

Effects of Thiamethoxam Seed Treatments on Soybean Aphid (Hemiptera: Aphididae) Feeding Behavior

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J. Econ. Entomol. 106(6): 2384–2390 (2013); DOI: <http://dx.doi.org/10.1603/EC13268>

ABSTRACT Since its discovery in North America in 2000, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), has rapidly become an important pest of soybean [*Glycine max* (L.) Merrill], sometimes resulting in significant yield losses. Previous research has documented the toxicity of neonicotinoid seed treatments to soybean aphids, but control under field conditions has been inconsistent. Imidacloprid, a popular neonicotinoid insecticide, has been shown to exhibit antifeedant effects on aphids. Antifeedant activity has not been demonstrated for other neonicotinoids, including thiamethoxam. This research investigated the effects of a thiamethoxam seed treatment on soybean aphid feeding behavior by using electronic penetration graphs (EPG) to visualize stylet penetration behavior. Soybean aphid feeding behavior was assessed for 9 h on thiamethoxam-treated and untreated soybeans (V2 and V4 stages). Because results were inconclusive from initial experiments, a study was conducted to document the effects of thiamethoxam-treated soybeans on soybean aphid survival. The seed treatment was shown to negatively affect aphid survival at 4, 8, and 11 d after aphid introduction. A subsequent EPG study then was designed to document soybean aphid feeding behavior for 15 h, after an initial exposure of 9 h to thiamethoxam-treated soybeans. In this study, the exposed aphids exhibited significant differences in feeding behavior compared with those aphids feeding on untreated soybeans. Soybean aphids on thiamethoxam-treated soybeans spent significantly less time feeding in the sieve element phase, with a greater duration of nonprobing events. These studies suggest soybean aphids are unable to ingest phloem sap, which may be another important element in seed treatment protection.

KEY WORDS soybean aphid, thiamethoxam, seed treatment, electronic penetration graph

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), has become an important pest of soybean, *Glycine max* (L.) Merrill, in North America (Ragsdale et al. 2004, 2011). Native to Asia, the soybean aphid first was discovered in the United States in Wisconsin in 2000. Its distribution currently includes 20 midwestern and eastern states, and three Canadian provinces (Ragsdale et al. 2004, 2011). The soybean aphid's dramatic increase over the past decade is due, in part, to its high reproductive potential. A soybean aphid population can double in 1.5 d at 25°C (McCornack et al. 2004). Additional factors influencing its rate of increase include environmental conditions (temperature, humidity, and precipitation), number of overwintering aphid eggs, cultural prac-

tices (cropping, sowing time, and soybean variety), control measures (type and time), natural enemies, and synchronization of soybean and aphid development (Ragsdale et al. 2004). As aphid infestations build, indirect damage can result from nonpersistent virus transmission (e.g., alfalfa mosaic virus [family *Bromoviridae*, genus *Alfavirus*], soybean mosaic virus [family *Potyviridae*, genus *Potyvirus*], and tobacco ringspot virus [family *Secoviridae*, genus *Nepovirus*]) and sooty mold formation from honeydew excretions (Clark and Perry 2002). Heavy infestations are highly detrimental to soybean plants, resulting in wrinkled foliage, underdeveloped roots, stunting, lower pod and seed counts, and reduced seed weight (Ragsdale et al. 2004, 2011; Wu et al. 2004).

In 2012, >31 million ha of soybeans were planted (U. S. Department of Agriculture–National Agricultural Statistics Service [USDA–NASS] 2013) in the United States. Production value exceeded US\$35 billion in 2011 (USDA–NASS 2012), making soybeans a key agronomic crop. Before the appearance of the soybean aphid in North America, only two of the 12 north central states reported insecticide use on soybean, with less than 1% of the acres receiving applications (Ragsdale et al. 2011). With its arrival, annual

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yield losses of >40% were reported in some areas (Ragsdale et al. 2007), and insecticides became an essential management option, with more producers beginning to rely on foliar application of a pyrethroid or organophosphate (USDA-NASS 2006, 2007). In 2006, >16% of soybean acres in the United States were treated with an insecticide (USDA-NASS 2007), resulting in a 160-fold increase in use and as much as US\$16–33/ha increase in production costs (Ragsdale et al. 2007).

