

# Effects of neonicotinoid seed treatments on soybean aphid and its natural enemies

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**Abstract** Insecticidal seed treatments are increasingly being applied to soybeans in North America, and several recent studies question what they add to current pest management. Here, we examine the effects of two neonicotinoid insecticidal seed treatments on insect populations (pest and natural enemies) in SD soybeans over 2 years. Moreover, we conducted laboratory experiments to determine the duration that seed treatments remained effective against the soybean aphid (*Aphis glycines*, Hemiptera: Aphididae) and how thiamethoxam affected survival of one of the aphid's predators, *Orius insidiosus* (Hemiptera: Anthracoridae) on soybean. Soybean aphids, thrips, and grasshopper populations were unaffected by the insecticidal seed treatments in the field. The laboratory trial revealed that all bioactivity of the seed treatments against soybean aphids was gone within 46 days after planting, prior to aphid populations damaging the crop. Bean leaf beetles, a sporadic pest in our area, were reduced by the seed treatments. But, there were no yield benefits of insecticidal seed treatments over the 2 years of the study at this location. Natural enemy communities were significantly reduced by thiamethoxam seed treatments relative to the untreated control, particularly populations of *Nabis*

*americoferus* (Hemiptera: Nabidae). *Chrysoperla* (Neuroptera: Chrysopidae) adults were reduced in the imidacloprid-treated plots. In the laboratory, rearing *O. insidiosus* on soybean plants treated with thiamethoxam resulted in higher mortality for both the nymphs and the adult stage. Offering the predator insect prey on the thiamethoxam-treated plants improved survival of the adult stage, but not the nymphal stage. This work confirms that insecticidal seed treatments offer little benefit to soybean producers of the Northern Great Plains and adds to the discussion by suggesting that generalist predators are adversely affected by the insecticides.

**Keywords** *Aphis glycines* · *Ceratomyza trifurcata* · Generalist predators · Omnivory · *Orius insidiosus* · Systemic insecticide · Seed treatment · Thrips

## Introduction

The use of insecticidal seed treatments is becoming more common in production agriculture. This delivery technology for crop protection chemicals has many benefits. In addition to directly protecting crops from seed and root feeders and early season foliar pests, these targeted applications can have other benefits such as decreasing applicator exposure and the amount of active ingredient used (Taylor et al. 2001) and less exposure to nontarget organisms than foliar applications (Albajes et al. 2003). Additionally, their ease of use can improve pest control in situations where foliar sprays are difficult to time or crop morphology prohibits adequate coverage (Nault et al. 2004). Seed treatments are also an efficient and efficacious delivery system for systemic insecticides that target insect vectors of plant pathogens where management timing is

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essential and difficult (Bradshaw et al. 2008; Strausbaugh et al. 2010). These benefits, along with the emergence of the neonicotinoid insecticide class, have led to an increase in this technology's use in row crops (Elbert et al. 2008; Gore et al. 2010).

The application of a broad-spectrum systemic insecticide can also have undesired effects. Like other preventative pest control technologies (e.g., transgenics), seed treatments do not always fit comfortably into the traditional integrated pest management (IPM) framework (Hutchins 2010). Producers incur a control cost prior to the manifestation of pest pressure, and this cost is not recouped with higher yield if economically damaging populations of herbivores do not occur prior to loss of bioactivity. Additionally, seed treatments may pose risk to beneficial species, such as ground and foliage dwelling predators (Al-Deeb et al. 2001; Mullin et al. 2005; Moser and Obrycki 2009) and pollinators (Girolami et al. 2009).

Insecticidal seed treatments on soybeans have recently come under scrutiny by the entomological community in the United States. Insecticidal seed treatments are currently widely applied to soybeans with claims of yield improvements for producers. Independent studies on the use of insecticidal seed treatments have revealed either no yield benefits (McCornack and Ragsdale 2006; Cox et al. 2008; Ohnesorg et al. 2009) or profit benefits below those that can be gained using treatment thresholds and foliar-applied insecticides (McCornack and Ragsdale 2006; Johnson et al. 2009). These studies have given particular attention to the key economic pest of soybeans, soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae), but the majority also examine yields, which incorporate the entire pest community. Some work suggests that the sporadic pest, bean leaf beetle *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), may be reduced by insecticidal seed treatments under some circumstances (Johnson et al. 2008). The goal of the current 2-year field study was to examine the effects of imidacloprid and thiamethoxam seed treatments on pest and beneficial insect populations and soybean performance, and to direct laboratory experiments at explaining the bioactivity of seed treatments against key pests and natural enemies. Specifically, the latter laboratory assay focused on *Orius insidiosus*, one of the key predators of soybean aphids in North America (Desneux et al. 2006; Harwood et al. 2007), that is also omnivorous to some degree on plant tissues (Lundgren et al. 2008; Seagraves and Lundgren 2010) and could thus directly ingest the systemic insecticides.

