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Research Article

Acute-Contact and Chronic-Systemic In Vivo Bioassays: Regional Monitoring of Susceptibility to Thiamethoxam in Soybean Aphid (Hemiptera: Aphididae) Populations From the North Central United States

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Abstract

The risks associated with soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in the North Central soybean systems has fostered the adoption of prophylactic chemical control practices, such as planting seeds treated with neonicotinoid insecticides, especially thiamethoxam. Consequently, there is a concern that increased selection pressure imposed on the arthropod–pest complex by this insecticide may lead to resistance. Therefore, in vivo bioassays were conducted to determine the susceptibility of soybean aphid to thiamethoxam among North Central U.S. populations. Concentration-mortality data were collected using contact glass-vial and detached-leaf systemic bioassays. The results of these experiments indicate that both bioassays were reliable to detect shifts in susceptibility between different soybean aphid clones. The LC_{50}s of field populations of soybean aphid were significantly different when mortality was recorded in contact and systemic exposure assays. Nevertheless, the magnitude of the resistance ratios was consistent in both methods. In addition, a significant increase in the LC_{50} and EC_{50} values was observed among field populations tested in detached-leaf systemic bioassays. These results represent the first extensive efforts to identify the variability in susceptibility of soybean aphid to thiamethoxam in the North Central United States Therefore, our results provide a baseline for future assessment and contribute to a better understanding of the applicability of in vivo bioassays for susceptibility monitoring and resistance detection of soybean aphid to thiamethoxam.

Key words: insecticide susceptibility, resistance monitoring, in vivo bioassay, thiamethoxam, soybean aphid

The soybean aphid is one of the most important insect pests of soybean in the North Central United States (Ragsdale et al. 2007). The feeding injury caused by soybean aphids can result in reductions of photosynthetic rates, plant height, the number of pods per plant, seeds per pod, seed coat quality, seed weight, and increased pod abortion (Lin et al. 1993, Macedo et al. 2003, Beckendorf et al. 2008). Yield losses of 40–50% have been reported (Wang et al. 1994, Ragsdale et al. 2007). Indirect injuries from aphid transmitted plant viruses are also associated with soybean aphid feeding (Hill et al. 2001).

In the United States, chemical control with insecticides is the most common management strategy employed to control soybean aphid populations (Myers et al. 2005, Ragsdale et al. 2007, Magalhaes et al. 2008, Chandrasena et al. 2011, Hodgson et al. 2012, Krupke et al. 2017). Over a period of ~6 years, the pest potential of this species throughout the North Central United States affected conventional management practices, increased scouting activities, increased production costs (Ragsdale et al. 2007), and increased insecticide use (Song and Swinton 2009, Ragsdale et al. 2011).

In recent years, neonicotinoid-treated soybean seeds have been planted over large areas as a prophylactic treatment targeting several early-season soybean pests (Magalhaes et al. 2008, Johnson et al. 2008). In addition, neonicotinoids represent an important active ingredient of several foliar-applied insecticides registered for use on soybean. This widespread and chronic exposure over consecutive generations and all instars of soybean aphid is likely to increase the risk for resistance development (Magalhaes et al. 2008). Although the potential for soybean aphid to cause significant loss is high and chemical control represents the only management option in this region, there is a general lack of information on the evolution

of insecticide resistance in this pest (Chandrasena et al. 2011). Insecticide resistance increases costs of pest management, human health, and environmental risks (Pimentel et al. 1992). Conversely, the rational and judicious use of insecticides may mitigate the economic and environmental impacts of chemical control, reduce the frequency of resistance alleles among pest populations, and increase profitability (ffrench-Constant and Roush 1990, Pimentel et al. 1992).

Insecticide resistance in soybean aphids have been reported in China, where resistance was reported at low frequency to the organophosphate insecticide, omethoate (Qian et al. 2012). In another study, laboratory selection with the pyrethroid lambda-cyhalothrin resulted in increased levels of resistance (76.6-fold) (Xi et al. 2015). In addition, the results from the synergism experiments, suggested high risk for cross-resistance to chlorpyriphos, acefate, esfenvarelate, cyfluthrin, cypermethrin, bifenthrin, carbofuran, and methomyl. However, in other aphid species, resistance to neonicotinoids has become common due to a reliance on foliar and seed treatments with these compounds (Bass et al. 2015). Resistance to thiamethoxam has been reported in *Myzus persicae* (Bass et al. 2011, Bass et al. 2015), *Aphis gossypii* (Herron and Wilson 2011), and *Brevicoryne brassicae* (Ahmad et al. 2013). In *M. persicae*, neonicotinoid resistance has been associated with multiple mechanisms, including the overexpression of a single cytochrome P450-dependent monooxygenase (CYP6CY3), and a conserved mutation in the insect β1-subunit of the nicotinic acetylcholine receptor (nAChR) known as R81T (Puinean et al. 2010, Bass et al. 2011, 2014) which is the target site for neonicotinoid insecticides. The R81T amino acid substitution was also found to be present in the β1-subunit of the nicotinic acetylcholine receptor of an imidacloprid-resistant strain of *A. gossypii* and in other field populations from Eastern Asia (Koo et al. 2014).

Pests resistant to insecticides of different modes of action, or with several mechanisms of resistance associated with a single mode of action, represents major limitations to chemical control (Jeschke et al. 2010, Fuentes-Contreras et al. 2013, Yu 2014, Bass et al. 2015). For this reason, management strategies based on insecticides require resistance monitoring to ensure efficacy and longevity (Forrester 1990, Siegfried et al. 2007). Monitoring for insecticide resistance is a fundamental component of insecticide resistance management (IRM) programs that provide early detection of resistance (Siegfried et al. 2007, Guedes 2017) and inform proactive pest management strategies that minimize selective pressures and prevent or at least delay field control failures (Mota-Sanchez et al. 2006, da Silva et al. 2012, Caballero et al. 2013, Guedes 2017).

The detection of changes in the insecticide susceptibility levels of pest populations commonly relies on the use of validated and sensitive in vivo bioassays (Siegfried et al. 2007, Magalhaes et al. 2008, Snodgrass et al. 2008, Galdino et al. 2011, Gore et al. 2013). Insecticide bioassays can guide the selection of the most cost-effective, efficient, and selective products and commonly serve as a basis for classification and labeling of insecticides (ffrench-Constant and Roush 1990). Although in vitro assays can be an important and complementary tool to classic in vivo bioassays (Siegfried and Ono 1993a,b; Moores et al. 1996; Foster et al. 2000; Criniti et al. 2008), they are not a replacement as they are normally specific to only one resistance mechanism (Nauen et al. 2015).