The release of neonicotinoid insecticides, such as thiamethoxam (CruiserMaxx, Syngenta Crop Protection, Greensboro, NC), as seed treatments provided growers another option for managing insect pests affecting seedling stage crops, including soybean aphids (McCornack and Ragsdale 2006). Its use has continued to increase in the following years and has remained popular with producers, regardless of their inconsistent performance against soybean aphids and minimal yield benefits (McCornack and Ragsdale 2006, Johnson et al. 2008, Magalhaes et al. 2009, Seagraves and Lundgren 2012).

Previous studies have reported that neonicotinoid insecticides negatively affect the feeding behavior of several hemipterans, including aphids (Nauen and Elbert 1994, Nauen 1995). However, the effect of neonicotinoid seed treated soybean on the feeding behavior of the soybean aphid by using the electronic penetrating graph (EPG) has yet to be reported. First described by McLean and Kinsey (1964) and Tjallingii (1978), EPG has become an increasingly popular tool for recording aphid feeding activity (Tjallingii 1978, 1985, 1988; Tjallingii and Esch 1993). The insect is wired into an electrical circuit with a host plant. The circuit is complete on insertion of the mouthparts into the plant, and changes in voltage (waveforms) over time are recorded. The objective of this study was to investigate the effects of a thiamethoxam seed treatment on soybean aphid feeding behavior by using EPG.

Materials and Methods

Plant Material and Seed Treatment. LG Seeds 2699RR (soybean aphid susceptible) seed was used in all studies. Seed was treated with the neonicotinoid insecticide, Cruiser 5FS (thiamethoxam, (E, Z)-3-(2-chloro-1, 3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine), at the labeled rate of 83 ml/100 kg of seed. Both seed-treated and untreated soybean plants were grown in plastic nursery pots (15.2 cm in diameter by 15.2 cm in depth; Reb Plastics Inc., Cleveland, OH) containing a Fafard Growing Media (Mix No. 3B; Conrad Fafard, Awawam, MA). Plants were maintained in a greenhouse (25 ± 2°C, 75 ± 5% RH, and a photoperiod of 16:8 [L:D] h) under 400-W high intensity discharge lamps, and received uniform daily watering throughout all studies. Growth rates were similar in the two EPG studies and screening assay.

Insect Colony. Apterous adult females (biotype I) were originally collected from infested fields near the

University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory (Dixon Co., NE) in 2007, with no previous exposure to neonicotinoid seed treatments. The colony was maintained in a growth chamber (25 ± 2°C, 75 ± 5% RH, and a photoperiod of 16:8 [L:D] h) on a continuous supply of vegetative (V4 to V6) 'KS4202' soybean plants. KS4202 was used because of its ability to tolerate significant aphid pressure for an extended period of time (Pierson et al. 2010, Prochaska et al. 2013). New plant material was introduced on a weekly basis.

EPG Recordings. Aphid feeding behavior was measured using the EPG-DC system (Giga-8 EPG model, EPG Systems, Wageningen, The Netherlands) with a 109-Ω resistance amplifier and adjustable plant voltage (Tjallingii 1978). Setup consisted of a copper plant electrode placed in the moist soil at the base of the potted plants. Output from the EPG was digitized at a sample rate of 100 Hz (100 samples per s) per channel by using a built-in data logger (DI-710, Dataq Instruments Inc., Akron, OH), and was recorded on the computer with EPG acquisition software (Stylet+, EPG Systems). The substrate voltage was monitored for fluctuations on the computer and adjusted at ± 5 V as needed. The gain was adjusted from 50 to 100× to improve the recording quality.

