained around each to minimize edge effects from other treatments. Soybeans were planted on 19 May, 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.

Soybean aphid sampling. The abundance of soybean aphids (*A. glycines*) was monitored at weekly intervals in each treatment from 7 June through 24 August. On 7 June plants were at the early unifoliate stage and by 24 of August, they were at the R5/R6 growth stage. Initially, 20 plants per plot were randomly selected each week for nondestructive aphid sampling; however, when $>$80% of the plants were infested with aphids (2 August), the number of plants per plot sampled was reduced to 10.

At lower aphid densities of 100 per plant or fewer, aphids were counted with high precision. At aphid densities from 100 to 500 per plant, aphid numbers were visually estimated in units of 100. Above 500 aphids per plant, numbers were visually estimated in units of 500. All parts of the plant (i.e., all foliage, stems, branches, pods, etc.) were inspected and both adult and immature aphids were counted. On each sampling date, the growth stages of the first three randomly selected plants per plot were also recorded.

Natural enemy sampling. The diversity and abundance of natural enemies in each treatment were assessed through two sampling techniques—visual inspection of plants and yellow sticky traps.

Visual counts of natural enemies were made from the first week of July through the first week of September. Ten plants were randomly selected throughout each plot excluding border rows. First, the plant was visually inspected without disturbing it, to count the mobile natural enemies. Then, the plants were gently manipulated to allow inspection of all parts of the plants. In order to increase counting efficiency, certain natural enemies were targeted for counting and recording: lady beetles (Coccinellidae) identified to species, *Oriu*s spp. (Anthocoridae), aphid parasitoid mummies (Braconidae/Aphelinidae), and syrphid flies (Syrphidae). Life history stages of these taxa were also recorded.

Sticky-card sampling was conducted weekly from the first week of July through the first week of September. Cards (Scentry® Multigard® unbaited AM trap, Gempler's, Janesville, WI) were fluorescent yellow and measured 27.9 cm by 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. As the plants grew taller, the sticky cards were placed higher on the wooden posts to avoid contact with plant foliage. Sticky cards were replaced each week, and old cards were wrapped in Reynolds Wrap® nonstick aluminum foil (Reynolds Consumer Products, Richmond, VA) with the sticky side of the card in contact with the no-stick side of the foil. Cards were labeled and stored in plastic Ziploc® bags (Johnson and Son, Racine, WI) in a −20°C freezer in the laboratory until natural enemies could be counted and identified under a dissecting microscope.

Yield. Soybeans were harvested with a combine on 10 October, and grain yield data were taken from the entire center two rows of each plot and adjusted to 13% moisture.

Statistical analysis. All count data (soybean aphids, individual natural enemy taxa, and total natural enemies), were log-transformed to satisfy the assumptions of normality. Then, transformed count data and soybean grain yield data were analyzed using PROC MIXED (SAS Institute 2008). The models included both fixed and random effects. In addition, the data were repeated measures, so we examined different variance–covariance structures. Based on the values of Akaike information criterion (AIC) and Bayesian information criterion (BIC), we determined the best model for the data analysis. The smaller the values of both AIC and BIC, the better the model. Treatments were compared at the 0.05 level of significance, using the least significant difference (LSD) test.

Pairwise correlation tests, PROC CORR (SAS Institute 2008), were conducted to test association of soybean aphid with natural enemies. Similarly, the same measure of correlation was used to test the relationship of soybean grain yield with each of the season-summed abundances of soybean aphid (cumulative aphid days). Cumulative aphid days were calculated by summing the aphid days accumulated during the growing season, using the following formula:

Aphid days = (mean aphids/plant at previous date + mean aphids/plant at current date)/2.

Results

Aphid abundance. Four treatments were tested for natural aphid colonization and population growth in the field: aphid-resistant (*Rag1*) and -susceptible soybean lines, with and without thiamethoxam seed treatment. Aphid population densities in the four treatments were low until the second week of July, but then began to increase (Fig. 1). Peak aphid populations were observed in the fourth week of August, after which no further aphid data were taken. The peak recorded aphid populations in the four treatments were 799.4 ± 174.8 aphids/plant in the susceptible control, 659 ± 164.9 aphids/plant in susceptible soybeans with seed treatment, 312.2 ± 121.3 aphids/plant in resistant soybeans, and 39.2 ± 9.2 aphids/plant in resistant soybeans with seed treatment (Fig. 1). These relative values are also reflected in the mean cumulative aphid days accrued in each