In vivo bioassays are commonly used to calculate the lethal concentration required to kill 50% of the insects tested (LC_{50}) but may vary for a variety of reasons including bioassay method (Robertson et al. 2007), arithmetical method (Saganuwan 2011), rearing conditions (Beranek 1974, Cueto et al. 2006), insect taxa (Stark and Sherman 1989), physiological development (Qu et al. 2015), prestress exposure (Maltby 1999), and symbiont composition (Ghanim and Kontsedalov 2009). In aphids, intraclonal genetic variation (Shufran et al. 2003) and epigenetics (Field and Blackman 2003) are also known to be associated with variation in responses to insecticides.

Bioassay methods should be tailored to aspects of a specific insect/insecticide system (Galdino et al. 2011), and it is essential to understand the inherent characteristics of the system (Gerami et al. 2013). Variation in bioassay response is always present and can even be observed between response curves obtained from the same sample, between samples of the same population and from samples of different populations (Robertson et al. 1995, Schaub et al. 2002). More importantly, significant differences between two populations with non-overlapping CL, do not always indicate resistance (Robertson et al. 2007). For this reason, estimating the 95% limits of intrapopulation variation is considered crucial for monitoring studies (Siegfried et al. 2007) and has been shown to guide the differentiation in response to toxicants between insect pest populations (Robertson et al. 1995).

The use of bioassays to monitor soybean aphid susceptibility to thiamethoxam is necessary to support the development and validation of a standard methodology for resistance monitoring in the North Central United States The present study was performed between 2012 and 2015 and documents the lethal and sublethal effects of systemic and contact exposure, as well as detection of potential shifts in response of soybean aphid populations to thiamethoxam.

Materials and Methods

Rearing Conditions

In total, 26 soybean aphid populations were obtained from collaborators between 2012 and 2014 as part of a North Central Soybean Research Program project (Table 1, Fig. 1). Infested leaves were gathered from soybean fields at different locations in South Dakota, North Dakota, Minnesota, Wisconsin, Iowa, Indiana, Michigan, Ohio, and Nebraska. In addition, two laboratory populations were obtained from USDA-APHIS National Biological Control Laboratory in Niles, MI, and from USDA-ARS laboratory in Brookings, SD. One potentially susceptible soybean aphid strain, originally collected in 2005, was maintained under pesticide-free laboratory conditions at Bayer Crop Science (Morrisville, NC). A subset from this strain, named laboratory reference colony (lab) was started in 2013 at the University of Nebraska and used as a reference strain in this study.

Prior to the establishment of soybean aphid colonies, the samples were inspected for parasitoid presence and quarantined until no parasitized aphids were found. An average of 200 aphids were used to establish each soybean aphid colony after quarantine. In addition, a sample of 100 aphids from each field population collected in 2012 were combined into a single colony, named Field_Pooled1 and in 2014 a set of 200 aphids were used to establish Field_Pooled2 population (Table 1). In total, 28 soybean aphid colonies were used as a source for all bioassays performed from 2012 to 2015. Soybean aphid populations collected in 2012 were bioassayed from 2012 to 2013, and those collected in 2014 were bioassayed from 2014 to 2015. The colonies were maintained on pesticide-free (*cv* KS4202) V2 soybean (Fehr et al. 1971) at 24 ± 3°C, 70 ± 5% relative humidity, and photoperiod of 16:8 (L:D) h inside individual plant growth chambers (model I-35VLX Percival Scientific Inc., Boone, IA) and transferred to non-infested seedlings every week.

Table 1. Source description of *A. glycines* populations, year of collection, collection sites, and origin

Soybean aphid populations	Collection date	Location	Origin*
SD1	2009	USDA-ARS, SD	Lab
SD2	June, 2012	Brookings, SD	Field
SD3	June, 2012	Brookings, SD	Field
SD4	June, 2012	Aurora, SD	Field
ND1	June, 2012	Fingal, ND	Field
ND2	June, 2012	Mapleton, ND	Field
MN1	June, 2012	Lamberton, MN	Field
NE	June, 2012	Dixon, NE	Field
WI1	June, 2012	Eau Claire, WI	Field
WI2	June, 2012	Monroe, WI	Field
WI3	June, 2012	Black River Falls, WI	Field
Field_Pooled1	2012	(100 Aphids from each other collection)	Field
Susceptible laboratory	April, 2013	Bayer Crop Sciences, NC	Lab
MI1	June, 2014	Ingham, MI	Field
MI2	June, 2014	Sanginaw Valley Research Center, MI	Field
MI3	2001	USDA-APHIS Niles, MI	Lab
MN2	July, 2014	Lamberton, MN	Field
IA1	July, 2014	Story County, IA	T-Field*
IA2	July, 2014	Story County, IA	NT-Field*
IA3	July, 2014	Ames city, IA	T-Field*
IA4	July, 2014	Ames city, IA	NT-Field*
SD5	July, 2014	Brookings, SD	Field
OH	July, 2014	OARDC - Wooster City, OH	Field
IN	Aug, 2014	West Lafayette, IN	Field
ND3	Aug, 2014	North Fargo	Field
ND4	Sep, 2014	Arvila, ND	Field
WI2 (2014)	Aug, 2014	Monroe, WI	Field
WI4	Aug, 2014	Monroe, WI	Field
Field_Pooled2	2014	(200 Aphids from each other collection)	Field

*T-Field: thiamethoxam seed-treated fields, and NT-Field: thiamethoxam non seed-treated fields.
Populations collected in 2012 were bioassayed from 2012 to 2013 and the populations collected in 2014 were bioassayed from 2014 to 2015.

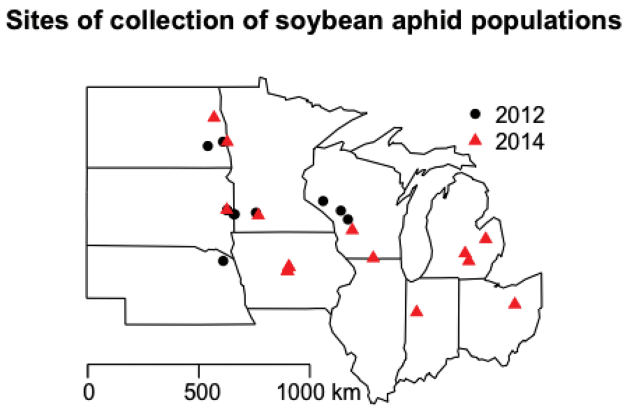

Fig. 1. Location of sites in North Central United States, where clones of soybean aphid monitored for thiamethoxam susceptibility were collected. For collection information, refer to Table 1.