## Methods

### Field study

Soybean (v'A 1702') was planted on 15 May 2009 and 27 May 2010 in 76.2 cm rows at a population of 465,000 per ha. Average planting date for east central SD generally falls between May 5 and 25. Plots were 5.33 by 7.62 m and separated by 6.1 m of bare cultivated ground. In each year, three treatments ( $n = 4$  replicates each) were arranged in a randomized complete block design. These treatments were an untreated control, an imidacloprid seed treatment (Gaucho 480FS, Gustafson LLC, Dallas, TX) applied at a rate of 62.5 g a.i./100 kg of seed, or a thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, Greensboro, NC) seed treatment applied at a rate of 50 g a.i./100 kg seed. The study was conducted at the Eastern South Dakota Soil and Water Research Farm near Brookings, SD (44.35, -96.81, latitude, longitude); the soil type was a Barnes loam with nearly level topography.

Beginning on 16 June 2009 and 22 June 2010, yellow sticky cards (7.62 × 12.7 cm; Great Lakes IPM, Vestaburg, MI) were placed just above the plant canopy on bamboo stakes at two randomly selected locations at least 1 m from the plot edge. Trapping continued until 12 August 2009 and 1 September 2010. Cards were removed approximately 7 days after placement, and new cards were placed in different random locations within the plot. Cards were wrapped in clear plastic and stored at -20°C until thrips (Thysanoptera: Thripidae) could be counted microscopically. The number of thrips collected during each sample period was standardized per card per day, and the sum of those means among treatments compared with an ANOVA (with treatment, sample year, and their interaction as parameters).

The numbers of soybean aphids on randomly selected whole soybean plants were counted approximately weekly from 16 June 2009 and 22 June 2010 (V1–V2 growth stage) until aphid numbers exceeded the economic injury level of 674 aphids per plant (Ragsdale et al. 2007) or leaf senescence. Twenty plants per plot were examined when 0–80% of plants were infested, ten plants when 81–99% were infested, and five plants at 100% infestation. Aphids per plant were square root transformed (to fulfill the assumptions of ANOVA), and treatments were compared with a repeated measures ANOVA. Ryan-Einot-Gabriel-Welsch tests were used to separate treatment means on dates with a significant treatment effect (SAS Institute, Cary, NC, USA, 27513). Aphid days were calculated for each experimental unit using the formula (Ruppel 1983):

$$\frac{(\text{Aphids per plant on previous sample date} + \text{Aphids per plant on current sample date})}{2 \times \text{number of days between sampling dates}}$$

Cumulative aphid days were generated by summing the aphid days in each experimental unit over the growing season. Sums per plot were compared using an ANOVA with treatment, year, and treatment  $\times$  year as parameters.

In addition to thrips and soybean aphids, predators and other herbivores of interest were monitored in each treatment. Each plot was sampled using a 38-cm diameter net with two 15-sweep samples on the days when aphids were sampled. Insects in the sweeps were emptied onto a ground cloth, counted, and released back into the plot. Seasonal counts of predatory arthropods and herbivores (species presented in Table 4) were summed within a plot across all sampling dates and compared using an ANOVA with treatment, year, and an interaction term as parameters.

Yields were assessed using the harvest from three 7.62 m two-row samples from each plot. Samples were collected with a combine and were weighed. Subsamples were isolated, and percent moisture was determined with a grain moisture tester (DICKEY-john Corporation, Auburn, IL). Kilograms per ha were determined, and treatment means were compared using ANOVA with treatment, year, and an interaction as parameters. Oil and protein contents and dry matter percentages were determined from each subsample using a near infrared spectrometer (Foss North America, Eden Prairie, MN). Arcsine square root transformed percentages were compared using separate ANOVAs with year and treatment as main factors.