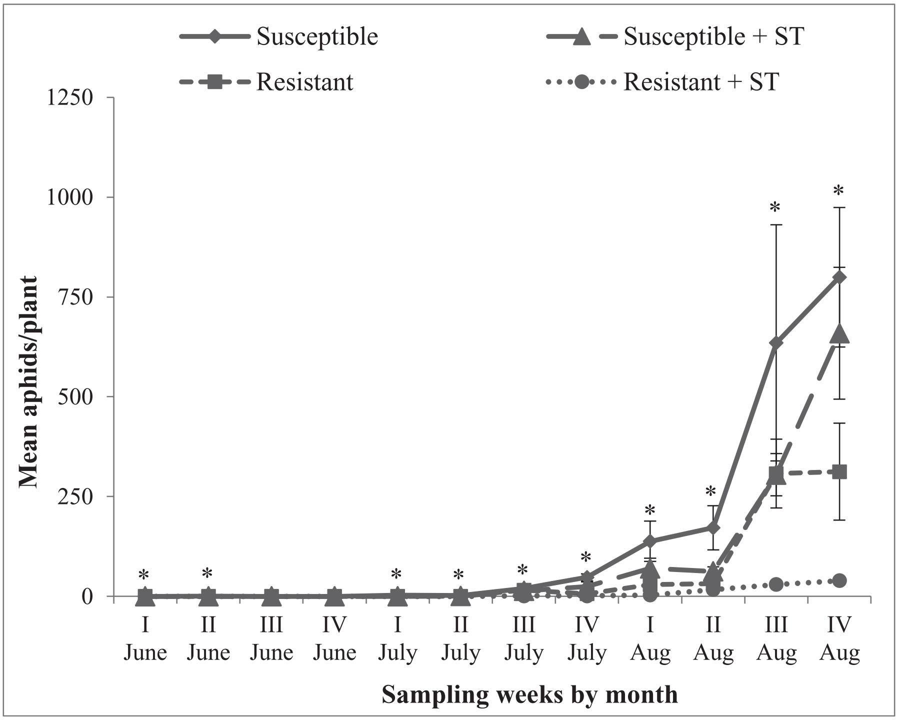

Fig. 1. Soybean aphid population growth in four treatments of soybean in the field (Volga, SD, 2011): aphid-susceptible and aphid-resistant (*Rag1*) isolines, with or without thiamethoxam seed treatment (ST). Error bars are standard errors of means. An asterisk (*) sign above the error bars indicates a significant difference among treatments on a given date ($P < 0.05$, least significant difference).

treatment (Fig. 2). Aphid populations exceeded economic threshold (250 aphids/plant; Ragsdale et al. 2007) during the third week of August in each treatment except in resistant, seed-treated soybeans, on which aphid densities were below threshold throughout the season (Fig. 1). Peak aphid populations exceeded the economic injury level (674 aphids/plant; Ragsdale et al. 2007) in the fourth week of August in the control, and nearly so in the seed-treated soybeans.

Fewer soybean aphids occurred on resistant than on susceptible soybean plants ($F = 122.58$; df = 1, 9; $P < 0.0001$), and also on seed-treated soybeans compared to soybeans without thiamethoxam seed treatment ($F = 65.65$; df = 1, 9; $P < 0.0001$). There was no interaction of variety (i.e., resistant versus susceptible) and seed treatment with respect to soybean aphid abundance. Soybean aphid densities varied by date ($F = 204.63$; df = 11, 132; $P < 0.0001$), and date interacted with both variety ($F = 2.6$; df = 11, 132; $P = 0.005$) and seed treatment ($F = 2.19$; df = 11, 132; $P = 0.018$). There was also a three-way interaction of these factors ($F = 3.17$; df = 11, 132; $P = 0.0008$). Significant variety effects were observed on 10 sampling

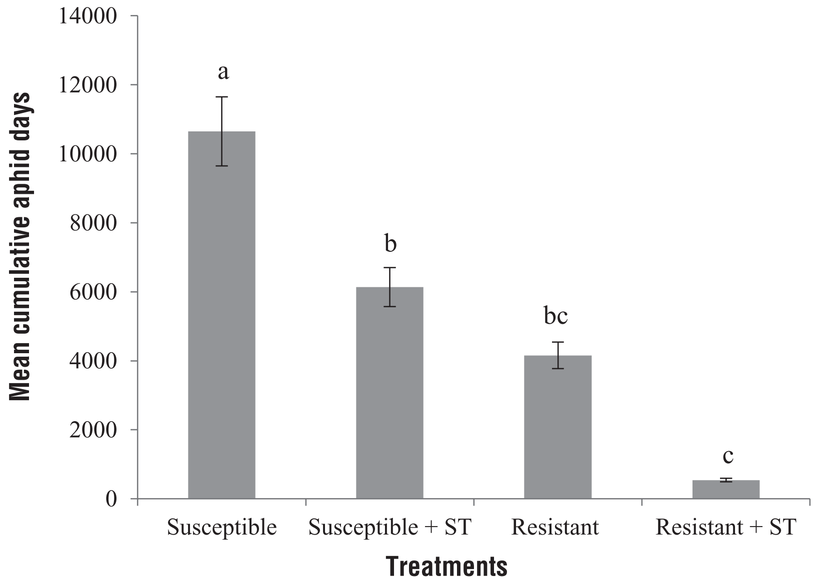

Fig. 2. Mean cumulative aphid-days in four treatments of soybean in the field (Volga, SD, 2011): aphid-susceptible and aphid-resistant (*Rag1*) isolines, with or without thiamethoxam seed treatment (ST). Error bars are standard errors of means. Bars with different letters are significantly different ($P < 0.05$, least significant difference).

dates, with lower aphid densities in resistant soybeans. Significant seed treatment effects were seen on nine sampling dates, with lower densities in seed treated soybeans compared to nontreated. Similarly, in an analysis of aphid pressure across the season (i.e., cumulative aphid days; Fig. 2), there were effects of both variety ($F = 22.04$; df= 1, 9; $P = 0.001$) and seed treatment ($F = 9.97$; df = 1, 9; $P = 0.01$). There were significantly fewer mean cumulative aphid days in *Rag1* resistant soybeans than in the susceptible soybeans, both with and without seed treatment (Fig. 2). There was no interaction between variety and seed treatment. This same pattern was seen during the overall aphid population peak at the end of August, when resistant soybeans with or without seed treatment had the lowest aphid populations (Fig. 1).