Insecticide

Technical grade thiamethoxam was purchased from Chem Service (West Chester, PA) and stored at –20°C until the preparation of the stock solutions and insecticidal serial dilutions.

Plant Material

Soybeans were planted at one seed per pot (15 cm diameter × 17 cm deep) using a commercial soil mixture Fafard 3b (Sungro Horticulture, Agawam, MA). Plants were grown in greenhouses at 25 ± 7°C (Alto 1000-W high-pressure sodium, Philips, Somerset, NJ) with a 16:8 (L:D) h photoperiod. The V2 plants served as host plants for the aphid colonies, and were replaced weekly, whereas V3–V4 soybean seedlings were used for the systemic detached-leaf bioassays.

Vial Bioassay

Stock solutions and serial dilutions of thiamethoxam were prepared in acetone. To examine the contact toxicity of thiamethoxam on soybean aphid populations, 0.5 ml of each concentration (0, 2, 20, 200, 2,000, and 20,000 ng/ml) was placed into 20-ml scintillation glass vials. Vials were then dried using a commercial hot dog roller grill (model 8045SXW NEMCO Food Equipment, Hicksville, OH) without heating to allow evaporation of acetone and provide a uniform coating on the inside of the vial. Once the vials were completely dry, they were capped and stored until the aphids were transferred. Each vial received 20 wingless aphids from all developmental stages. Mortality was recorded at 24 h after treatment. Each bioassay consisted of three replicates per thiamethoxam concentration.

Detached-Leaf Bioassay

Insecticidal stock solutions were prepared in acetone and diluted to desired concentrations in distilled water. To avoid mortality from acetone, the highest concentration of acetone in the insecticide solutions was <0.01%. The detached-leaf systemic bioassays followed methods described by Magalhaes et al. (2008). Each plastic tray (CD International, Pitman, NJ) with eight square cells per tray (8 cm in depth × 10 cm in length) was used, and 5-ml glass tubes were

attached on the bottom of each tray cell. The petioles of excised V3–V4 soybean trifoliates were immersed in the 5-ml glass tubes containing different thiamethoxam concentrations. The trifoliates were held for ~12 h in the absence of aphid pressure until the trifoliates regained turgidity, thereby assuring proper insecticide uptake. Thirty aphids of all instars were transferred to the trifoliates using a fine paintbrush (model 00 Connoisseur 367 W-Talklon Round, Beaverton, Oregon). Each cell was then sealed with a transparent and porous plastic lid to avoid aphid escape. The number of dead and living aphids was recorded after 7 d. The experiment included five insecticide concentrations (1, 5, 10, 50, and 100 ng/ml) plus the controls (water and 0.01% acetone), with three replications per treatment.

Statistical Analysis

The results of both bioassay methods were analyzed by Probit (Finney 1947) using Polo-Plus (LeOra Software, 2002). The program estimates the lethal concentrations (LCs) with its 95% CLs, slope, and SE. A chi-square goodness-of-fit test (χ^2) was employed to verify the proper fit of toxicity data to the probit model. The mortality data were corrected for control mortality using Abbott's formula (Abbott 1925). Resistance ratios (RRs) were calculated by dividing the LC_{50} and LC_{90} of each field population by LC_{50} and LC_{90} of the lab colony within the same year of monitoring, and it estimates the magnitude of differences in susceptibility between tested aphid populations. The RRs were classified as equal (RR ≤ 1), very low (RR = 2 - 10), low (RR = 11–20), moderate (RR = 21–50), high (RR = 51 - 100), and very high (RR > 100) (Ahmad et al. 2010, Ahmad and Akhtar 2013). The vial bioassay with the lab colony was replicated twice in 2012 and the data were pooled and analyzed, resulting in a composite LC_{50}. In 2014, a composite LC_{50} was calculated from 12 replications with the lab colony. Statistical differences occurred when the 95% CLs did not overlap. The differences in the mean LC_{50}s and EC_{50}s of all field collections between years of monitoring were analyzed with analysis of variance (ANOVA) and least square means (LSMEANS) at 0.05 probability level using PROC GLIMMIX (SAS, v. 9.3; SAS Institute Inc.). The latter statistical procedure was also used to determine differences in fitness between the lab colony and field populations by using the survivorship data recorded after 7 d of systemic exposure to untreated controls. The number of nymphs produced were transformed to percentage population growth inhibition relative to controls and used to calculate the effective concentrations (EC_{50}s) in the detached-leaf systemic bioassays. The data were then analyzed by non-linear regression using PROC NLIN (SAS, v. 9.3; SAS Institute Inc.) fitted to a probit model as described by Marçon et al. (1999).

Results

Vial Bioassay

A significant interaction was detected between the years of monitoring (2012 and 2014) and the mean LC_{50} of thiamethoxam calculated for all tested populations, including the lab colony ($F_{3, 38}$ = 13.23; P < 0.0001). In both years, the mean LC_{50} of field populations differed from that of the lab colony (t = 2.30; d.f. = 38; P = 0.0269; and t = 5.82; d.f. = 38; P < 0.0001 for 2012 and 2014, respectively). There was no significant difference between the mean LC_{50} of tested field populations between 2012 and 2014 (t = –1.26; d.f. = 38; P = 0.2163) (Fig. 2).

Toxicity of thiamethoxam measured as mortality after 24 h of exposure is shown in Tables 2 and 3. In 2012, the LC_{50} of the field strain ND2 was significantly higher than the LC_{50} of the lab colony (5.0-fold) (Table 2). Out of 16 populations of soybean aphid evaluated in 2014, IA1, IA3, IA4, IN, and Field_Pooled2 showed significantly higher LC_{50} when compared with the lab colony (Table 3). In 2014, there was no significant difference in susceptibility to thiamethoxam between populations originating from treated fields (IA1 and IA3), or non-treated fields (IA2 and IA4) (Table 3). The high chi-square obtained for MN1, NE, Field_Pooled1, and ND3 populations indicate a significant departure (α = 95%) from the probit model.

Differences in susceptibility were also observed between 2012 and 2014 at the LC_{90} level (Tables 2 and 3). In 2012, the LC_{90} of SD4, NE, and Field_Pooled1 were significantly higher than the LC_{90} of the lab colony. In 2014, except for MI1, MI2, MI3, OH, and WI4, all other populations differed from the LC_{90} of the lab colony (RRs ranged from 4.7- to 10.8-fold).

