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Method Development for Monitoring Bean Leaf Beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), Susceptibility to Thiamethoxam Seed Treatments on Soybeans[1]

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ABSTRACT The increased use of thiamethoxam seed treatments for controlling pests such as the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), suggests the need for methods to measure and monitor the development of resistance to thiamethoxam. The objectives of this study were to develop a bioassay method that can be used to monitor bean leaf beetle susceptibility to thiamethoxam, and to quantify the relative concentrations of thiamethoxam and clothianidin in early growth stage soybean tissue treated with thiamethoxam as a seed treatment. Overwintered and F1 bean leaf beetles were collected from alfalfa and soybean fields and used in excised soybean leaf laboratory bioassays to measure susceptibility to thiamethoxam. Petioles of excised leaves were immersed in 0, 10, 50, 100, 500 and 1000 ng a.i./ml thiamethoxam solutions, beetles were placed on the leaves, and beetle mortality and defoliation levels were recorded. The bioassay method proved adequate to calculate an LC_{50} and LC_{90}, and an EC_{50} based on defoliation level. The quantification of insecticide residues in soybean leaves from different vegetative stages indicates that the thiamethoxam concentration declines rapidly as the plant grows, and concentrations are at lethal levels for bean leaf beetles through V2. These results provide a method for monitoring bean leaf beetle susceptibility to thiamethoxam that is more representative of the pathway of exposure encountered by beetles in the field, particularly with respect to seed treatments, and provides an estimate of baseline susceptibility for future thiamethoxam resistance monitoring efforts.

KEY WORDS Neonicotinoid, bioassay, insecticide resistance, resistance monitoring

The bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), is a common pest of legumes, primarily cultivated soybeans, *Glycine max* (L.) Merr., across the United States. The insect is native to North America and is

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predominately found east of the Rocky Mountains (Kogan et al. 1980). The bean leaf beetle was historically more common as a soybean pest in the southern states (Kogan et al. 1980) before populations expanded their range into North Central and Midwestern states due to milder winter temperatures and the expansion of soybean cultivation (French & Hammack 2007).

The management of the bean leaf beetle is important to soybean growers across the United States (Krell et al. 2004). Adult bean leaf beetles feed on soybean leaves resulting in defoliation. Pod feeding is also a significant concern for soybean growers, which can cause direct yield loss (Smelser & Pedigo 1992) and increase the risk of disease transmission by the feeding bean leaf beetles (Shortt et al. 1982, Smelser & Pedigo 1992).

Chemical control is the primary method of management. Many insecticides have been used to manage bean leaf beetles, and the use of neonicotinoid seed treatments has become a common practice to manage early season populations (Johnson et al. 2008). Today, neonicotinoid insecticides are the most commonly used insecticides on soybean in the Midwest, primarily through seed treatments (Douglas & Tooker 2015). However, the short and long-term effects on insect pest populations from widespread and continuous use of neonicotinoids on soybeans are unknown.

One of the most commonly used neonicotinoids in seed treatment is thiamethoxam. Thiamethoxam was developed in 1992 and commercialized as a seed treatment in 1998 (Maienfisch et al. 2001). Thiamethoxam, along with its metabolite clothianidin, are second-generation neonicotinoids (Maienfisch et al. 2001, Nauen et al. 2003). Between 2008 and 2012, thiamethoxam was applied as a seed treatment to more soybean acres in the United States than any other neonicotinoid (i.e. imidacloprid and clothianidin) (US EPA 2014).

Continuous exposure of insect pests to plants systemically translocating thiamethoxam may pose a risk for the development of resistance to neonicotinoids (Bass et al. 2015). As demonstrated by the rapid reduction in susceptibility of bean leaf beetle populations to frequent pyrethroid applications (targeting stink bugs instead of bean leaf beetles) in Mississippi and Louisiana (Musser et al. 2012), repeated exposure to neonicotinoids may also reduce the susceptibility of the bean leaf beetle to this important group of insecticides. Development of reliable bioassay methods are necessary to establish a baseline of variation among field populations of bean leaf beetle, as well as to estimate exposure based on stage-specific concentrations of select neonicotinoids in soybean plants. The objectives of this study were to 1) develop a bioassay method that can be used to monitor bean leaf beetle susceptibility to thiamethoxam, and 2) to quantify the relative concentrations of thiamethoxam and clothianidin in early growth stage soybean tissue treated with thiamethoxam as a seed treatment.