Yield and relationship to soybean aphid abundance. Soybean grain yield (Fig. 3) differed significantly by both variety ($F = 27.88$; df = 1, 9; $P = 0.0005$) and seed treatment ($F = 50.19$; df = 1, 9; $P < 0.0001$). There was no seed treatment by variety interaction. Both *Rag1* resistant and seed-treated susceptible soybeans produced significantly greater grain yield than the susceptible soybean control (at 13.5% and 19.1% greater, respectively), though they did not differ significantly from each other (Fig. 3). Highest yield was observed in *Rag1* resistant soybeans with seed treatment, with 38.1% greater yield than in the control. Soybean aphid

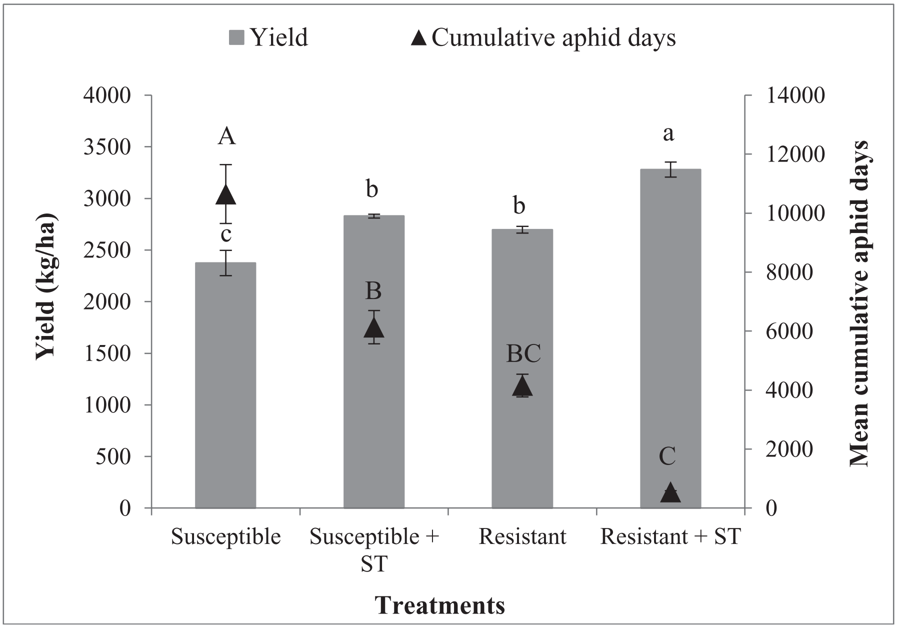

Fig. 3. Soybean grain yield and mean cumulative aphid days in four different treatments of soybean in the field (Volga, SD, 2011): aphid-susceptible and aphid-resistant (*Rag1*) isolines, with or without thiamethoxam seed treatment (ST). Error bars are standard errors of means. Yields are represented by bars and lowercase letters. Cumulative aphid days are represented by squares and capital letters. Error bars with different letters are significantly different ($P < 0.05$, least significant difference).

populations measured by cumulative aphid days were found to have a significant negative association with soybean grain yield ($P < 0.0001$, $R^2 = 0.67$; Fig. 4).

Natural enemy abundance in visual observation. Natural enemies were counted by visual inspection of plants in the field in each of the four treatments. All life stages of lady beetles (i.e., eggs, larvae, pupae, and adults), larvae of predatory flies and adults of syrphids, nymphs and adults of *Orius* spp. (the predators), and aphid mummies (the parasitoids) were recorded (Table 1). Generalist predators were the dominant natural enemies (84.4% of the total) found during the visual inspection of plants (1,287 counted throughout the season on 1,440 plants). Among them, sessile lady beetle eggs accounted for 10.3% of the predators observed, whereas postnatal (i.e., nonegg) predators (larvae, pupae, and adults of lady beetles; predatory fly larvae; adults of syrphid flies; and nymphs and adults of *Orius* spp.) accounted for 89.7% (1,155 out of 1,287 of total predators) (Table 1). Among them, coccinellids (i.e., lady beetles) were the most abundant group. Of the total postnatal predators observed (1,155), lady beetle adults, larvae, and pupae accounted for 17%, 25.8%, and 18.1%, respectively. Among adult lady beetles, *H. axyridis* was the most abundant, accounting for 46.2% of the total adult lady beetle