Fitness of Tested Populations After 7 d of Systemic Exposure (Untreated Controls)

There was no significant difference on the mean number of living aphids after systemic exposure to untreated controls when comparing field and lab colonies between 2012 (t = -0.2; d.f. = 24; P = 0.8398) and 2014 (t = -1.09; d.f. = 24; P = 0.284). However, the population numbers of field populations were significantly higher in 2014 when compared with 2012 (t = -2.37; d.f. = 24; P = 0.0263) (Fig. 4).

Detached-Leaf Bioassay

There was a significant interaction between year of monitoring and the mean LC_{50} of thiamethoxam generated from all tested populations ($F_{1, 24}$ = 10.55; P = 0.0034). The mean LC_{50} increased from 2012 to 2014 (t = -7.98; d.f. = 24; P = 0.0034) (Fig. 3A). The lethal effects of thiamethoxam measured after 7 d of systemic exposure are shown in Tables 4 and 5 for 2012 and 2014, respectively. In 2012, the field strains SD4, WI1, WI2, and Field_Pooled1 differed significantly from the LC_{50} of the lab colony with RRs of 5.7, 3.6, 6.3, and 3.7-fold, respectively. The high chi-square calculated for

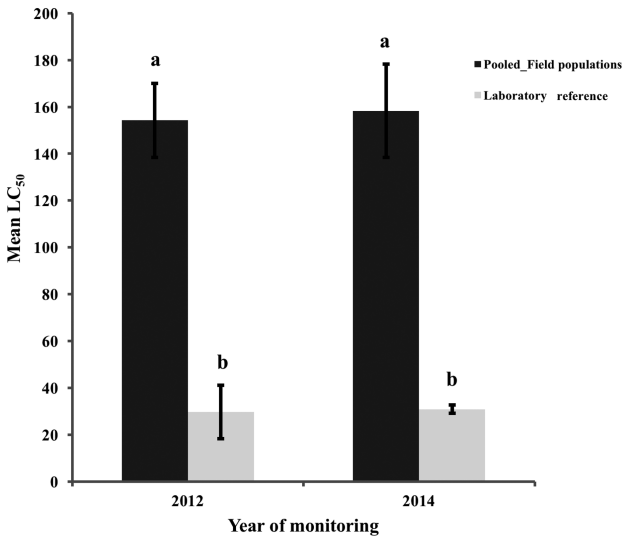

Fig. 2. Mean LC_{50} of *A. glycines* populations exposed to thiamethoxam in contact vial bioassay. Mortality was measured after 24 h of exposure. Error bars represent the standard error of the mean. Means with different letters between columns indicate significant differences (P < 0.05) between field and laboratory populations across and within years of monitoring. Field populations (2012) n = 3,960, Field populations (2014) n = 5,400, Susceptible laboratory colony (2012) n = 720, Susceptible laboratory colony (2012) n = 4,320.

Table 2. Thiamethoxam contact vial bioassays performed on all group ages of *A. glycines* collected from soybean fields, North Central United States in 2012

Colonies of SBA	*n**	Slope ± SE	LC_{50}† (95% CL)	RR‡ (LC_{50})	LC_{90}† (95% CL)	RR‡ (LC_{90})	χ^2 (d.f.)§
SD1	360	0.835 ± 0.118	115.5 (17.5–349.8)	4.0	3963.1 (1215.5–41501.0)	5.3	3.2 (3)
SD3	360	0.870 ± 0.117	188.6 (20.6–674.7)	6.5	5597.5 (1433.123–143577.6)	7.6	4.8 (3)
SD4	360	0.948 ± 0.147	247.7 (23.3–758.7)	8.6	5565.4 (1678.5–0.14083E +06)‖	7.5	4.2 (3)
ND1	360	0.774 ± 0.076	99.1 (28.5–316.4)	3.4	4483.9 (1091.3–78143.0)	6.1	5.1 (3)
ND2	360	0.868 ± 0.087	142.4 (56.0–306.0)‖	5.0	4268.1 (1231.2–63940.0)	5.8	4.0 (3)
WI1	360	0.919 ± 0.129	171.3 (15.8–611.1)	6.0	4253.4 (1125.2–0.10634E+06)	5.7	4.8 (3)
WI2	360	0.758 ± 0.110	102.3 (2.4–485. 9)	3.5	5008.7 (995.5–0.5673E+06)	6.8	5.6 (3)
WI3	360	0.793 ± 0.099	114.7 (8.7–512.5)	3.9	4728.9 (969.4–0.25422E+06)	6.4	6.0 (3)
MN1	360	0.761 ± 0.090	149.76 (14.4–814.6)	5.2	7218.7 (1191.0–0.22567E+07)	9.8	9.9 (3)§
NE¶	720	0.796 ± 0.068	211.5 (28.3–945.0)	7.3	8617.2 (1658.7–0.10984E+07)‖	11.7	13.5 (3)§
Field_Pooled1 #	720	0.631 ± 0.059	82.2 (20.1–229.3)	2.8	8843.7 (2538.4–77613.0)‖	12.0	21.1 (3)§
Laboratory**	720	0.741 ± 0.066	28.7 (15.4–48.7)	NA	736.0 (402.5–1557.1)	NA	0.1 (3)

*Number of tested insects.

†LC_{50} and LC_{90} values are presented in ng/ml.

‡RR, resistance ratio = $LC_{50/90}$ field colony/$LC_{50/90}$ Susceptible laboratory colony.

§High chi-square values indicate a significant deviation from the regression model $P < 0.05$.

‖Significant differences ($P < 0.05$) between the LC_{50} and LC_{90} values of *A. glycines* field populations when compared to the LC_{50} and LC_{90} values of susceptible laboratory colony.

¶Pooled data of two bioassays on the NE colony.

#Pooled data of two bioassays on the Field_Pooled1 colony.

**Pooled data of two bioassays on the Susceptible laboratory colony (2012).

NA, not applicable.