Materials and Methods

Bean leaf beetle response to lethal and sublethal doses of thiamethoxam. Preliminary bioassays were performed to determine an appropriate range of insecticide concentrations. Technical grade thiamethoxam (99.5%) was purchased from Chem Service Inc. (West Chester, PA) and stored at −20°C. A stock solution of 1 μg thiamethoxam/ml acetone was used to prepare the dilutions

for the systemic bioassay, which consisted of five treatments (10, 50, 100, 500 and 1000 ng a.i./ml) and the control (0 ng/ml).

A complete randomized block design with 10 replications was used for the bioassays. Glass shell vials (16 mm × 50 mm) were glued into place with hot glue in the bottom right corner of each cell in a plastic tray consisting of 8 separate cells (10 cm wide × 8 cm depth) (C–D International, Pitman, NJ). Ten plastic trays were used as replications, with each cell as an experimental unit. The separate insecticide treatments were dispensed in 5 ml into the vials and covered with either rubber caps with a hole in the middle or Parafilm® with a slit cut in the center. Both were used to prevent beetles from falling into the solutions and to minimize evaporation.

Soybeans were grown in the greenhouse at the University of Nebraska – Lincoln, Lincoln, NE. Greenhouse conditions were maintained at 24–27°C with a photoperiod of 16:8 (L:D) h. Soybean variety NK2752R was planted in 15.2-cm pots containing a soil mixture of peat-perlite-vermiculite-soil mix at ratio of 34-31-31-4%. Soybeans were grown, four plants per pot, to at least the V1 stage (one fully expanded trifoliate) (Fehr & Caviness 1977). A single trifoliate with petiole attached was excised from the soybean plant, placed in a plastic Ziploc bag, and held in a cooler with ice for transport back to the lab. The excised soybean petioles were inserted into the rubber cap holes or slits in the Parafilm® so that the end of the petiole was immersed in the treatment solutions. Soybeans were allowed to regain turgidity for at least 15 h before beetles were released into each treatment cell.

Overwintered beetles were collected on 10 May 2011 from an uncut alfalfa field in Gage County (southeastern NE). Beetles were collected using the "Lazy 8" sweep net method (Kogan et al. 1980) along the edge of the field. Beetles were separated from other insects using an aspirator and placed in plastic containers with vented lids. Cut alfalfa was placed in the containers to provide a food and moisture source for the beetles. Containers were cleaned daily to remove frass and moisture build-up, and provided with fresh alfalfa cuttings. Sweet clover was sometimes used as an alternative forage. Beetles were separated by sex (3 females: 2 males) and held for 10 d in a growth chamber maintained at 14°C to limit feeding and mating.

First generation (F1) beetles were collected from soybean fields in Butler County (southeastern NE) using a single row cross sweep with a net (Kogan et al. 1980) in late July 2011. Beetles were provided with soybean leaves upon collection and for transfer back to the laboratory, and were held for 5 d under the same conditions as described above until needed for each bioassay. Beetles were sexed and separated (4 females: 1 male) just prior to bioassay.

Overwintered and F1beetles were starved for 24 h before being released into each cell of the trays. Adhesive plastic covers were placed on the top of the cells to prevent beetle escape. Mortality was recorded at 24-h intervals up to 96 h. Beetles were considered dead when they were on their elytra and no movement was detected. Beetles that exhibited slight leg and antennae movement were considered morbid and recorded as dead.

Termination of the study occurred when defoliation reached levels that would not support another 24-h period of beetle feeding, and the leaflets with the highest defoliation still allowed accurate leaf area measurements. Leaf area measurements and defoliation damage was assessed using ImageJ software (Wayne Rasband, National Institutes of Health, USA).

Mortality data were analyzed by probit analysis (Finney 1971) using POLO-Plus (LeOra Software 2002) and corrected using the Abbott's formula (Abbott 1925) to determine LC_{50} and LC_{90} values with confidence intervals. Defoliation data were analyzed by non-linear regression (Marçon et al. 1999) to calculate EC_{50} values (the concentration that caused 50% of defoliation compared to the control) with confidence intervals.