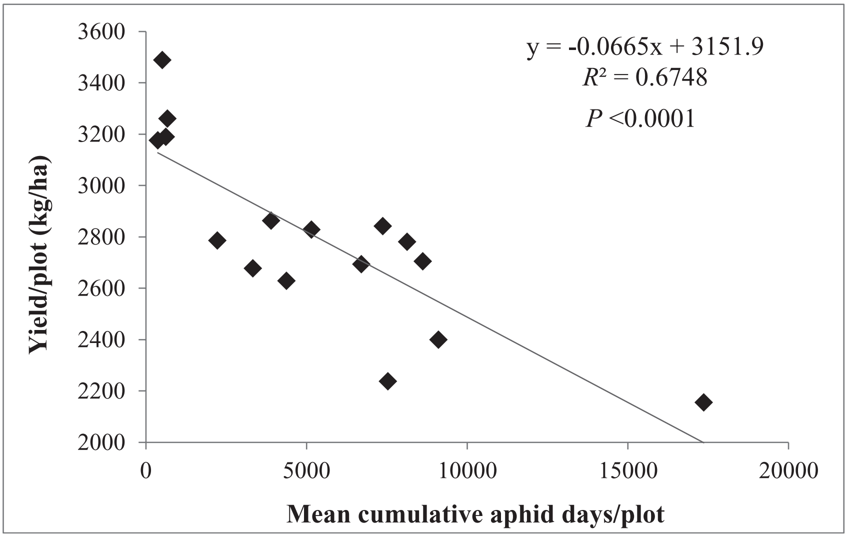

Fig. 4. Relationship of cumulative aphid-days with soybean grain yield across all treatments in the field (Volga, SD, 2011): aphid-susceptible and aphid-resistant (*Rag1*) isolines, with or without thiamethoxam seed treatment (ST).

observations. (Lady beetle larvae and pupae were not identified to species due to the limitations of visual counting in the field). After coccinellids, *Orius* spp. and predatory fly larvae accounted for 23.1% and 9.9% of the observed postnatal predators, respectively. Besides predators, 238 parasitoid aphid mummies were also detected out of the total 1,525 natural enemies observed in the field (15.6% of the total natural enemies).

A significant positive relationship was observed between the mean number of natural enemies per plant and mean number of soybean aphids per plant across all treatments ($P < 0.0001$; $R^2 = 0.44$; Fig. 5). Fig. 6a summarizes the abundance and trends of natural enemies across treatments throughout the soybean growing season in visual plant inspections. Across all treatments, there was initially a low abundance of natural enemies until the fourth week of July, at which point populations began to increase. They achieved their overall peak in the last day of sampling (the first week of September). Looking at the average natural enemies per plant in each treatment for the whole season, most of the factors analyzed were significant: variety ($F = 25.80$; df = 1, 12; $P = 0.003$), seed treatment ($F = 10.5$; df = 1, 12; $P = 0.0069$), date ($F = 54.6$; df = 8, 96; $P < 0.0001$), date by variety interaction ($F = 5.30$; df = 8, 96; $P < 0.0001$), date by seed treatment interaction ($F = 2.00$; df = 8, 96; $P = 0.05$), and date by variety by seed treatment interaction ($F = 2.9$; df = 8, 96; $P = 0.005$). However, there was no interaction between soybean variety and seed treatment.

Significantly fewer natural enemies were observed in resistant than in susceptible soybeans. Significant variety effects were observed on four sampling dates, with lower densities of natural enemies in resistant soybeans than in

Table 1. Diversity and abundance of natural enemies inspected in visual counts during summer 2011 at Volga, SD. A total of 1,440 plants were visually inspected for natural enemies throughout the season.

Order	Family	Identity	Number Observed
Coleoptera	Coccinellidae	*Harmonia axyridis* adults	91
		Harmonia axyridis larvae	134
		Coccinella septempunctata adults	35
		Hippodamia convergens adults	37
		Other adult lady beetles	34
		Other lady beetle larvae excluding *Harmonia axyridis*	164
		Lady beetle pupae	210
		Lady beetle eggs	132
Diptera	Syrphidae	Syrphid adults	68
		Predatory fly larvae	115
Hemiptera	Anthocoridae	*Orius* spp. adults	189
		Orius spp. nymphs	78
Hymenoptera		Aphid parasitized mummies	238
Total natural enemies inspected in visual counts			1,525

susceptible. There were significant seed treatment effects on natural enemies on three sampling dates, with lower densities of natural enemies in thiamethoxam seed-treated soybeans than in untreated soybeans. Averaged across the season, resistant soybeans with or without thiamethoxam seed treatment had lower densities of natural enemies than the susceptible soybeans with or without thiamethoxam seed treatment (Fig. 6a).

Fig. 6b summarizes the abundance of adult lady beetles throughout the season. In all treatments, adult lady beetles were observed in the first week of July but did not begin to generally increase until the first week of August. Beetles peaked at different times in different treatments, peaking the latest in the susceptible control in the first week of September (Fig. 6b). There were significantly fewer adult lady beetles in resistant soybeans ($F = 16.93$; df $= 1, 12$; $P = 0.001$). Besides variety, abundance of adult lady beetles were also significantly influenced by date ($F = 10.68$; df $= 8, 96$; $P < 0.0001$), and there was a date by variety interaction ($F = 4.06$; df $= 8, 96$; $P = 0.0003$). Other tested factors did not have significant effect. Averaged across the season, resistant soybeans both with and without seed treatment had significantly lower densities of adult lady beetles than the susceptible soybeans with or without seed treatment (Fig. 6b).