#### Bioactivity of insecticides against aphids

The seasonal bioactivity of field-collected soybean vegetation from plants treated with the insecticidal seed treatments was evaluated against soybean aphids in the laboratory in 2009 using methods similar to those employed by McCornack and Ragsdale (2006). In each trial, three randomly selected soybean plants were carefully uprooted from each plot ( $N = 36$ , 12 per treatment). These insect-free plants were immediately taken to the laboratory, and the youngest fully expanded trifoliolate (except on 22 July when the 4th node was used) was removed with a razor and placed in a 15-ml glass test tube with water, which was then sealed around the stem using Parafilm and placed in a 0.71-l plastic cup (15 cm deep, 10.5 cm top diam., 6 cm bottom diam; Solo Cup Company, Urbana, IL). Ten soybean aphids from a laboratory culture (originating from a collection near Brookings, SD in 2008) were placed on the foliage using a camel hair paintbrush, and survival was confirmed prior to the assay. Each cup was sealed with plastic wrap, and cups were placed in an environmental chamber with conditions of 25°C, 50% relative humidity, and 14:10 h (light/dark). The number of aphids surviving in each unit was recorded after 7 days.

Aphids were considered alive if they reacted with motion when prodded with a sharp needle. For each assay date, aphid counts were square root transformed and treatment means compared using ANOVA with a Ryan-Einot-Gabriel-Welsch means separation test.

#### Insecticide toxicity to *Orius insidiosus*

Untreated soybean (var. Asgrow 1702) seeds and seeds treated with thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, Greensboro, NC) at a rate of 50 g a.i./100 kg seed were planted in the greenhouse (80 plants per treatment). The oldest trifoliolate nodes from V2 plants were removed and were individually placed in 15-ml glass test tubes, sealed around the stem with Parafilm. Foliage tubes were individually placed into 0.71-l plastic cups (15 cm deep, 10.5 cm top diam., 6 cm bottom diam; Solo Cup Company, Urbana, IL). Plants from treated or untreated seeds were randomly assigned to an appropriate treatment involving fed or unfed *O. insidiosus* adults or nymphs (i.e., a total of eight treatments; Table 1). *Orius insidiosus* were collected from alfalfa near Brookings, SD and were reared to the desired life stage (neonate nymphs or <5-day-old enclosed adults) under conditions described in Lundgren and Fergen (2006). Adults and nymphs that received prey were fed eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) (Beneficial Insectary, Redding CA), and all treatment received water as a saturated cotton wick. Each plant received its respective *O. insidiosus* (one per plant) and diet treatment, and the cups were sealed with plastic wrap. *O. insidiosus* were exposed to the treatments under conditions of 25°C, 50% relative humidity, and 14:10 (Light/Dark) for 72 h. Mortality was recorded, and the proportions of adults or nymphs surviving in each treatment were arcsine square root transformed and analyzed using separate ANOVA for adults and nymphs.

**Table 1** Treatment combinations of *Orius insidiosus* stages, food availability, and systemic insecticide tested in the laboratory bioassay conducted on greenhouse-produced soybean plants

<i>O. insidiosus</i> stage	Prey ( <i>Ephestia kuehniella</i> eggs)	Systemic insecticide
Adult	Present	Thiamethoxam
Adult	Present	Untreated
Adult	Absent	Thiamethoxam
Adult	Absent	Untreated
Nymph	Present	Thiamethoxam
Nymph	Present	Untreated
Nymph	Absent	Thiamethoxam
Nymph	Absent	Untreated

## Results

### Field experiment

#### Herbivores

There were no consistent effects of insecticidal seed treatments on herbivores in soybeans. Seed treatments did not affect *A. glycines* season-long abundance compared to the control in 2009 ( $F_{2,9} = 1.07$ ,  $P = 0.38$ ) or 2010 ( $F_{2,9} = 1.97$ ,  $P = 0.19$ ) (Table 2). However, more soybean aphids were present on untreated plants on two of the sample dates, 6 and 17 July 2009, compared to the insecticide-treated plants (6 July:  $F_{2,9} = 9.84$ ,  $P < 0.01$ ; 17 July:  $F_{2,9} = 10.97$ ,  $P < 0.01$ ) (Table 2). Aphid densities were low (less than 1% of economic injury levels) on these dates where differences were observed. In 2009, aphid populations exceeded the action threshold level (250 aphids per plant; Ragsdale et al. 2007) on the same sampling dates (12 Aug 2009) in all three treatments. In 2010, aphid numbers were low and never reached the economic injury level. When years were examined together, there was no difference among treatments in cumulative aphid days (treatment:  $F_{2,23} = 0.40$ ,  $P = 0.67$ ; year:  $F_{1,23} = 168.69$ ,  $P < 0.01$ ; treatment  $\times$  year:  $F_{2,23} = 0.21$ ,  $P = 0.81$ ). Mean (SEM) cumulative aphid days were  $4,718.94 \pm 1,566.07$ ,  $4,347.04 \pm 1,535.30$ , and  $4,074.49 \pm 1,466.14$  in the untreated, imidacloprid, and thiamethoxam-treated plots (pooled across years).