Translocation and detection of thiamethoxam applied as seed treatments in soybean tissue. Soybeans were planted on 13 May 2011 in 76.2-cm rows and grown under regular agronomic practices at the University of Nebraska Northeast Research and Extension Center Haskell Agricultural Laboratory, Concord, NE. Soil type was Baltic silty clay, and the soybean variety was Garst 25-F2 with the Rag 1 gene [resistant to the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae)] and commercially seed-treated with CruiserMax (3.0 fl oz per 100 lb seed or 0.0762 mg thiamethoxam per seed, mefenoxam, and fludioxonil).

Plots were 9.14 m long and 4 rows wide. Thirty random plant samples were taken from each plot at vegetative growth stages VC, V1, V2, V3, and V4. Whole plants were pulled from the ground, bagged by plot, and taken back to the lab in a cooler with ice. Cotyledon, unifoliate, and trifoliate leaves were removed at the node and separated. Because of the very low levels of insecticide in the plant tissues, leaflet samples from the same node were pooled to provide at least a 5-g sample (Magalhaes et al. 2009). Soybean samples were stored in a freezer at -20°C until insecticide residue assay.

Untreated soybean plants were grown in greenhouse conditions to determine neonicotinoid levels from a calculated LC_{50} (240 ng/ml). Eighty soybean plants were grown in greenhouse conditions described above. The plants were grown to V3, and the second trifoliate was harvested at the node leaving the petiole intact. Leaves were transported to the lab in a plastic bag in a cooler containing ice. Thirty excised soybean petioles were immersed in 150 ml of a 250 ng/ml thiamethoxam solution in two separate replications (60 total plants). After immersion for 48 h, soybean samples were removed from the solution and frozen until insecticide residue assay.

The following thiamethoxam assay procedures were adapted from Magalhaes et al. (2009). Individual stock solutions of all neonicotinoid analytes, the surrogate, and internal standard were prepared at concentrations of 5 μg μl^{-1} in methanol from analytical grade clothianidin (99.4% [a.i.]), imidacloprid (99.5% [a.i.]), thiamethoxam (99.5% [a.i.]) (Chem Service Inc., West Chester, PA), terbutylazine (surrogate, Sigma-Aldrich, Milwaukee, WI), and $^{13}C_3$-labeled atrazine (internal standard, Merck & Co., Whitehouse Station, NJ). Analyte, surrogate, and internal standard calibration solutions were prepared from the stock solutions diluted to 50 ng μl^{-1} in methanol. Calibration standard samples were prepared from the calibration solutions in a sample matrix obtained from the method extraction of untreated soybean trifoliates. Analytes and surrogate were added to individual calibration samples in amounts of 250, 1000, and 2500 ng to create a three-point calibration curve. Internal standard (2500 ng) was added to all calibration standards and samples to quantify analyte concentrations. Mean percent recovery of the surrogate from the 47 samples was 109 $\pm$ 4%. Analyte detection limits were estimated from instrument signal to noise to be 32 ng ml^{-1} in the final injection matrix, corresponding to an analyte concentration in soybeans of 0.01 μg g^{-1}.

Soybean samples were taken from the freezer and 5-g samples were weighed from the leaflet samples. The frozen samples were ground with a mortar and pestle in liquid nitrogen. The leaf material was placed in a 10-ml plastic tube and terbutalyzine was added as a surrogate at 2500 ng (50 μl of a 50 ng μl^{-1} solution) with 30 ml acetonitrile HPLC grade as an extraction reagent. After extraction, samples were shaken for 30 min at 4°C on a multi-purpose rotator (Model #2314, Thermo Scientific, Waltham, MA) and centrifuged at 3500 rpm, 16°C (IEC Multi-RF, Thermo Electron, Milford, MA) for 20 min. A 10-ml aliquot of the supernatant was mixed with 90 ml of reagent grade water. Aqueous extracts were passed through a 200-mg solid phase extraction (SPE) cartridge (Oasis HLB, Waters, Milford, MA) connected to a vacuum manifold. The Oasis HLB cartridge used for SPE was previously prepared by sequential washing with 5 ml of acetonitrile, methanol, and reagent grade water.

Insecticide residues were eluted from the SPE cartridge with 2 ml of methanol into a disposable culture tube (13 mm in width × 100 mm in depth) (Fisher Scientific, Pittsburgh, PA) and 2500 ng (50 μl of a 50 ng μl^{-1} solution) of ^{13}C-labeled atrazine was added as an internal standard. The eluant was then evaporated at room temperature under a nitrogen flow to approximately 200–300 μl. The concentrated solution was diluted to a final volume of 500 μl with double distilled water and filtered with a Mini-UniPrep™ Syringeless Filter (0.45 μm, pore size) (Whatman, Florham Park, NJ).