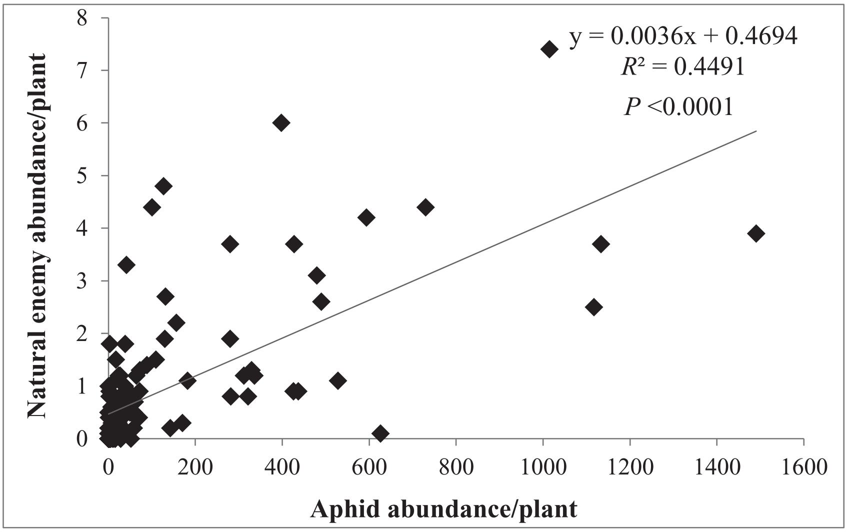

Fig. 5. Relationship of natural enemy abundance per plant with soybean aphid abundance per plant across all treatments in the field (Volga, SD, 2011): aphid-susceptible and -resistant (*Rag1*) isolines, with or without thiamethoxam seed treatment (ST).

Orius spp. (minute pirate bugs) were observed earlier during the season than lady beetles (Fig. 6c). Populations of *Orius* spp. began to increase in the second week of July and significantly varied by date ($F = 8.59$; df = 8, 96; $P < 0.0001$), reaching a peak during the first week of August and declining thereafter. As did lady beetles, *Orius* spp. differed significantly by variety ($F = 5.29$; df = 1, 9; $P = 0.04$), with higher populations in the aphid-susceptible variety than the aphid-resistant variety. There was neither a seed treatment effect nor a seed treatment by variety interaction for *Orius* spp.

Natural enemy abundance on sticky-card traps. Sticky-card traps deployed weekly in each plot captured more natural enemies numerically than were recorded in the visual inspections. A total of 2,793 natural enemies were recorded from 288 sticky card traps throughout the soybean growing season, compared to 1,525 observations on 1,440 plants made through visual inspection (Table 2).

Orius insidiosus was the most common natural enemy trapped on the sticky cards (34.4%) followed by the parasitoid group Aphelinidae (26.9%). Adult lady beetles accounted for 2.7% of sticky trap natural enemy captures. It has been noted that lady beetles, and *H. axyridis* in particular, may be underrepresented in sticky trap sampling because they are large, strong, and have a hemispheroid body shape that enables them to escape (Stephens and Losey 2004). Among lady beetle adults, *Hippodamia convergens* Guerin-Meneville was the most abundant (63.6% of the total lady beetles) followed by *H. axyridis* (20.7% of the total lady beetles). This was in contrast to the numbers observed through visual inspection, where *H. axyridis* accounted 46.3% of total adult lady beetles compared to 18.8% of the *H. convergens* adults. Other lady beetles species observed in the sticky traps were *C.*

septempunctata, *Coleomegilla maculata* DeGeer, and *Hippodamia tredecimpunctata* L. Other natural enemies and their proportional abundance identified in sticky cards were spiders (12.8%), chrysopids (9.2%), syrphid flies (5.3%), hemerobiids (3.1%), braconid parasitoids (1.3%), *Orius tristicolor* White (0.93%), and *Orius* spp. not identified to the species level (3.0%). (Because of detached wings and body parts on sticky cards, some of the *Orius* individuals were difficult to identify to the species level and thus were placed in a category of *Orius* spp.)

The total number of natural enemies collected from sticky traps in the different treatments is summarized in Fig. 7. There were significant effects of variety ($F =$ 23.47; df $= 1, 9$; $P = 0.0009$) and seed treatment ($F = 12.6$; df $= 1, 9$; $P = 0.0062$). Whether seed-treated or not, significantly fewer natural enemies were captured in resistant soybeans than in susceptible soybeans (Fig. 7). There was no variety by seed treatment interaction.

Discussion

Host plant resistance and thiamethoxam seed treatment were both found to have an effect on soybean aphid abundance and yield, though resistance and seed treatment did not interact. These treatments did not differ from each other in either cumulative aphid days or yield, though both differed from the control and from the combined resistance–thiamethoxam treatment.