Insect electrodes consisted of a gold wire (10 μm in diameter and 2–3 cm in length; Sigmund Cohn Corp., Mount Vernon, NY) attached to the dorsum of the aphid by using silver conductive glue (4-ml water with one drop of Triton X-100 [Sigma-Aldrich, St Louis, MO], 4-g water soluble glue [clear paper glue, non-toxic; 3M, St. Paul, MN], and 4-g silver flake [purity: 99.95%; size: 8–10 μm]; Inframat Advanced Materials, Manchester, CT). The opposite end of the gold wire was attached to a copper wire (0.51 mm in diameter and 2 cm in length), which was soldered to a copper nail (1.6 by 19.0 mm). The electrode was inserted into the EPG probe once the aphid was securely attached. The EPG probe was an amplifier with a 1 giga-ohm input resistance and 50× gain (Tjallingii 1985, 1988).

All plants, EPG probes, and insect and plant electrodes were placed inside one of two Faraday cages to protect the EPG's internal conductors from electrical and environmental noise (Crompton and Ode 2010). The Faraday cages were constructed from aluminum mesh, which formed an aluminum frame and base (61 by 61 by 76 cm).

Initial Effect of Thiamethoxam Seed Treatments on Soybean Aphid Feeding Behavior. Thiamethoxam-seed treated and untreated soybean plants were grown to the V2 (fully developed trifoliate at second node) and V4 (fully developed trifoliate at fourth node) stages (Fehr and Caviness 1977). Plants were selected based on uniformity and transferred from the greenhouse to the laboratory (23 ± 5°C), and allowed to acclimate for ≈2 h.

Apterous adult females (biotype I) were collected from the laboratory colony and held without food in a petri dish for 1 h. During this time, the selected individuals were attached to an electrode. Following the 1-h starvation period, the wired aphid was care-

fully situated on the adaxial side of a V2 or V4 trifoliolate. Placement was considered successful if the aphid was able to freely move about on the leaf surface. Aphid feeding behavior was recorded for 9 h on thiamethoxam-treated V2 and V4 (thiamethoxam-V2 and thiamethoxam-V4) and untreated V2 and V4 soybeans (untreated-V2 and untreated-V4) under continuous light. The experimental design was an unbalanced block design with 20 replications per treatment. Each aphid recording represented a replicate. Plants were discarded after each 9-h recording.

Analyses of EPG recordings were based on the experimental design procedures described by van Helden and Tjallingii (2000). EPG waveforms were differentiated and categorized according to Reese et al. (2000). For analysis purpose, the waveforms are grouped into three main behavioral phases: pathway, xylem, and phloem or sieve element (Prado and Tjallingii 1994, Lei et al. 1999, Jiang and Walker 2001). The pathway phase (waveforms A, B, and C) is indicative of intercellular stylet penetration and withdrawal, no stylet movement, and brief intracellular punctures by stylet tips, known as potential drops (waveform pd; Prado and Tjallingii 1994, Jiang and Walker 2001). The three waveforms that constitute the pathway phase were categorized as waveform C for simplicity. The xylem phase (waveform G) occurs when the stylet tips are in xylem tissue (Janssen et al. 1989, Spiller 1990). The sieve element phase reflects salivary secretions and ingestion of phloem sap (waveforms E1 and E2, respectively). In some cases, these two waveforms are difficult to distinguish (Annan et al. 1997), so they were labeled as waveform E in both EPG studies. Waveforms F (stylet penetration problems) were not found in these recordings.

EPG feeding behavior parameters were selected from the Sarria Excel Notebook (Sarria et al. 2009). Parameters of interest in both EPG studies included time to first probe (elapsed time between placement of aphid on the plant to insertion of the mouthparts) and first sieve element phase. The total number of potential drops, pathway phases, sieve element phases, xylem phases, and nonprobing events also were recorded. Finally, the total duration (in minutes) of pathway phases, sieve element phases, xylem phases, and nonprobing events was calculated.