The prepared aliquots (containing analyte, terbutylazine and $^{13}C_3$-labeled atrazine) were analyzed by reverse phase HPLC/MS/MS utilizing a Waters 2695 HPLC autosampler/pump coupled to a Finnegan LCQ (Thermo Scientific, Waltham, MA) ion-trap mass spectrometer. HPLC separation utilized a Luna C8 (5 μm particle size) column (250 mm × 2 mm i.d.) (Phenomenex, Torrance, CA). The mobile phase was a 90:10 ratio of 0.1% (v/v) ammonium formate in water and 0.1% (v/v) ammonium formate in methanol for 2 min, followed by a 8 min linear gradient to a 20:80 mobile phase ratio, held for 12 min, then returned to a 90:10 ratio and held for another 10 min to re-equilibrate the column for a total run time of 30 min. The flow rate was 0.3 ml min^{-1} and sample injection volume was 25 μl. The LCQ mass spectrometer was operated in atmospheric pressure chemical ionization (APCI) mode with the vaporizer temperature at 350°C, the discharge current at 5.0 μA, the sheath gas as 80 (arbitrary units), the auxiliary gas at 1 (arbitrary units), the tube lens voltage at −5.0 V, the capillary voltage at 3.0 V, the capillary temperature at 150°C, the lens voltage at −36.0 V, the multipole 1 offset at −3.0 V, the multipole 2 offset at −5.0 V, and the multipole RF amplitude at 500 $V_{p\text{-}p}$. The daughter ion transitions and percent collision energies used in the analysis were for each analyte: imidacloprid (m/z = 256−>210, 30%), clothianidin (m/z = 250−>169, 25%), thiamethoxam (m/z = 292−>211, 25%), terbutylazine (m/z = 230−>174, 35%), and $^{13}C_3$-labeled atrazine (m/z = 219−>177, 35%). The isolation width was 3 amu and the activation time was 30 msec for all analytes. The collision gas was helium.

Results

Bean leaf beetle response to lethal and sublethal concentrations of thiamethoxam. Thiamethoxam had significant lethal effects on bean leaf beetles in bioassays involving treated soybean foliage, although the overwintering

population was generally more susceptible than the F1 generation. The estimated LC_{50} and 95% confidence limits (CL) for the overwintering population was 240.8 ng/ml (95% CL: 161.4–327.1) (Table 1) and the LC_{50} for the F1 generation was 750.2 ng/ml (95% CL: 396.0–3279.4).

Thiamethoxam also caused a significant sublethal response based on defoliation differences. The EC_{50} (effective concentration causing 50% defoliation compared to the untreated control) was 60.1 ng/ml (95% CL: 37.2–93.2) for overwintering beetles (Table 1) and 92.2 ng/ml (95% CL: 52.3–188.2) for F1 beetles.

Translocation and detection of thiamethoxam applied as seed treatments in soybean tissue. The highest concentrations for thiamethoxam per leaflet or structure was detected in the cotyledon of node 1 (N1) of the VC plant (2668 ng/g of tissue), which was the first growth stage sampled at 24 d after planting (DAP) (Table 2). Although thiamethoxam concentrations decreased within cotyledon samples over time, these concentrations were still almost 10-fold higher than any of the other samples collected from other vegetative stages and nodes. Because the cotyledon had senesced by 34 DAP, no further samples were obtained.

The thiamethoxam concentration in the leaf at node 2 (N2) of V1 plants was approximately 10-fold lower (108.8 ng/g) than the concentration in cotyledons at N1. A similar concentration was detected at N2 leaves of V2 plants (106.4 ng/g). Leaves at N2 were the oldest leaves sampled at 39 and 43 DAP, and the thiamethoxam concentration declined to a concentration of 30.3 and 25.9 ng/g of tissue, respectively.

At N3, the first trifoliate leaves were present and thiamethoxam was detected at 77.4 ng/g of tissue 31 DAP. At 34 DAP, the thiamethoxam concentration at N3 had declined to 49.7 ng/g of tissue, and this trend continued through 39 and 43 DAP.

Leaves at node 4 (N4) were sampled 34, 39, and 43 DAP. The highest concentration was found at the earliest sample date (V2 plant) at 27.6 ng/g of tissue. For V3 plants sampled 5 d later, the leaves at N4 thiamethoxam concentration dropped to 6.4 ng/g of tissue, but a slightly higher concentration of 9.8 ng/g of tissue was detected in V4 plants.