There were no treatment differences in grasshopper (Orthoptera: Acrididae) abundance among treatments (treatment:  $F_{2,23} = 0.14$ ,  $P = 0.87$ ; year:  $F_{1,23} = 4.53$ ,  $P = 0.05$ ; treatment  $\times$  year:  $F_{2,23} = 0.69$ ,  $P = 0.51$ ) (Table 3). Likewise, there were no differences in thrips abundance among the treatments (treatment:  $F_{2,23} = 0.41$ ,  $P = 0.67$ ; year:  $F_{1,23} = 2.82$ ,  $P = 0.11$ ; treatment  $\times$  year:  $F_{2,23} = 0.01$ ,  $P = 0.99$ ). Mean (SEM) (pooled across years) seasonal densities were  $31.33 \pm 3.03$ ,  $27.11 \pm 4.06$ , and  $28.26 \pm 3.00$  thrips per card per day for untreated, imidacloprid, and thiamethoxam-treated plots. Bean leaf beetle adults were more abundant in the untreated soybeans when data were combined from both years ( $F_{2,23} = 15.28$ ,  $P < 0.01$ ) (Table 3). There were significantly more *C. trifurcata* adults captured in 2009 than in 2010 ( $F_{1,23} = 5.09$ ,  $P = 0.04$ ). The significant interaction between year and treatment ( $F_{2,23} = 3.63$ ,  $P = 0.05$ ) is the result of there being no treatment effect in 2009 ( $F_{2,11} = 3.83$ ,  $P = 0.06$ ; this marginal effect was driven by a higher density of bean leaf beetle adults in the untreated plots) and there being more bean leaf beetle adults in the untreated soybeans in 2010 ( $F_{2,11} = 11.67$ ,  $P < 0.01$ ). This difference was created by elevated densities of bean leaf beetle adults on one of the late season

**Table 2** Per plant mean (SEM) season-long abundances per plot of *Aphis glycines* in soybean plots treated with insecticidal seed treatments or left untreated

	2009										2010					
	30 June	6 July	17 July	23 July	30 July	5 Aug	12 Aug	18 Aug	10 Aug	17 Aug	30 July	20 July	13 July	6 July	29 June	22 June
Untreated control	0.41 $\pm$ 0.35	1.44 $\pm$ 0.55a	4.14 $\pm$ 0.85a	13.57 $\pm$ 3.88a	36.70 $\pm$ 6.95a	108.62 $\pm$ 8.11a	697.35 $\pm$ 84.53a	1,062.25 $\pm$ 128.61a								
Imidacloprid	0.00 $\pm$ 0.00	0.12 $\pm$ 0.11b	2.06 $\pm$ 0.57b	4.94 $\pm$ 1.04a	23.62 $\pm$ 8.58a	85.27 $\pm$ 25.26a	576.5 $\pm$ 145.32a	1,202.35 $\pm$ 126.94a								
Thiamethoxam	0.00 $\pm$ 0.00	0.05 $\pm$ 0.05b	0.74 $\pm$ 0.19b	8.61 $\pm$ 2.88a	26.32 $\pm$ 3.72a	107.25 $\pm$ 23.24a	537.25 $\pm$ 136.5a	1,093.75 $\pm$ 111.63a								
Untreated control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.31 $\pm$ 0.12a	0.52 $\pm$ 0.30a	0.29 $\pm$ 0.06a	7.15 $\pm$ 1.07a	38.80 $\pm$ 4.50a	58.07 $\pm$ 11.40a								
Imidacloprid	0.01 $\pm$ 0.01	0.00 $\pm$ 0.00	0.05 $\pm$ 0.05a	0.46 $\pm$ 0.29a	0.81 $\pm$ 0.41a	4.69 $\pm$ 0.80a	39.70 $\pm$ 7.95a	46.92 $\pm$ 4.85a								
Thiamethoxam	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.02 $\pm$ 0.02a	0.34 $\pm$ 0.05a	0.70 $\pm$ 0.13a	4.04 $\pm$ 0.66a	28.77 $\pm$ 1.30a	38.52 $\pm$ 6.91a								