Table 3. Thiamethoxam contact vial bioassays performed on all group ages of *A. glycines* collected from soybean fields, North Central United States in 2014

Colonies of SBA	*n**	Slope ± SE	LC_{50}† (95% CL)	RR‡ (LC_{50})	LC_{90}† (95% CL)	RR‡ (LC_{90})	χ^2 (d.f.)§
MI1	360	0.910 ± 0.091	40.4 (16.6–89.6)	1.3	1035.9 (383.8–5547.3)	1.1	3.1 (3)
MI2	360	0.870 ± 0.117	61.1 (5.06–152.8)	2.0	859.6 (236.0–54496.0)	0.9	7.3 (3)
MI3	360	1.049 ± 0.119	30.1 (10.7–69.8)	1.0	502.3 (189.29–3023.1)	0.5	3.5 (3)
IA1	360	0.213 ± 0.083	268.0 (68.7–1167.8)‖	9.0	9375.5 (1884.5–0.40346E+06)‖	10.1	7.3 (3)
IA2	360	0.920 ± 0.127	272.7 (29.3–934.7)	9.2	6751.4 1773.1–55413.0)‖	7.2	5.4 (3)
IA3	360	0.845 ± 0.099	155.6 (38.24–445.4)‖	5.2	5117.0 (1504.0–59183.0)‖	5.5	3.9 (3)
IA4	360	0.914 ± 0.086	235.4 (89.961–632.4)‖	7.9	5940.0 (1803.0–53963.0)‖	6.4	4.4 (3)
SD5	360	0.959 ± 0.150	261.4 (21.22–811.0)	8.8	5671.3 (1679.5–0.20306E+06)‖	6.1	4.5 (3)
OH	360	0.855 ± 0.115	137.4 (12.85–484.03)	4.6	4331.6 (1109.8–0.14711E+06)	4.6	5.2 (3)
IN	360	0.857 ± 0.081	139.1 (55.7–346.8)‖	4.7	4354.3 (1378.6–32426.0)‖	4.7	3.6 (3)
ND3	360	0.774 ± 0.085	155.1 (6.27–1093.0)	5.2	10025.0 (1346.1–0.11654E+08)‖	10.8	8.8 (3)§
ND4	360	0.738 ± 0.080	143.6 (18.49–738.06)	4.8	7823.4 (1313.7–0.98818E+06)‖	8.4	7.7 (3)
WI2 (2014)	360	0.783 ± 0.100	171.1 (12.61–7411.7)	5.8	7411.7 (1471.8–0.82614E+06)‖	8.0	6.5 (3)
WI4	360	0.804 ± 0.086	138.5 (25.94–540.13)	4.6	5443.4 (1183.5–0.18983E+06)	5.8	6.2 (3)
MN2	360	0.829 ± 0.101	165.0 (18.7–656.9)	5.5	5801.8 (1297.1–0.26215E+06)‖	6.2	6.0 (3)
Field_Pooled2	360	0.862 ± 0.081	300.6 (80.08–1273.4)‖	10.1	9232.1 (1940.3–0.34944E+06)‖	9.9	7.3 (3)
Laboratory¶	4,320	0.856 ± 0.027	29.6 (25.1–34.6)	NA	929 (743.8–1185.9)	NA	7.0 (3)

*Number of tested insects.

†LC_{50} and LC_{90} values are presented in ng/ml.

‡ RR, resistance ratio = $LC_{50/90}$ field colony/$LC_{50/90}$ Susceptible laboratory colony.

§High Chi-square values indicate a significant deviation from the regression model $P < 0.05$.

¶Pooled data of 12 bioassays on the Susceptible laboratory colony.

‖Significant differences ($P < 0.05$) between the LC_{50} and LC_{90} values of *A. glycines* field populations when compared with the LC_{50} and LC_{90} values of susceptible laboratory colony.

NA, not applicable

SD3, WI3, and NE populations indicate a significant departure (α = 95%) from the probit model. In 2014, the LC_{50} of all tested populations were significantly higher when compared with the LC_{50} of the lab colony. The mortality data of MI2, MI3, IA4, and ND4 did not fit the probit model as indicated by high chi-square values (Table 5). In 2012 the field strains of SD4, WI1, WI2, ND2, and Field_Pooled1 and the laboratory strain of SD1 were significantly higher than the LC_{90} of the lab colony (Table 4). In 2014, all populations differed from the LC_{90} of the lab colony with RRs ranging from 3.0- to 9.2-fold (Table 5).

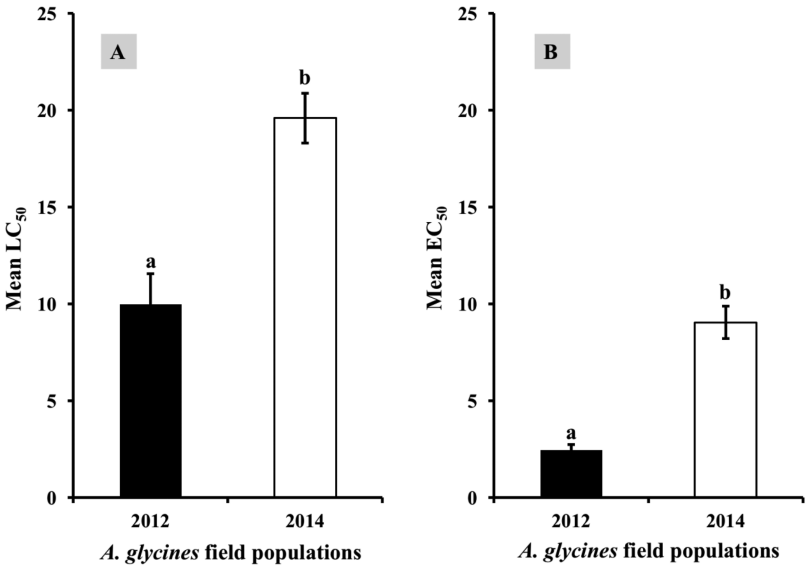

Fig. 3. Mean LC_{50}, and EC_{50} of field *A. glycines* populations exposed to thiamethoxam in systemic detached-leaf bioassays. Mortality (**A**) and percent population growth inhibition relative to controls (**B**) were recorded after 7 d. Error bars represent the SEM. LC_{50} and EC_{50} means with different letters between columns indicate significant differences among treatments ($P < 0.05$). Field populations (2012) $n = 12,856$, Field populations (2014) $n = 22,557$.