Detection of thiamethoxam within leaves in the newer foliage at node 5 and 6 were below 10 ng/g of tissue and just above detection limits. Leaves from V3 plants sampled 39 DAP had thiamethoxam concentration of 6.4 ng/g of tissue. The leaves at N5 and N6 of V4 plants sampled 43 DAP had thiamethoxam concentrations of 4.1 ng/g and 4.3 ng/g of tissue. In general, lower concentrations were detected in the newer foliage.

Clothianidin, an active metabolite of thiamethoxam, was consistently detected only in the cotyledons at N1. In VC soybean (24 DAP) the concentration of clothianidin was 72.3 ng/g of tissue (Table 2), approximately 37-fold lower than thiamethoxam. Clothianidin was also detected in the cotyledons at N1 of V1 soybean (31 DAP) and in V2 plants (34 DAP), with only trace amounts detected in leaves at N2–N4 for V3 plants and in N5 and N6 leaves for V4 plants.

Concentrations of thiamethoxam and clothianidin were also quantified from soybean trifoliates obtained from untreated seeds and immersed in a 250 ng/ml thiamethoxam solution for 48 h which is comparable to the LC_{50} of 240.8 ng/ml determined from the systemic bioassays described previously. The thiamethoxam concentration was 844.0 ng/g of tissue. This concentration is lower than thiamethoxam levels detected in cotyledons at N1, but higher than concentrations

Table 1. Estimated LC_{50}[a], LC_{90}[b] and EC_{50}[c] with 95% confidence limits of the overwintering and F1 bean leaf beetles.

Beetle population	LC_{50} (ng/ml)	LC_{90} (ng/ml)	EC_{50} (ng/ml)
Overwintering	240.8 (95% CL: 161.4–327.1)	937.8 (95% CL: 666.1–1568.4)	60.1 (95% CL: 37.2–93.2)
F1	750.2 (95% CL: 396.0–3279.4)	4021.0 (95% CL: 1450.5–294053.0)	92.2 (95% CL: 52.3–188.2)

[a]LC_{50} (lethal concentration) is the concentration required to kill 50% of the population tested.
[b]LC_{90} (lethal concentration) is the concentration required to kill 90% of the population tested.
[c]EC_{50} (effective concentration) is the concentration required to cause 50% defoliation compared to the untreated control.

Table 2. Residue profile of seed-treatment applied thiamethoxam and active metabolite clothianidin in soybean leaflets at each node up to growth stage V4 with corresponding days after planting.

	Thiamethoxam (ng/g)						Clothianidin (ng/g)					
	Node						Node					
Growth stage (days after planting)	1	2	3	4	5	6	1	2	3	4	5	6
VC (24)	2668.4						72.3					
V1 (31)	1821.1	108.8	77.4				43.6	0.0	0.0			
V2 (34)	1655.6	106.4	49.7	27.6			60.0	0.0	0.0	0.0		
V3 (39)		30.3	20.0	7.1	6.4			3.5	3.3	3.1	0.0	
V4 (43)		25.9	4.0	9.8	4.1	4.3		0.0	0.0	0.0	5.2	2.5

detected at N2, or any other detection within the soybean leaves. Clothianidin from the same trifoliates was detected at 19.0 ng/g of tissue.

Discussion

Thiamethoxam was highly active against bean leaf beetles in bioassays with an LC_{50} of approximately 250 ng/ml for overwintering beetles. Based on quantification of thiamethoxam from soybean leaves treated with LC_{50}, the concentration of thiamethoxam in treated leaf tissue corresponds to 844.0 ng/g of leaf tissue. Therefore, the LC_{50} was much lower than the concentrations of thiamethoxam in soybean cotyledons throughout the first three vegetative growth stages before cotyledons senesced and fell from the plant. These results suggest that seeds treated with thiamethoxam should be highly effective in controlling overwintering beetles as they attempt to colonize soybean fields. However, the estimated LC_{50} and corresponding tissue concentration was higher than any concentrations of thiamethoxam detected among different leaflets during later stages of plant development. This suggests beetles would have to colonize a soybean field prior to V2 to be subjected to a thiamethoxam concentration equivalent at or above the LC_{50}. It should be noted that although exposure after V2 may not cause high mortality, reduced defoliation could still be achieved as the EC_{50} was well below the LC_{50}. Thiamethoxam at sublethal concentrations affected feeding and reduced defoliation. This suggests that soybeans are protected from bean leaf beetle defoliation at lower concentrations of insecticide than necessary to cause significant adult mortality.