Effect of Thiamethoxam Seed Treatments on Soybean Aphid Survival. The results from the EPG study prompted a screening assay designed to determine whether the seed treatments impacted soybean aphid survival. Soybean plants were initially grown in the greenhouse. Approximately 1 wk before the soybeans reached V2 and V4, the plants were transferred to a walk-in growth chamber and allowed to acclimate. Ten apterous adult females (biotype I) were transferred to the adaxial side of the top trifoliolate. Ten replications of each treatment were arranged in a completely randomized design. Plants were measured 48 h after introduction to assess aphid survival. Additional aphids were added if fewer than 10 were present. Aphid numbers were measured 4, 8, and 11 d after introduction.

Effect of Thiamethoxam Seed Treatment on Soybean Aphid Feeding Behavior Following 9 h of Exposure. A second EPG study was conducted to further assess the effect of thiamethoxam-treated soybeans on soybean aphid feeding. For this study, 15–20 aphids were transferred from KS4202 soybeans (colony plants) and allowed to feed on thiamethoxam-treated or untreated V2 soybeans for 9 h under continuous light conditions. Following exposure, aphids were selected and placed in a petri dish and transferred to the laboratory for testing. Individuals were deemed acceptable for the study if they were capable of moving on their own. This was rarely an issue on untreated plants. However, uncoordinated movements suggesting intoxication were routinely observed with aphids collected from seed-treated plants. These individuals were discarded.

It was determined from the previous EPG study and screening assay that longer recordings were needed to assess soybean aphid feeding. After the initial 9-h exposure, surviving aphids randomly selected from the thiamethoxam-treated soybeans were transferred to another thiamethoxam-treated plant for a subsequent 15 h (thiamethoxam–thiamethoxam) for continued seed treatment exposure. Aphids selected as the controls were transferred from untreated soybean plants to an untreated test plant for 15 h (untreated–untreated). A third treatment (thiamethoxam–untreated) was included to determine whether aphid feeding, after the initial 9-h exposure, would more closely resemble that of those aphids fed on treated or untreated soybean plants. Individual aphids were randomly selected from thiamethoxam-treated soybeans and placed on untreated plants for 15 h. Tethering and EPG techniques were used as described previously. The experimental design was an unbalanced block design with 13 replications per treatment. Plants were discarded after each 15-h recording.

Statistical Analyses. The annotated EPG files were transferred into a Microsoft Excel Workbook spreadsheet (Microsoft Corporation, Redmond, WA) and waveform durations were calculated. For the first EPG study, data from all recordings were combined, separated by treatment (thiamethoxam-V2, thiamethoxam-V4, untreated-V2, and untreated-V4), replicate number (randomly selected), and waveform duration before converting to comma-separated values (CSV). Separately, data from the second EPG study were prepared in the same manner by treatment (thiamethoxam–thiamethoxam, thiamethoxam–untreated, and untreated–untreated), replicate number (1–20 or 1–13), and waveform duration. The CSV files were checked for errors by using a beta-program designed for SAS software (SAS Institute 2006, Cary, NC). Once errors in waveform labeling were corrected, treatments were tested for significance differences by using analysis of variance (ANOVA), implemented in SAS PROC GLIMMIX. When appropriate, means were separated using Fisher least significant difference (LSD) test ($\alpha = 0.05$).

The residuals from both EPG studies were assessed for normality by using graphical analysis of the resid-

Table 1. Mean number and duration of EPG feeding variables (\pm SEM) for soybean aphid feeding on thiamethoxam-treated and untreated V2 and V4 soybeans for 9 h