There were fewer soybean aphids over time and lower peak densities on *Rag1* resistant soybeans, with or without thiamethoxam seed treatment, compared to the corresponding near-isoline susceptible control. The *Rag1*-containing line maintained aphid densities below the average economic injury level of 674 aphids/plant calculated in 2007; this is the point where yield loss average equals control cost (Ragsdale et al. 2007). The economic injury level using today's higher crop values may be lower, but a functional economic threshold of 250 aphids/plant is still widely used and considered applicable. Aphid populations on the *Rag1* line just exceeded this economic threshold during the third and fourth weeks of August. The aphid population in the susceptible control was significantly greater during the same period of time, suggesting that *Rag1* resistant soybeans still possess resistance against soybean aphid in South Dakota, even though aphid biotypes that are virulent on *Rag1* have been found elsewhere in the Midwest (Kim et al. 2008). However, peak aphid population just above the economic threshold suggests that *Rag1* resistance might not prevent producers from needing to spray in certain years when soybean aphid pressure is heavy. In this study the *Rag1* resistant treatment had higher yield than the untreated control but lower yield than *Rag1* combined with thiamethoxam.

Soybeans with thiamethoxam seed treatment on both susceptible and *Rag1* lines also had lower aphid densities throughout the season than on the nontreated control. As in the resistant soybeans, the aphid population in the seed-treated susceptible line exceeded the economic threshold. Unlike in the *Rag1* soybeans, the aphid population on seed-treated soybeans also approached the economic injury level during the third and fourth weeks of August (though the cumulative aphid days in the two treatments did not differ significantly). This result is in contrast with Megalhaes et al. (2009) in which aphids on seed-treated soybeans were kept below

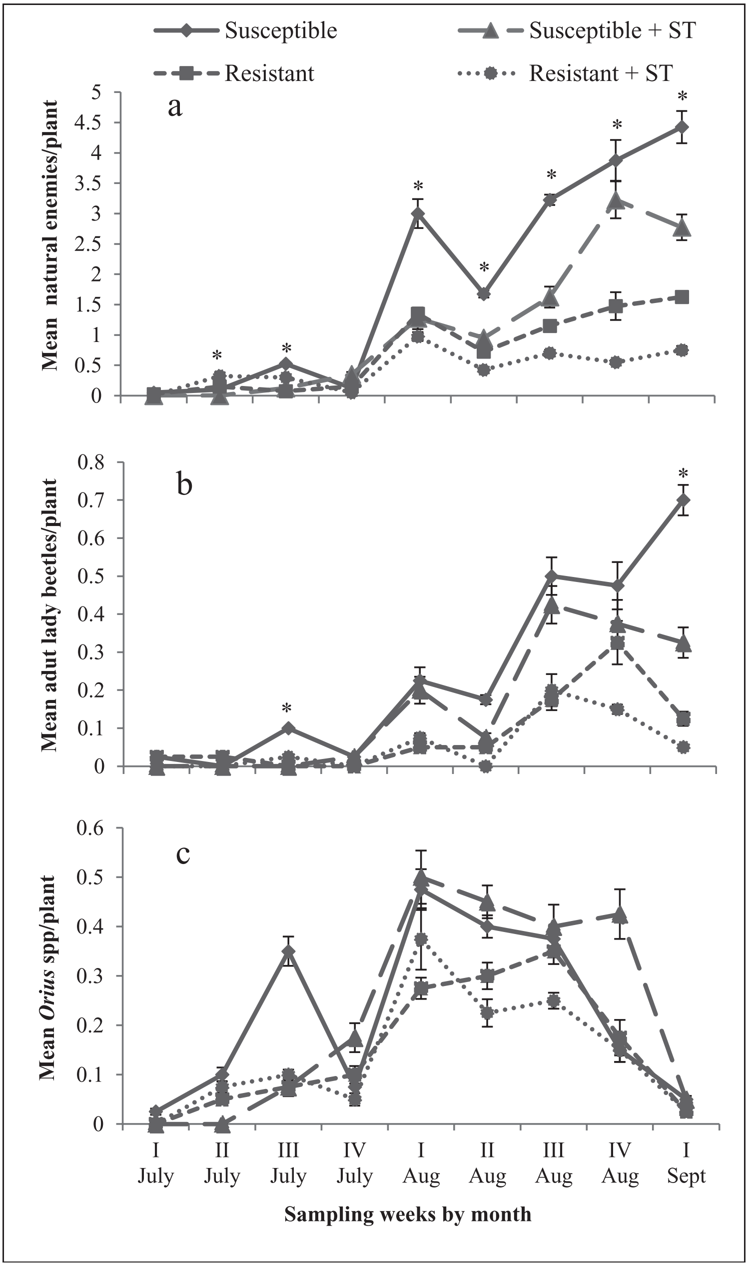

Fig. 6. Abundance of (a) natural enemies, (b) adult lady beetles, and (c) *Orius* spp., counted through visual inspection in four treatments of soybean in the field (Volga, SD, 2011): aphid-susceptible and aphid-resistant (*Rag1*) isolines, with or without thiamethoxam seed treatment (ST).