Concentrations of thiamethoxam and its active metabolite, clothianidin, rapidly decrease in each new soybean leaf under field conditions. The concentration of insecticides is high in cotyledons, but is much lower in other soybean leaves. Both compounds decreased in concentration over time. As the plant matures, less insecticide is available for uptake and translocation within the plant and concentrations in plant material rapidly decline and are not evenly distributed among the plant structures. At the end of the sampling period, 41 DAP, the thiamethoxam concentration barely exceeded the detection limit, and it seems unlikely that control could not be achieved in new trifoliates arising from node 4 and later. These results support those reported by Magalhaes et al. (2009) who reported a rapid decline in neonicotinoid concentrations in soybean foliage from seed treatments. These results indicate that the level of bean leaf beetle control is likely to decrease over time after soybean emergence and control of F1 adults from seed treatment insecticide in leaf tissue is unlikely. These results are in agreement with those reported by McCornack & Ragsdale (2006) who concluded mortality of soybean aphids was significantly higher in older leaves than in new trifoliates.

Bioassay methods should be designed considering the characteristics of the insect/insecticide system under question (Galdino et al. 2011). Other bioassay methods, such as glass-vial bioassays, have been widely used to monitor and detect insecticide resistance, but systemic-uptake bioassays have also been used (e.g., Cahill et al. 1996, Prabhaker et al. 1996, Prabhaker et al. 2005, Castle et al. 2014, Matsuura & Nakamura 2014). Although methods such as the glass-vial bioassay are reliable, more rapid, and require less insecticide when compared to the detached-leaf method, the systemic detached-leaf bioassay is more

representative of the pathway of exposure encountered by bean leaf beetles in the field. The detached-leaf bioassay also allows detection of the effects of sub-lethal concentrations (Magalhaes et al. 2008).

The present studies provide a foundation for determining baseline susceptibility of bean leaf beetles to systemic neonicotinoid insecticides. This is becoming increasingly important as the number of acres planted with neonicotinoid-treated soybeans has increased significantly since 2000 (Douglas & Tooker 2015). The implications of these results when choosing a pest management strategy also are important, especially when considering resistance management. Integrated pest management (IPM) strategies call for management tactics such as crop rotation, crop refuse destruction, tillage, plant spatial arrangements (Quisenberry & Schotzko 1994), and use of economic thresholds before employing curative (often chemical) methods. While use of seed treatments can be an effective tool in an IPM program, particularly for other hard to scout or manage early season pests, choosing seed treatments with this high level of control before bean leaf beetle populations can be assessed is not generally recommended because there are procedures for scouting and estimating risk of bean leaf beetle injury, the decision is made before populations are present, and overuse will increase selective pressures (Krupke et al. 2015). Monitoring bean leaf beetle response to thiamethoxam seed treatments and supplementary neonicotinoid foliar applications to detect the development of resistance is therefore advisable. In addition, as beetles colonize a newly emerged soybean field, there should be adequate amounts of insecticide available to cause significant mortality; but as soybeans grow new vegetative material, the amount of insecticide available decreases. This suggests that beetles may have exposure to neonicotinoids below lethal levels, which could also contribute to the development of resistance.

Other considerations for future research include investigating the variability of adult beetle response to treatments across generations. The present results suggest overwintered beetles are more susceptible than the F1 generation. Increased susceptibility of the overwintering population may have been associated with stress associated with overwintering, mating, age, and reproduction and other physiological differences compared to the F1 generation. Although there is little insecticide available to control the F1 generation, there may be an effect on the F1 larvae, which feed in the root zone. In addition, water availability and aspects of soil composition and chemistry likely affect insecticide uptake and translocation within the plant. This field study was conducted at one location with adequate moisture, but because these compounds are water soluble, the availability of neonicotinoids could vary depending on soil moisture. Soil composition and chemistry may also affect how the insecticide binds to soil particles and is available for uptake by the plant.

Continued integration of neonicotinoid seed treatments into soybean pest management is expected for the control of various pests. The bioassay method developed here provides an efficient method for monitoring bean leaf beetle susceptibility to neonicotinoids and a foundation for future resistance monitoring efforts.

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