Feeding variable	Mean \pm SEM ^a			
	Thiamethoxam-V2	Thiamethoxam-V4	Untreated-V2	Untreated-V4
Time to first probe ^b	26.7 \pm 7.9a	19.9 \pm 5.3a	23.2 \pm 6.1a	30.0 \pm 9.8a
Time to first sieve element phase	267.9 \pm 47.9a	251.9 \pm 42.8a	278.8 \pm 39.6a	158.1 \pm 24.2a
No. potential drops	126.8 \pm 15.9a	120.7 \pm 11.1a	142.3 \pm 13.5a	107.4 \pm 9.0a
No. pathway phases	19.4 \pm 2.5ab	25.1 \pm 3.6a	28.7 \pm 4.3a	15.2 \pm 2.0b
Duration of pathway phases ^b	144.7 \pm 16.6a	139.4 \pm 16.5a	177.3 \pm 20.8a	123.8 \pm 12.2a
No. xylem phases	1.3 \pm 0.3a	1.7 \pm 0.4a	1.8 \pm 0.6a	1.2 \pm 0.2a
Duration of xylem phases	63.5 \pm 13.9a	67.2 \pm 12.2a	76.3 \pm 11.2a	54.3 \pm 20.5a
No. sieve element phases	1.1 \pm 0.2a	1.0 \pm 0.2a	1.0 \pm 0.2a	1.0 \pm 0.2a
Duration of sieve element phases	177.3 \pm 37.1a	155.7 \pm 33.9a	154.3 \pm 37.6a	239.5 \pm 33.7a
No. nonprobing events	17.8 \pm 2.6ab	23.3 \pm 3.7a	26.7 \pm 4.3a	13.8 \pm 2.1b
Duration of nonprobing events	218.1 \pm 27.6a	253.9 \pm 26.0a	199.5 \pm 28.1a	214.1 \pm 28.1a

^a Treatment means within the same row followed by the same letter indicate no significant differences ($P \leq 0.05$), LSD test.

^b Time and duration calculated in minutes.

uials and a Shapiro–Wilk test (Shapiro and Francia 1972). A log transformation was performed for the EPG data that did not exhibit a normal distribution. Transformed data were reconverted to the original scale for summarization in the tables.

Data from effect of thiamethoxam seed treatments on soybean aphid survival were analyzed using repeated measures ANOVA, implemented in SAS PROC GLIMMIX. The Akaike Information Criterion corrected (AICc) fit statistic was used to determine the most appropriate covariance structure, and the Toeplitz structure was selected. Treatment and evaluation date were considered fixed effects with one-way interactions for both being significant, and therefore, simple effects were used to determine whether differences existed among treatment means. Means were separated using Fisher LSD test ($\alpha = 0.05$).

Results and Discussion

Initial Effect of Thiamethoxam Seed Treatments on Soybean Aphid Feeding Behavior. EPG feeding variables from the first EPG study of the four treatments (thiamethoxam-V2, thiamethoxam-V4, untreated-V2, and untreated-V4) are reported in Table 1. Of the EPG feeding variables of interest, there was a significant treatment effect for the number of pathway phases ($F = 3.08$; $df = 3, 76$; $P = 0.0324$) and nonprobing events ($F = 2.96$; $df = 3, 76$; $P = 0.0375$) were detected. For the number of pathway phases, untreated-V4 was significantly less than thiamethoxam-V4 ($t = 2.33$; $df = 76$; $P = 0.0226$). Similarly, the number of nonprobing events with aphids on untreated-V4 was significantly less than thiamethoxam-V4 ($t = 2.15$; $df = 76$; $P = 0.0349$).

Previous studies found significant less sieve element feeding on resistant versus susceptible soybean genotypes (Diaz-Montano et al. 2007, Crompton and Ode 2010, Zhu et al. 2011). This difference was not observed in our study. This difference suggested that the initial feeding (9 h) of the soybean aphid was not strongly affected by the thiamethoxam seed treatment and was insufficient to cause intoxication. Because

changes in sieve element feeding were not observed during this period of time, it is unlikely that the aphids were able to detect the presence of the insecticide.