Table 4. Thiamethoxam detached-leaf systemic bioassays performed on all group ages of *A. glycines* collected from soybean fields, North Central United States in 2012

Colonies of SBA	*n**	Slope ± SE	LC_{50}† (95% CL)	RR ‡ (LC_{50})	LC_{90}† (95% CL)	RR‡ (LC_{90})	EC_{50}† (95% CL)	χ^2 (d.f.)§
SD1	861	1.24 ± 0.120	7.9 (4.1–16.7)	1.8	84.8 (32.7–872.3)	4.6	1.08 (0.6–1.5)	2.5 (3)
SD2	844	1.60 ± 0.125	5.7 (3.4–8.8)	1.3	35.7 (20.7–88.1)	1.9	1.29 (1.1–1.4)	5.0 (3)
SD3	1046	2.03 ± 0.206	7.1 (3.1–12.3)	1.6	30.3 (16.3–89.5)	1.6	2.85 (1.6–4.3)	9.8 (3)§
SD4‖	4271	1.05 ± 0.067	24.7 (20.7–30.2)	5.7	410.0 (267.6–703.7)	22.5	8.86 (5.4–15.0)	1.4 (3)
WI1	1147	1.34 ± 0.137	15.4 (12.1–20.2)	3.6	138.9 (86.1–277.3)	7.6	4.44 (3.1–5.9)	0.2 (3)
WI2#	4691	1.056 ± 0.058	27.2 (22.9–33.1)	6.3	445.7 (298.3–729.5)	24.4	8.90 (2.2–39.4)	1.8 (3)
WI3	1199	1.53 ± 0.108	9.1 (3.5–24.9)	2.1	62.5 (23.4–1299.0)	3.4	4.84 (2.3–7.3)	20.3 (3)§
ND1	912	2.01 ± 0.173	5.0 (4.1–6.0)	1.1	21.7 (16.9–30.0)	1.1	1.44 (1.3–1.5)	1.9 (3)
ND2	1012	1.301 ± 0.127	8.3 (6.5–10.8)	1.9	80.8 (51.9–150.7) *	4.4	2.04 (1.5–2.5)	1.7 (3)
MN	1250	1.50 ± 0.116	9.3 (4.2–33.1)	2.1	66.8 (22.5–2717.0)	3.6	4.30 (1.5–8.5)	6.4 (3)
NE	877	1.28 ± 0.104	8.0 (1.2–27.4)	1.8	79.7 (24.1–10895.0)	4.3	1.29 (0.7–1.8)	21.6 (3)§
Field_Pooled1#	1085	1.36 ± 0.138	15.9 (12.5–20.8)	3.7	138.4 (86.1–274.4)	7.6	4.05 (2.9–5.3)	0.1 (3)
Laboratory	971	2.06 ± 0.176	4.3 (3.6–5.3)	NA	18.2 (14.3–24.6)	NA	1.55 (1.4–1.6)	0.9 (3)

*Number of tested insects.

†LC_{50}, EC_{50}, and LC_{90} values are presented in ng/ml.

‡RR, resistance ratio = $LC_{50/90}$ field colony/$LC_{50/90}$ Susceptible laboratory colony.

§High chi-square values indicate a significant deviation from the regression model $P < 0.05$.

‖Pooled data of three bioassays on the SD4 colony.

¶Significant differences ($P < 0.05$) between the LC_{50} and LC_{90} values of *A. glycines* field populations when compared to the LC_{50} and LC_{90} values of susceptible laboratory colony.

#Pooled data of three bioassays on the WI2 colony.

NA, not applicable.

The sublethal effects of thiamethoxam measured as the concentration to cause 50% inhibition of population growth (EC_{50}) generated from all tested populations indicated a significant interaction between the year of monitoring and mean percentage population growth inhibition ($F_{1, 24} = 16.78$; $P = 0.0004$). An increase of EC_{50} values were observed from 2012 to 2014 (6.03 ± 1.47%) ($t = -4.10$; d.f. = 24; $P = 0.0004$) (Fig. 3B). The EC_{50}s of all soybean aphid populations to thiamethoxam for 2012 and 2014 are shown in Tables 4 and 5, respectively. In 2012, the field strains from SD4, WI1, WI2, WI3, and Field_Pooled1 were significantly higher than the EC_{50} of the lab colony. In 2014, the EC_{50} of all tested populations differed significantly from the EC_{50} of the lab colony with RRs ranging from 2.5 to 15.8.

Discussion

Both bioassay techniques employed in this monitoring study were shown to be sensitive in detecting shifts in insecticide susceptibility among soybean aphid populations. The glass-vial bioassay has been used to monitor and detect resistance to insecticides in a multitude of insect-insecticide systems, including sap-sucking pests, such as *Bemisia tabaci* (Prabhaker et al. 1996), *Bemisia argentifolii*

Table 5. Thiamethoxam detached-leaf systemic bioassays performed on all group ages of *A. glycines* collected from soybean fields, North Central United States in 2014

Colonies of SBA	*n**	Slope ± SE	LC_{50}† (95% CL)	RR‡ (LC_{50})	LC_{90}† (95% CL)	RR‡ (LC_{90})	EC_{50}† (95% CL)	χ^2 (d.f.)§
MI1	1,566	2.27 ± 0.161	18.9 (12.7–27.4)‖	4.1	69.1 (44.3–150.3)‖	3.8	9.95 (6.0–20.1)‖	7.1 (3)
MI2	1,501	2.16 ± 0.148	20.1 (12.9–30.6)‖	3.3	78.6 (47.8–197.9)‖	4.4	9.12 (6.6–13.8)‖	8.9 (3)§
MI3	1,260	1.69 ± 0.155	19.0 (10.4–32.1)‖	4.1	108.0 (55.6–541.9)‖	6.0	5.73 (4.6–6.8)‖	8.1 (3)§
IA1	1,574	1.81 ± 0.143	23.7 (15.9–35.7)‖	5.1	121.1 (70.2–333.6)‖	6.7	11.18 (7.8–19.1)‖	5.8 (3)
IA2	1,261	1.85 ± 0.143	12.9 (10.8–15.3)‖	2.8	63.7 (50.1–86.3)‖	3.5	4.76 (3.9–5.6)‖	2.5 (3)
IA3	1,962	1.95 ± 0.131	19.8 (15.7–25.1)‖	4.3	89.5 (63.0–149.0)‖	4.9	11.59 (10.1–12.5)‖	3.2 (3)
IA4	1,570	2.21 ± 0.160	20.7 (11.8–34.2)‖	4.5	78.5 (44.8–265.0)‖	4.3	11.48 (7.9–18.7)‖	11.9 (3)§
SD5	1,399	2.70 ± 0.192	15.2 (11.2–21.4)‖	3.3	45.4 (30.3–92.4)‖	2.5	8.73 (4.1–22.1)‖	6.5 (3)
OH	1,774	1.80 ± 0.123	32.0 (24.2–43.5)‖	6.9	164.9 (104.7–339.9)‖	9.2	20.39 (6.7–66.5)‖	4.3 (3)
IN	1,124	2.03 ± 0.179	12.7 (8.3–18.2)‖	2.7	54.6 (35.0–120.4)‖	3.0	3.26 (2.5–4.0)‖	4.8 (3)
ND3	1,329	1.51 ± 0.115	14.4 (9.3–28.5)‖	3.1	101.7 (55.1- 307.8)‖	5.6	5.98 (3.3–9.6)‖	5.7 (3)
ND4	1,672	2.63 ± 0.160	21.8 (15.4–30.3)‖	4.7	67.1 (45.8–123.5)‖	3.7	14.2 (8.8–31.4)‖	7.9 (3)§
WI2 (2014)	1,698	1.53 ± 0.116	22.0 (15.2–34.0)‖	4.8	153.4 (83.5–474.1)‖	8.5	12.22 (3.7–52.5)‖	5.9 (3)
WI4	1,402	1.88 ± 0.143	16.6 (11.8–23.7)‖	3.6	79.8 (49.0–187.8)‖	4.4	7.56 (6.3–9.1)‖	5.3 (3)
MN2	1,465	2.00 ± 0.145	23.4 (16.7–32.5)‖	5.0	101.7 (65.7–208.9)‖	5.6	9.13 (7.1–11.8)‖	5.0 (3)
Field_Pooled2	1,361	2.27 ± 0.210	15.2 (9.8–29.3)‖	3.3	56.0 (29.1–419.1)‖	3.1	7.61 (6.9–8.3)‖	3.8 (3)
Laboratory	1,284	2.17 ± 0.197	4.6 (3.7–5.5)	NA	17.9 (14.2–24.3)	NA	1.29 (1.1–1.4)	1.2 (3)