Column means within a year followed by the same letter are not significantly different ( $\alpha = 0.05$ , Ryan-Einot-Gabriel-Welsch test). No statistics were possible for dates on which no aphids were collected

**Table 3** Mean (SEM) seasonal sums of soybean arthropods collected per plot (pooled across 2 years) using sweep nets

	Untreated control	Imidacloprid seed treatment	Thiamethoxam seed treatment
<b>Herbivores</b>			
<i>Cerotoma trifurcata</i> (Coleoptera: Chrysomelidae)	29.87 ± 6.95a	12.50 ± 1.94b	3.00 ± 0.78b
Grasshoppers (Orthoptera: Acrididae)	6.50 ± 0.98a	6.75 ± 0.80a	7.75 ± 2.94a
<b>Predators</b>			
<i>Orius insidiosus</i> (Hemiptera: Anthocoridae)	18.25 ± 2.54a	11.87 ± 1.62a	14.87 ± 2.59a
<i>Nabis americanoferus</i> (Hemiptera: Nabidae)	14.25 ± 1.88a	8.62 ± 1.54b	8.50 ± 0.57b
Spiders (Araneae)	24.37 ± 3.79a	22.87 ± 4.09a	18.12 ± 2.59a
Harvestmen (Opiliones: Phalangiidae)	8.62 ± 1.29a	9.00 ± 1.45a	8.00 ± 1.66a
Lacewing adults ( <i>Chrysoperla</i> sp.; Neuroptera: Chrysopidae)	5.00 ± 0.53a	2.00 ± 0.53b	2.75 ± 0.98ab
<i>Chrysoperla</i> sp. larvae	2.87 ± 0.89a	1.75 ± 0.70a	1.75 ± 0.77a
Lady beetle adults (Coleoptera: Coccinellidae)	4.87 ± 1.17a	3.37 ± 0.92a	4.25 ± 0.75a
Lady beetle larvae	14.50 ± 4.32a	15.00 ± 4.94a	8.87 ± 3.13a
Total predators <sup>a</sup>	105.75 ± 6.00a	87.50 ± 7.85ab	76.37 ± 2.78b

Means within a row followed by the same letter are not significantly different (Tukey’s HSD test,  $\alpha = 0.05$ )

<sup>a</sup> *O. insidiosus* nymphs and adults, *Nabis* sp. adults and nymphs, *Geocoris* sp., spiders, harvestmen, lacewing adults and larvae, coccinellid adults and larvae, and ants

sampling dates (17 August) in the untreated control plots ( $F_{2,11} = 10.33, P < 0.01$ ) (data not shown).

*Natural enemies*

When all predator taxa were examined as a group, abundance was significantly greater in the untreated control than from soybeans treated with thiamethoxam (treatment:  $F_{2,23} = 6.73, P < 0.01$ ; year:  $F_{1,23} = 2.41, P = 0.14$ ; treatment × year:  $F_{2,23} = 1.08, P = 0.36$ ) (Table 3). More nabids (Hemiptera: Nabidae) were found in the untreated plots (treatment:  $F_{2,23} = 4.74, P = 0.02$ ; year:  $F_{1,23} = 0.96, P = 0.34$ ; treatment × year:  $F_{2,23} = 0.59, P = 0.56$ ). Adult lacewings (Neuroptera: Chrysopidae) were more abundant in the untreated than the imidacloprid seed treatment (treatment:  $F_{2,23} = 0.54, P = 0.01$ ; year:  $F_{1,23} = 1.15, P = 0.30$ ; treatment × year:  $F_{2,23} = 2.26, P = 0.13$ ).

Although the other predators followed a similar trend in having greater abundance in the untreated plots than in at least one of the insecticide treatments, these effects were not always significant (Table 3). No differences were found in abundances of *O. insidiosus* (treatment:  $F_{2,23} = 1.81, P = 0.19$ ; year:  $F_{1,23} = 0.53, P = 0.47$ ; treatment × year:

$F_{2,23} = 0.60, P = 0.56$ ), spiders (Araneae) (treatment:  $F_{2,23} = 1.27, P = 0.30$ ; year:  $F_{1,23} = 13.06, P < 0.01$ ; treatment × year:  $F_{2,23} = 0.27, P = 0.76$ ), *Phalangium opilio* (Opiliones: Phalangiidae) (treatment:  $F_{2,23} = 0.17, P = 0.85$ ; year:  $F_{1,23} = 11.85, P < 0.01$ ; treatment × year:  $F_{2,23} = 0.02, P = 0.98$ ), *Chrysoperla* sp. larvae (Neuroptera: Chrysopidae) (treatment:  $F_{2,23} = 0.82, P = 0.45$ ; year:  $F_{1,23} = 1.72, P = 0.21$ ; treatment × year:  $F_{2,23} = 3.06, P = 0.07$ ), coccinellid larvae (Coleoptera: Coccinellidae) (treatment:  $F_{2,23} = 2.72, P = 0.09$ ; year:  $F_{1,23} = 66.39, P < 0.01$ ; treatment × year:  $F_{2,23} = 1.26, P = 0.31$ ), or coccinellid adults (treatment:  $F_{2,23} = 0.91, P = 0.42$ ; year:  $F_{1,23} = 10.80, P < 0.01$ ; treatment × year:  $F_{2,23} = 1.22, P = 0.32$ ) (Table 3).

*Crop performance*

Soybean yields were similar in all treatments and were significantly higher in 2009 than in 2010 (treatment:  $F_{2,18} = 0.14, P = 0.88$ ; year:  $F_{1,18} = 18.22, P < 0.001$ ; treatment × year:  $F_{2,18} = 0.03, P = 0.97$ ) (Table 4). Although there were differences in seed quality between years (Dry matter:  $F_{1,23} = 14.62, P < 0.01$ ; Oil:  $F_{1,23} = 150.08, P < 0.01$ ; Protein:  $F_{1,23} = 9.78, P < 0.01$ ),

**Table 4** Soybean yield and quality per plot from insecticide-treated seed and an untreated control

Seed treatment	kg/ha	Dry matter	Oil	Protein
Untreated	3,346 ± 169a	93.56 ± 0.39a	18.54 ± 0.15a	38.02 ± 0.28a
Imidacloprid	3,438 ± 195a	93.87 ± 0.36a	18.61 ± 0.17a	37.93 ± 0.30a
Thiamethoxam	3,338 ± 227a	93.68 ± 0.37a	18.56 ± 0.18a	38.04 ± 0.29a

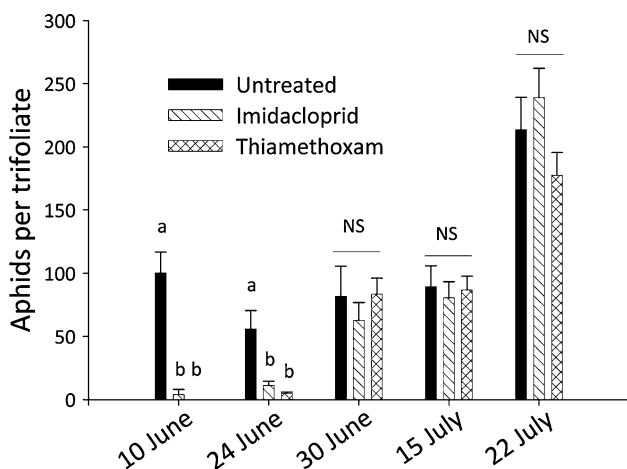
there were no significant interactions between treatment and year (dry matter:  $F_{2,23} = 0.73$ ,  $P = 0.49$ ; Oil:  $F_{2,23} = 0.33$ ,  $P = 0.72$ ; Protein:  $F_{2,23} = 0.001$ ,  $P = 0.99$ ). When years were combined, there were no differences among treatments in seed quality (dry matter:  $F_{2,23} = 0.28$ ,  $P = 0.76$ ; Oil:  $F_{2,23} = 0.37$ ,  $P = 0.69$ ; Protein:  $F_{2,23} = 0.11$ ,  $P = 0.90$ ).