Effect of Thiamethoxam Seed Treatments on Soybean Aphid Survival. The comparison of mean aphid numbers among the four treatments is presented in Table 2. ANOVA detected a significant one-way interaction for insecticidal seed treatment ($F = 15.11$; $df = 3, 35.30$; $P < 0.0001$) and evaluation date ($F = 20.14$; $df = 2, 70.03$; $P < 0.0001$). Two-way interactions were not significant ($F = 1.01$; $df = 6, 71.21$; $P = 0.4235$). As one-way interactions were significant, simple effects were used to determine whether differences existed among treatment means. At 4 d after aphid introduction, the mean number of aphids on thiamethoxam-V2 was significantly lower than on untreated-V2 ($t = 2.99$; $df = 52.34$; $P = 0.0042$) and untreated-V4 ($t = 2.19$; $df = 52.34$; $P = 0.0334$). No significant differences in aphid numbers were observed among thiamethoxam-treated soybeans (thiamethoxam-V2 and thiamethoxam-V4; $t = 1.18$; $df = 52.34$; $P = 0.2444$).

Between 4 and 8 d after aphid introduction, there was a significant increase in aphid numbers on the thiamethoxam-V4 ($t = 2.49$; $df = 87.49$; $P = 0.0148$), untreated-V2 ($t = 5.96$; $df = 90.53$; $P < 0.0001$), and untreated-V4 ($t = 5.31$; $df = 92.38$; $P < 0.0001$) treatments. Changes in aphid numbers were not significant for thiamethoxam-V2 between 4 and 8 d after aphid introduction ($t = 0.43$; $df = 72.88$; $P = 0.6673$). At 8 d

Table 2. Mean number of soybean aphids (\pm SEM) for thiamethoxam-treated and untreated soybeans (V2 and V4 stage plants) after initial infestation of 10 aphids per plant

Treatment-V stage	Mean no. aphids \pm SEM ^a		
	4 DAI ^b	8 DAI	11 DAI
Thiamethoxam-V2	5.6 \pm 1.6a	4.2 \pm 2.3a	11.9 \pm 6.7a
Thiamethoxam-V4	17.0 \pm 3.2a	38.2 \pm 11.3b	69.9 \pm 24.5b
Untreated-V4	38.0 \pm 7.5b	124.4 \pm 33.1c	299.4 \pm 77.3c
Untreated-V2	71.3 \pm 9.8c	185.3 \pm 36.4c	453.8 \pm 73.0d

^a Treatment means within the same column followed by the same letter indicate no significant differences ($P \leq 0.05$), LSD test.

^b DAI, days after aphid introduction.

Table 3. Mean number and duration of EPG feeding variables (\pm SEM) for soybean aphid feeding on thiamethoxam-treated and untreated V2 soybeans for 15 h

Feeding variable	Mean \pm SEM ^a		
	Thiamethoxam–thiamethoxam ^b	Thiamethoxam-untreated	Untreated-untreated
Time to first probe ^c	11.0 \pm 4.4a	33.0 \pm 18.3a	6.1 \pm 2.5a
Time to first sieve element phase	144.6 \pm 31.5a	232.2 \pm 90.1a	140.9 \pm 19.9a
No. potential drops	109.2 \pm 29.3a	135.2 \pm 24.1a	139.3 \pm 27.3a
No. pathway phases	21.2 \pm 5.8a	18.7 \pm 4.2a	22.5 \pm 4.4a
Duration of pathway phases ^c	227.2 \pm 51.2a	223.4 \pm 40.9a	158.6 \pm 29.6a
No. xylem phases	0.9 \pm 0.3a	1.2 \pm 0.4a	0.8 \pm 0.3a
Duration of xylem phases	60.7 \pm 14.5a	105.9 \pm 19.3a	68.6 \pm 25.8a
No. sieve element phases	1.2 \pm 0.4a	2.0 \pm 0.7a	2.5 \pm 0.5a
Duration of sieve element phases	112.0 \pm 31.6a	354.6 \pm 96.6b	633.7 \pm 49.8b
No. nonprobing events	19.6 \pm 5.9a	16.2 \pm 3.6a	19.9 \pm 4.0a
Duration of nonprobing events	557.4 \pm 64.6a	403.9 \pm 79.2a	65.4 \pm 16.6b

^a Treatment means within the same row followed by the same letter indicate no significant differences ($P \leq 0.05$), LSD test.