Table 2. Diversity and abundance of adult natural enemies inspected in sticky-card trap counts during summer 2011 at Volga, SD. A total of 288 sticky-card traps deployed were inspected for natural enemies throughout the season.

Order	Family	Identity	Number Collected
Coleoptera	Coccinellidae	*Harmonia axyridis*	16
		Coccinella septempunctata	2
		Coleomegilla maculata	4
		Hippodamia convergens	49
		Hippodamia tredecimpunctata	5
		Other adult lady beetles	1
Diptera	Syrphidae	Syrphid fly	149
Hemiptera	Anthocoridae	*Orius insidiosus*	962
		Orius tristicolor	26
		Orius spp.	84
Neuroptera	Chrysopidae	*Chrysoperla carnea*	258
	Hemerobiidae	Brown lace wing	87
Araneae		Spider	359
Hymenoptera	Braconidae		37
	Aphelinidae		754
Total natural enemies collected from sticky traps			2,793

the economic threshold throughout the season. In another study, McCarville et al. (2014) documented a reduction in cumulative aphid days of 38% in neonicotinoid seed-treated soybeans compared to untreated ones; however, such seed treatment did not provide yield advantage. In this study the thiamethoxam treatment had higher yield than the untreated control but lower yield than *Rag1* combined with thiamethoxam.

Aphid populations were still increasing when the study was terminated. Thus, the failure of thiamethoxam seed treatment to keep aphid populations below the economic threshold during the time of peak infestation suggests that the systemic

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Sampling dates are reported by subsequent weeks in each month. Error bars are standard errors of means. An asterisk (*) sign above error bars indicates a significant difference among treatments on a given date ($P < 0.05$, least significant difference).

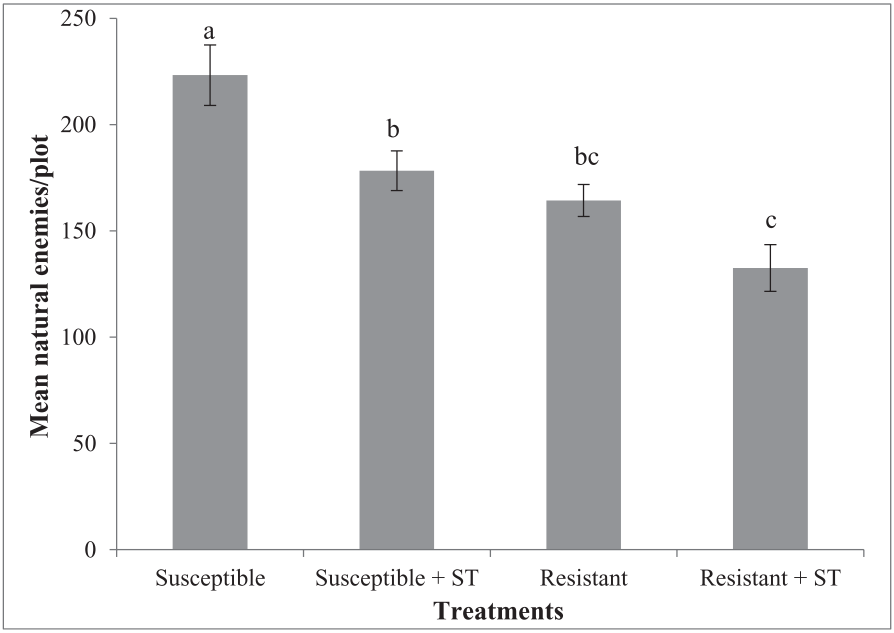

Fig. 7. Mean number of the natural enemies collected from sticky-card traps in four treatments of soybean in the field (Volga, SD, 2011): aphid-susceptible and aphid-resistant (*Rag1*) isolines, with or without thiamethoxam seed treatment (ST). Error bars are standard errors of means. Bars with different letters are significantly different ($P < 0.05$, least significant difference).

insecticidal toxicity might have degraded and thus lost effectiveness against later aphid infestations. McCornack and Ragsdale (2006) and McCarville and O'Neal (2013) also found the thiamethoxam seed-treatment effect diminished in the field on 49 and 42 d after planting, respectively. Similarly, Seagraves and Lundgren (2012) documented the loss of insecticidal seed treatment efficacy in the laboratory after 46 d. Thus, while thiamethoxam seed treatment reduced soybean aphid infestation relative to the nontreated control, it did not reduce aphid populations below the point where most producers would choose to manage the pest.

Rag1 resistance combined with thiamethoxam seed treatment had the fewest cumulative aphid days and the highest yield in this study. These factors did not interact, and their effects were thus additive; also, they did not differ from each other when deployed alone. All three management approaches in this study (susceptible soybeans with seed treatment, resistant soybeans without seed treatment, and resistant soybeans with seed treatment) returned higher yield than the untreated control, though we did not compare yield with an aphid-free treatment. In one multilocation, multiyear study with 23 location-years, McCarville et al. (2014) observed a 5% reduction in yield due to aphid feeding on single-gene-resistance soybeans (compared to aphid-free controls), and a 14% yield reduction on susceptible soybeans. In the same study, the addition of thiamethoxam seed treatment significantly reduced cumulative aphid days, but did not increase yield. A pyramided resistant line with two genes (*Rag1* and *Rag2*), did not lose the yield compared to aphid-free controls, and provided the greatest yield protection.