*Number of tested insects.

†LC_{50}, EC_{50} and LC_{90} values are presented in ng/ml.

‡RR, resistance ratio = $LC_{50/90}$ field colony/$LC_{50/90}$ Susceptible laboratory colony.

§High chi-square values indicate a significant deviation from the regression model $P < 0.05$.

‖Significant differences ($P < 0.05$) between the LC_{50} and LC_{90} values of *A. glycines* field populations when compared to the LC_{50} and LC_{90} values of susceptible laboratory colony.

NA, not applicable.

(Sivasupramaniam et al. 1997), stink bugs complex (Willrich et al. 2003, Snodgrass et al. 2005, Nielsen et al. 2008) and other aphid species, such as *Myzus persicae* (Shean and Ranshaw 1991), *Aphis craccivora*, *Rhopalosiphum maidis* (Tang et al. 2013), and *Diuraphis noxia* (Bayoun et al. 1995). To a lesser extent than the vial assay, toxicological studies were performed using detached-leaf bioassays with *Aphis glycines* (Magalhaes et al. 2008) and *Cerotoma trifurcata* (Tietjen et al. 2017). Nonetheless, other similar systemic uptake bioassays have been extensively used in other pest programs (Cahill et al. 1996, Prabhaker et al. 1996, Prabhaker et al. 2005, Castle et al. 2014, Matsuura and Nakamura 2014). In general, the glass-vial bioassay was shown to be reliable, more rapid, and requires less insecticide when compared with the detached-leaf method. Another advantage of the glass-vial bioassay is that it allows resistance monitoring of populations collected directly from the field (McCutchen et al. 1989) because the vials can be pretreated and no insect rearing is necessary prior to experiments (Sivasupramaniam et al. 1997). However, the systemic detached-leaf bioassay is more representative of the route of exposure encountered under field conditions. This assay also allows the measurement of chronic effects of sublethal concentrations at a population level (Magalhaes et al. 2008). The disadvantages of the systemic bioassay include increased labor and dependence on plant material, and it is much longer in duration. Although these attributes of systemic bioassays limit utility for standardized resistance monitoring, the assay still is able to detect shifts in susceptibility levels, such as performed by Magalhaes et al. (2008) and Tietjen et al. (2017).

Our data suggest that the magnitude of the RRs of all tested populations were similar in range for both assays when significant differences were observed between soybean aphid field populations and the lab colony (Tables 2–5). For example, in both bioassays and years of monitoring and at both lethal concentration levels (LC_{50} and LC_{90}), the great majority of RRs were categorized as very low (RR = 2 - 10). However, in 2012, the LC_{90} RRs of SD4 and WI2 populations achieved moderate levels (RR = 21–50).

Regardless of their origin (laboratory, treated, or non-treated field populations) or year of evaluation, soybean aphid populations were highly susceptible to contact and systemic exposure to thiamethoxam. Our results demonstrate significant effects of this insecticide against soybean aphids at lethal concentrations in both bioassays, and at sublethal concentrations in the detached-leaf bioassay. Subtle changes associated with sublethal exposure to insecticides may affect development, reproduction, longevity, feeding behavior, and dispersal (Guedes et al. 2016). Importantly, these subtle changes may also possess stimulatory effects on pest populations. For example, soybean aphids exposed to sublethal concentrations of imidacloprid showed no negative effects as well as higher reproduction and population growth rates when compared with untreated controls (Qu et al. 2015). This stimulatory effect or response to sublethal concentrations of insecticides is defined as hormesis (Wang et al. 2016), and the identification of such changes may help guide pest management decisions and explain insecticide-induced pest outbreaks (Guedes et al. 2016). In our investigation, no indication of hormesis was detected in soybean aphid populations exposed to sublethal concentrations of thiamethoxam, and nymph production was always higher under control treatments (data not shown).

Although the EC_{50}s RRs were all categorized as very low (RR = 2–10), there was a substantial increase in nymph production among soybean aphid populations between the 2 years of monitoring. However, no significant differences were observed in the EC_{50}s calculated for the lab colony during the same period (Tables 4 and 5), suggesting a possible decrease in susceptibility of field populations. This increase in the EC_{50}s could also be associated with other factors, such as the weight of insects (Robertson et al. 2007), seasonal environmental conditions related to the growing season sampling date, host plant quality, and rearing conditions (Godfrey and Fuson 2001). In addition, when aphid survivorship and population numbers at untreated detached-leaves were compared between the lab and field populations across years of monitoring, no significant

differences were recorded. However, the number of living aphids of field populations was higher in 2014 (Fig. 4) when compared with 2012. Those results indicate that there is no difference in fitness between the lab and field aphids; therefore, the EC_{50}s calculated in 2014 represent a significant decrease in susceptibility when compared with 2012 estimates. In general, for both bioassay methods, the differences observed in susceptibility to thiamethoxam between 2012 and 2014 revealed increased but small levels of resistance from 2012 to 2014.