^b Soybean aphids exposed to thiamethoxam-treated soybean plant for 9 h and subsequently transferred to another thiamethoxam-treated soybean plant where feeding was recorded for 15 h.

^c Time and duration calculated in minutes.

after aphid introduction, the thiamethoxam-treated soybeans continued to affect aphid survival. The mean number of aphids on thiamethoxam-V2 was significantly fewer than on untreated-V2 ($t = 3.96$; $df = 108$; $P = 0.0002$) and untreated-V4 ($t = 3.53$; $df = 52.34$; $P = 0.0009$). Further, thiamethoxam-V4 had significantly less aphids compared with untreated-V2 ($t = 4.59$; $df = 52.34$; $P < 0.0001$) and untreated-V4 ($t = 3.30$; $df = 52.34$; $P = 0.0018$). Interesting, significant differences also were observed between thiamethoxam-V2 and thiamethoxam-V4 ($t = 2.22$; $df = 52.34$; $P = 0.0309$).

From 8 to 11 d after aphid introduction, there was a significant increase in aphid numbers on the thiamethoxam-V4 ($t = 2.79$; $df = 81.58$; $P < 0.0065$), untreated-V2 ($t = 9.05$; $df = 89.45$; $P < 0.0001$), and untreated-V4 ($t = 7.28$; $df = 89.08$; $P < 0.0001$) treatments. Again, aphid numbers did not significantly increase on the thiamethoxam-V2 treatment ($t = 1.57$; $df = 91.67$; $P = 0.1202$). At 11 d after aphid introduction, the mean number of aphids for thiamethoxam-V2 was significantly less than thiamethoxam-V4 ($t = 2.92$; $df = 52.34$; $P = 0.0052$), untreated-V2 ($t = 6.41$; $df = 52.34$; $P < 0.0001$), and untreated-V4 ($t = 5.64$; $df = 52.34$; $P < 0.0001$). Although more aphids were present on the thiamethoxam-V4 versus thiamethoxam-V2 treatment (11.9 ± 6.7 and 69.9 ± 24.5 , respectively), thiamethoxam-V4 was significantly different than untreated-V2 ($t = 7.52$; $df = 52.34$; $P < 0.0001$) and untreated-V4 ($t = 5.66$; $df = 52.34$; $P < 0.0001$).

This study clearly demonstrates that thiamethoxam-treated soybeans negatively affect aphid survival. The findings are similar to that of McCornack and Ragsdale (2006) who observed significant soybean aphid mortality 24–48 h after exposure to thiamethoxam-treated soybeans.

Effect of Thiamethoxam Seed Treatment on Soybean Aphid Feeding Behavior Following 9 h of Exposure. The EPG feeding variables for the three treatments (thiamethoxam–thiamethoxam, thiamethoxam–untreated, and untreated–untreated) are reported in Table 3. There were no significant differ-

ences among treatments for time to first probe and first sieve element phase. Once feeding was initiated, there were no significant differences observed among treatments for mean number and duration of pathway phases, and number of potential drops. Further, no significant differences were observed among treatments for the mean number and duration of the xylem phases.

Although the mean number of sieve element phases was not significantly different among the three treatments ($F = 2.68$; $df = 2, 36$; $P = 0.0823$), significant differences in duration were detected ($F = 10.68$; $df = 2, 27$; $P = 0.0003$). Aphids on the thiamethoxam–thiamethoxam treatment spent significantly less time ingesting phloem sap from sieve element tissues than aphids on the thiamethoxam–untreated ($t = 2.50$; $df = 27$; $P = 0.0189$) and untreated–untreated ($t = 4.67$; $df = 27$; $P < 0.0001$) treatments. Significant differences were not observed between the aphids on the thiamethoxam–untreated and untreated–untreated treatments ($t = 1.80$; $df = 27$; $P = 0.0830$).