Both resistance and seed treatments were associated with lower natural enemies populations, and the lowest natural enemy population occurred in the seed-treated *Rag1* soybeans, an additive effect of resistant variety and seed treatment. The relative total abundance of natural enemies recorded in both visual observations and on sticky cards closely tracked aphid population growth trends and cumulative aphid days. In most treatments, there was an approximate 1-wk lag between when soybean aphids (prey) and natural enemies began to increase. Soybean aphids started to increase after the third week of July, and total natural enemies began to build after the fourth week of July, though this varied by species. Except in seed-treated susceptible soybeans, natural enemies peaked 1 wk after aphids peaked in the three other treatments. Increased immigration and/or increased reproduction in response to greater prey resources might explain this pattern, though this study does not allow us to distinguish the two.

Aphid abundance varied among treatments in this study, and there was a significant pattern of lower natural enemy abundance where aphids were less abundant, whether in aphid-resistant or thiamethoxam-treated plots. This study does not allow us to determine the mechanisms behind this pattern, which could be due to a population-level response by natural enemies to prey availability, or a direct negative impact of resistant soybeans or thiamethoxam on natural enemies (or both). There was overall lower abundance of natural enemies in *Rag1* soybeans. Trichome density, leaf structure, and phytochemistry have been documented to affect the foraging and fitness of natural enemies (Kennedy 2003; Lundgren et al. 2009a, b; van Emden 1995), and many predators are known to engage in host-plant feeding when prey are scarce. The specific resistance mechanism of *Rag1* has not been determined, though the resistance genes have been isolated and shown to have elements of antibiosis and antixenosis (Hill et al. 2006a, b). Traits that confer resistance to soybean aphid may also have adverse effect on lady beetles and *Orius* spp., the dominant predators in this study. Lundgren et al. (2009a) found reduced longevity of adult *H. axyridis* reared on resistant soybeans.

As in aphid-resistant soybeans, overall populations of natural enemies were lower in seed-treated soybeans than in the untreated ones. This result is consistent with Seagraves and Lundgren (2012), who found lower populations of generalist predators on thiamethoxam-treated soybeans. Moser and Obrycki (2009) found higher mortality of *H. axyridis* larvae exposure to corn seedlings treated with thiamethoxam. Conversely, even though *Orius* spp. is also regarded as a user of plant-based food (Lundgren et al. 2009b), their abundance did not differ in the thiamethoxam treatment compared to non-seed-treated soybeans.

Regardless of overall natural enemy abundance, generalist predators were most abundant. In the visual survey of natural enemies, they represented the largest share (i.e., 84.4%) of total natural enemies counted. Among aphid parasitoids, aphilinids were also found in abundance; on sticky cards they comprised 26.9% of the total natural enemies counted, showing that this parasitoid taxon is present in soybean fields, which may open the possibility for a greater scope of this parasitoid group for aphid management.

Coccinellids and *Orius* spp. were the most abundant predators, accounting for 84% of the total postnatal predators in visual counts (comprising 61% and 23% of the total nonegg predator count, respectively). *Harmonia axyridis* and *O. insidiosus* were the dominant species in the study area. The numerical dominance of *H. axyridis* and *O. insidiosus* agrees with Rutledge et al. (2004) who found this in soybean fields in Michigan, and with Brosius et al. (2007), who found *O. insidiosus* to be the most common predator of soybean aphid in Nebraska.

There was a disparity in natural enemy species distribution between visual counts and sticky cards, suggesting that choice of sampling method is important in this system. Among coccinellids, *H. axyridis* was the most common species in visual counts but the second most abundant lady beetle on sticky traps, suggesting that this lady beetle species may be more efficient in escaping sticky traps or they may have a lower preference for the yellowish green color of the sticky cards. Stephens and Losey (2004) documented that *H. axyridis* escaped from sticky cards significantly better than other coccinellids in alfalfa. Sticky-card traps captured a greater proportion of *Orius* spp. (mostly *O. insidiosus*) than observed in visual counts; these small insects may have escaped notice to some degree in the visual counting, and are probably underrepresented by this sampling method.

Our study suggests that in a year of heavy soybean aphid pressure where aphids exceed the economic injury level, seed treatments, single-gene resistance, or a combination provide greater yield protection than taking no action (as in the susceptible control), with yield protection the highest in seed-treated *Rag1* soybeans. However, it is unclear whether any of these tactics, particularly when used alone, would provide greater yield protection or economic return than a traditional approach of scouting and applying foliar insecticide after the threshold is reached (a treatment not included in this study). Both resistant traits and seed treatments add to input costs and may not always prevent the need for further investment in pest management later in the season in years of heavy aphid pressure. Future work should examine the relative costs and yield advantages of single- and multiple-gene-resistant varieties and seed treatments relative to scouting and thresholds, particularly over time. Soybean aphid populations fluctuate from year to year and cannot be accurately predicted in advance of planting decisions; longer-term studies can better assess the probability of net return. This study presents 1 yr of data and should be interpreted in that light.

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