#### Bioactivity of insecticides against aphids

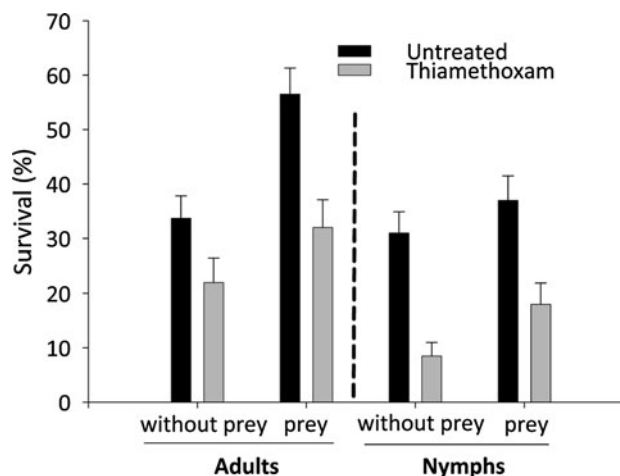
In 2009, bioactivity of the field-collected foliage with seed treatments had dissipated against soybean aphids by 30 June, which was approximately 45 days after planting. There were more aphids on soybean trifoliates taken from the untreated plots on 10 and 24 June after 7 days than trifoliates from either seed treatment (10 June:  $F_{2,33} = 96.06$ ,  $P < 0.01$ ; 24 June:  $F_{2,33} = 15.06$ ,  $P < 0.01$ ) (Fig. 1). Aphids survived and reproduced equally well on treated and untreated plants after 30 June (30 June:  $F_{2,33} = 0.50$ ,  $P = 0.61$ ; 15 July:  $F_{2,33} = 0.12$ ,  $P = 0.88$ ; 22 July:  $F_{2,33} = 1.71$ ,  $P = 0.20$ ) (Fig. 1).

#### Insecticide toxicity to *Orius insidiosus*

*Orius insidiosus* adults provided prey had higher survival than those with only water after 72 h ( $F_{1,76} = 12.38$ ,  $P < 0.01$ ) (Fig. 2). More adults exposed to soybean foliage from plants treated as seeds with thiamethoxam died than those exposed to untreated control plants ( $F_{1,76} = 14.02$ ,  $P < 0.01$ ) (Fig. 2); however, there was no interaction between the presence of food and thiamethoxam seed treatment on adult mortality ( $F_{1,76} = 1.34$ ,  $P = 0.25$ ). Nymphal survival after 72 h was not affected by the



**Fig. 1** Mean (SEM) number of surviving aphids after exposure for 7 days to soybean foliage from field-collected plants from the experimental plots. Bars within a date with the same letter are not significantly different ( $\alpha = 0.05$ ; Ryan-Einot-Gabriel-Welsch multiple comparison test). NS indicates treatments with similar means (not significant)



**Fig. 2** Survival of *Orius insidiosus* adults and nymphs on soybean grown from thiamethoxam-treated and untreated seed in the absence and presence of prey (*Ephestia kuehniella* eggs). Prey-fed adults survived better than those without prey, and those exposed to thiamethoxam experienced higher mortality than those that were not exposed. Nymphs fed prey survived as well as those without prey, but thiamethoxam increased mortality for both prey treatments. See text for supporting statistics

presence of prey ( $F_{1,76} = 3.42$ ,  $P = 0.07$ ) (Fig. 2). Nymphs exposed to soybean from thiamethoxam-treated seeds had lower survival than those placed on untreated soybean ( $F_{1,76} = 27.99$ ,  $P < 0.01$ ) (Fig. 2). There was no interaction between the presence of food and neonicotinoid seed treatment affecting nymphal survival ( $F_{1,76} = 0.44$ ,  $P = 0.51$ ).

#### Discussion

The current study confirms earlier reports that insecticidal seed treatments on soybean provide minimal or no benefits to producers, and both the laboratory and field results add to the discussion by suggesting that generalist predators may be reduced by thiamethoxam seed treatments. Given that conserving and combining endemic generalist predators with compatible pest management tactics is a cornerstone of IPM, prescriptive use of systemic insecticides when they have little or no effects on target pests seems a poor strategy. Moreover, our estimates are that current insecticidal seed treatments unnecessarily cost producers approximately \$12–15 per acre (M.P.S. personal communications with local seed dealers). We recommend that producers avoid insecticidal seed treatments in soybeans and apply foliar insecticides only when pests exceed regionally recommended treatment thresholds (Ragsdale et al. 2007).

Populations of soybean aphids, grasshoppers, and thrips were unaffected by insecticidal seed treatments; bean leaf beetle populations were reduced by insecticidal seed

treatments, but these reductions did not provide any yield benefits. Since its invasion of SD in 2001, soybean aphid typically establishes in soybean fields of our region in late June at the earliest and typically will only exceed economic thresholds after August 1 (for example, Table 2). The bioassay using field-collected plant material indicates that all bioactivity of the insecticidal seed treatments against soybean aphids had largely disappeared from plants between 15 and 22 July (Fig. 1), which suggests that the insecticide is gone before economically threatening populations occur. This supports a previous trial by McCornack and Ragsdale (2006), which showed all bioactivity of the seed treatments had left the plants in the field within 49 days of planting. Moreover, aphid populations in the field were similar in all three treatments of the current study, reconfirming that the insecticide treatments had no measurable bioactivity against aphid populations in the field. Other work, conducted throughout North America, has shown that soybean aphid populations are either unaffected by insecticide seed treatments or that yield benefits are achieved more economically using foliar applications of insecticides as needed (McCornack and Ragsdale 2006; Cox et al. 2008; Johnson et al. 2009; Ohnesorg et al. 2009).