The concentration–response curves used to compare susceptibility of different populations are generally considered insensitive for detecting resistance at low frequencies (ffrench-Constant and Roush 1990). Consequently, using a single diagnostic insecticide concentration has shown to be more efficient and practical in detecting resistance (or small shifts in tolerance) at low frequencies (Marçon et al. 2000). However, it is critical that baseline susceptibility data are generated in order to support the calculation of such concentrations. It should be noted that diagnostic bioassays based on a single lethal concentration or lethal dose provide limited information on population level responses (Stark and Banks 2003) as the slope of the response curve cannot be estimated. Therefore, the use of techniques based on bio-demographic parameters that are informative of an insect's population life span, prereproductive period, and fertility rates should be incorporated in toxicological studies (Walthall and Stark 1997). As a result, the detection of the total effects of insecticides at lethal and sublethal levels can be investigated (Kammenga and Riksen 1996, Santos et al. 2016).

Genetic analysis of soybean aphid population structure in North America has revealed low allelic polymorphism and moderate genotypic diversity (Michel et al. 2009). The narrow differences in the RRs obtained in the present study may reflect this lack of genetic diversity among soybean aphid populations as variability in susceptibility during both years was minimal (Tables 2–5). Furthermore, Michel et al. (2009) reported that the genetic variation in soybean aphid populations is strongly associated with time of collection, but not with geography. As a result, collecting soybean aphids for insecticide monitoring bioassays early in the season may improve the likelihood of detecting small changes in susceptibility that are linked with genotypic variability and minimize effects of clone selection in bioassay

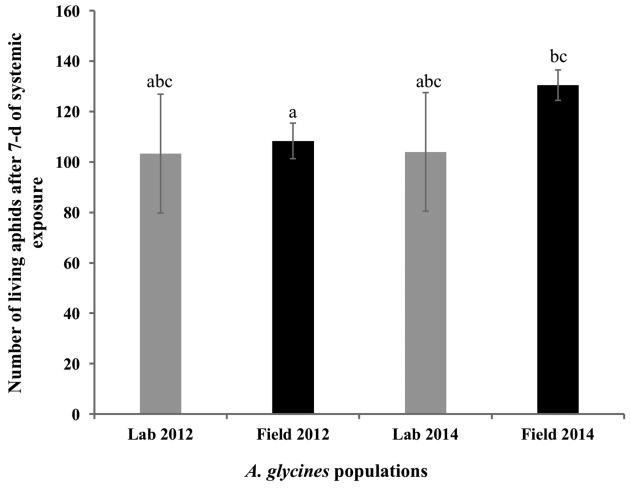

Fig. 4. Mean number of living *A. glycines* exposed to thiamethoxam in systemic detached-leaf bioassays recorded after 7 d of exposure to untreated detached-leafs. Error bars represent the SEM. Different letters between columns indicate significant differences ($P < 0.05$) between field and laboratory populations across and within years of monitoring.

responses (Roush and McKenzie 1987, Robertson et al. 2007). For example, Godfrey and Fuson (2001) reported higher tolerance of *A. gossypii* to bifenthrin, chlorpyrifos, and triazamate when aphids were exposed to early-season conditions in comparison to aphids reared under the late-season conditions. In our study, all field aphid samples were collected between mid-June and early-September (mid- to late-season); however, as described by Wenger and Michel (2013), widespread gene flow during sexual reproduction associated with high interpopulation migration between soybean aphids may reduce the chances for significant differentiation between populations separated by geographic locations.

Because geography does not fully explain variation in response among soybean aphid populations, we combined subsamples of aphids from all field populations into two distinct pooled colonies in an attempt to increase their genetic diversity. The comparison of the LC_{50}s obtained for multiple bioassays of the Field_Pooled populations from 2012 to 2014 represents 1.10- and 3.65-fold increase in resistance for the systemic detached-leaf and contact vial bioassays, respectively. In part, the differences in response observed in our study may be simply associated with natural variation among tested populations, such as observed by Gerami et al. (2013) with *A. gossypii* in Iran. In detached-leaf bioassays, both the LC_{50}s and EC_{50}s were in the range of concentrations reported in another study with soybean aphids and thiamethoxam (Magalhaes et al. 2008). Herron and Wilson (2011) documented a reduction in susceptibility of *A. gossypii* to thiamethoxam using treated cotton leaf discs on field collections associated with control failures. Initially, a discriminatory concentration of 0.02 g/liter (20,000 ng/ml) of thiamethoxam were applied for each aphid cohort (25 aphids with 3 replications each), and subsequently, the populations with higher survivorship (%) were submitted to concentration–response bioassays. The authors observed an increase of 13.2-fold in resistance (ranging from 8.8 to 22) in a 2-year period (2007–2009). We observed slightly lower variation in susceptibilty among the tested field populations (RRs ranged from 2.98 to 10.15 in the vial bioassay, and from 1.16 to 6.94 in the detached-leaf bioassays from 2012 to 2014). In our investigation, no previous selection was made to discriminate individuals by percent survivorship such as described in the study by Herron and Wilson (2011). However, it is important to consider that although none of our calculated LC_{50} RRs were categorized as moderate (RR = 21–50), high (RR = 51–100), or very high (RR = >100), it seems plausible that the high use of this mode of action in the North Central US soybean systems may be associated with the low but apparent increase in resistance observed in our investigation from 2012 to 2014.

Thiamethoxam has been highly effective against a broad-spectrum of crop pests, including sap-sucking arthropods such as aphids. For this reason, it provides a valuable tool in integrated pest management (IPM) and insecticide resistance management (IRM) programs, especially in cases where resistance to other modes of action has adversely affected pest management practices (Nauen and Denholm 2005). To date, no field control failures or resistance to neonicotinoid compounds has been reported in soybean aphids from the North Central United States. However, decreasing susceptibility of thiamethoxam in acute-contact bioassays and decreased inhibition of nymph production after chronic-systemic exposure advise for the importance of reducing the selection pressure imposed by neonicotinoid seed-treated soybean.

The concentration–response data generated in this study will be useful for monitoring susceptibility of soybean aphid field populations to thiamethoxam in future IRM programs. The implementation of such programs may increase the longevity and efficacy of

neonicotinoids against soybean aphids and promote more sustainable pest management approaches in North Central US soybean systems.

Our results will also serve as a foundation to develop a standardized technique for monitoring the susceptibility of soybean aphids to neonicotinoid insecticides in North America. Further research is being conducted to elucidate the risks of short-term adaptations, and resistance evolution on soybean aphid field populations, and to identify the presence of a potential fitness-differential after multigeneration of systemic selection with thiamethoxam.

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