For nonprobing EPG parameters, there were no significant differences among treatments for the mean number of nonprobing events ($F = 0.10$; $df = 2, 36$; $P = 0.9033$). However, there were significant differences among treatments in the duration of nonprobing events ($F = 24.31$; $df = 2, 36$; $P < 0.0001$). Aphids on the thiamethoxam–thiamethoxam treatment had a significantly greater duration of nonprobing events than the untreated–untreated treatment ($t = 6.52$; $df = 36$; $P < 0.0001$). Significant differences also were detected between the thiamethoxam–untreated and untreated–untreated treatments ($t = 5.40$; $df = 36$; $P < 0.0001$). No significant differences were observed between the thiamethoxam–thiamethoxam and thiamethoxam–untreated treatments ($t = 1.12$; $df = 36$; $P = 0.2694$). Significant differences in these two parameters suggest >9 h of exposure to thiamethoxam-treated soybeans will negatively impact feeding behavior, resulting in aphid intoxication.

Our studies did not examine where and when the aphids imbibed the insecticide in the thiamethoxam-

treated plants. The chemical properties of neonicotinoid insecticides cause the majority of parent compound and secondary metabolites to be loaded into the xylem tissues of a plant (Sur and Stork 2003). In the second EPG study, over half (seven total) of the aphids exposed to the thiamethoxam-treated plants ingested xylem sap, and likely, the insecticide. This suggests that ingestion of thiamethoxam and its metabolites (e.g., clothianidin [Nauen et al. 2003]), through the plant xylem, may ultimately affect the aphid's ability to reach the sieve element tissues. For the aphids that did not achieve xylem ingestion, it is possible that a small amount of insecticide may be present in the phloem sap, resulting in reduced feeding (Nauen and Elbert 1994). Our findings are supported by the work of Costa et al. (2011), who observed similar feeding behavior in greenbugs, *Schizaphis graminum* Rondani, exposed to imidacloprid-treated wheat seed. Greenbugs exposed to treated plants exhibited significantly less phloem ingestion. Unlike our study, feeding was affected within 8 h of imidacloprid exposure (Costa et al. 2011).

In the second EPG study, soybean aphids spent less time probing on treated plants than on the control plants. When this observation is combined with those aphids of the first EPG study, it appears that after 9 h of thiamethoxam seed treatment exposure, aphids may reject the plant and terminate feeding. Similar behavior was reported by Nauen (1995), who observed that neonicotinoid insecticides appeared to cause an antifeedant response in green peach aphids, *Myzus persicae* (Sulzer), at sublethal exposure levels. This led to a failure to ingest plant nutrients, resulting in reduced weight gain, reduced honeydew excretions, starvation, and death. Arguably, the antifeedant nature of this chemistry was a factor in the greater duration of nonprobing events, and inability to feed following exposure to thiamethoxam seed treatments.

This study also documented the ability of the soybean aphid to recover after sublethal exposure to a thiamethoxam seed treatment. Aphids subjected to the thiamethoxam-untreated treatment exhibited more phloem ingestion than those exposed to thiamethoxam-thiamethoxam treatment, suggesting >9 h of exposure were needed before the detrimental effects of the thiamethoxam seed treatment could be observed. Neonicotinoid insecticides have been shown to influence EPG parameters of hemipteran feeding behavior, including stylet probing, xylem ingestion, and nonprobing activities (Nauen and Elbert 1994). Our results found no differences in these EPG feeding variables. Sieve element ingestion, however, was affected. This suggests the inability of soybean aphids to ingest phloem sap may be another important element in seed treatment protection. Additional research is needed to document the translocation of neonicotinoid insecticides within specific soybean vascular tissues. This information would improve our understanding of soybean aphid feeding behavior, and help to better explain the inconsistencies commonly associated with soybean aphid control under field conditions.

Acknowledgments

We gratefully acknowledge W. Freddy Tjallingii, Elaine Backus, and Predeesh Chandran for their technical support, and Timothy Ebert for assistance with the statistical analysis.

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Received 4 June 2013; accepted 6 October 2013.