Other pest populations varied in their responses to insecticidal seed treatments, but none of these interactions led to differences in crop yield. Populations of thrips and grasshoppers, two sporadic or occasional pests of soybeans were entirely unaffected by the insecticidal seed treatment. Bean leaf beetle populations were reduced by insecticidal seed treatments, but only during the latter part of the season. The timing of the observed reductions is coincident with the emergence of the summer generation of bean leaf beetles in our region (Riedell et al. 2005). Because much of the bioactivity of the seed treatments had left the plants by this stage, we speculate that the insecticidal seed treatment had some benefits against the immature stage of bean leaf beetles that feeds subterraneously on soybean nodules (Lundgren and Riedell 2008). Soybean seed treatments have been observed to reduce bean leaf beetle populations in soybeans in other regions as well (Dr. Eileen Cullen, personal communications). In spite of the higher bean leaf beetle populations in the untreated plots, populations were below economic thresholds in both treatments in both years and we found no effects of soybean seed treatments on soybean yield or quality (Table 3).

Although pest populations were largely unaffected by the insecticidal seed treatments, we found that the generalist predator community in the soybean foliage was reduced by approximately 25% by the thiamethoxam treatment (Table 3). To our knowledge, this is the first documentation of this reduction in predator populations brought about by insecticidal seed treatments. Ohnesorg et al. (2009) did not find a similar pattern when they examined the effects of

seed applied insecticides, but in this study, foliar insecticides were also applied and reduced natural enemy abundance. Within this guild of generalist predators, nearly all taxa were reduced by some degree by at least one of the insecticidal seed treatments. However, the only taxa which were significantly reduced when  $\alpha = 0.05$  were Nabidae (likely *Nabis americanoferus* Carayon, J.G. Lundgren, personal observation) and *Chrysoperla* sp. Other studies have found that imidacloprid and thiamethoxam systemic insecticides can harm beneficial predators in other study systems by reducing survival, decreasing development rates, and reducing fecundity (Smith and Krischik 1999; Stapel et al. 2000; Al-Deeb et al. 2001; Krischik et al. 2007; Rogers et al. 2007; Lundgren 2009). Given that background prey populations were largely equivalent among the treatments (see above), we suspect that the systemic insecticides were having direct toxic effects on the predators, either through contact or through facultative omnivory on the soybean plants themselves (Lundgren 2009). *Chrysoperla* and *Nabis* (as well as many other generalist predators) are both strongly omnivorous (Stoner 1972; Canard 2001), and the laboratory assay with *O. insidiosus* suggests that a major mechanism for why predators were reduced in the thiamethoxam-treated soybeans is that they were feeding on soybean that contained insecticide. In the laboratory assay, *O. insidiosus* adults and nymphs had lower survival on plants with thiamethoxam seed treatments than on untreated plants. Two experimental observations lend credence to our hypothesis that this mortality is largely based on plant consumption by the predators. First, we found that adding prey to the plants increased the survival of *O. insidiosus* adults, suggesting that this insect shifted its diet to prey from plant tissue thus reducing insecticidal exposure (Fig. 2). Also, the nymphal stage of *O. insidiosus* is strongly dependent on plant tissue for nutrition (Lundgren et al. 2008; Seagraves and Lundgren 2010), and we found that nymphal survival was more affected by the thiamethoxam treatment (Fig. 2). Prey availability did not affect the survival of these nymphs, which likely are more reliant on plant nutrients than the adult stage. The avoidance of a systemic insecticide by *O. insidiosus* adults when prey was available suggests toxicity is due to plant feeding and has been observed in other studies (Feese and Wilde 1975; Al-Deeb et al. 2001). It is important to note that while *O. insidiosus* field populations were reduced by approximately 35 and 19% by imidacloprid and thiamethoxam seed treatments, these deviations from the untreated control were not significant. This research not only confirms that insecticidal seed treatments have little effect on the key pest of soybeans, but also suggests that this prescriptive use of some of these insecticides may harm long-term IPM of soybean pests by reducing the abundance of their key natural enemies.

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