An Aphid-Dip Bioassay to Evaluate Susceptibility of Soybean Aphid (Hemiptera: Aphididae) to Pyrethroid, Organophosphate, and Neonicotinoid Insecticides

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ABSTRACT Since the discovery of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in North America in 2000, chemical control has been the most effective method to manage aphid outbreaks. Increased insecticide use in soybean raises the possibility of developing insecticide resistance in soybean aphid, and monitoring insecticide susceptibility is essential to maintain pesticide tools. We developed a simple and reliable aphid-dip bioassay by using a tea strainer that resulted in ≥90% survival in controls. Using this technique, we tested susceptibility of a greenhouse strain of soybean aphid that has never been exposed to insecticides, and field-collected aphid strains from two counties in Michigan. Aphid susceptibility was tested for five insecticides by dipping groups of five aphids in each insecticide dose for 10 s. After 48 h, aphids were classified as dead or alive, and counted. Aphids from all strains were highly susceptible to chlorpyrifos, λ-cyhalothrin, esfenvalerate, and dimethoate, with LC_{50} and LC_{90} values well below the recommended application rates. However, aphids showed less susceptibility after 48 h to neonicotinoid imidacloprid, with higher LC_{90}s and wider fiducial limits. This illustrated the potential limitation of using a 48-h assay to evaluate insecticides with longer-term, sublethal impacts. Nevertheless, this study made use of a simple aphid-dip method to test and compare insecticide susceptibility of soybean aphid. In the event of a field failure, the aphid populations involved can be tested in comparison to a susceptible greenhouse strain to determine the extent of resistance development.

KEY WORDS soybean aphid, *Aphis glycines*, insecticide susceptibility, aphid-bioassay

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is native to Asia, and is one of the most serious insect pests of soybean, *Glycine max* (L.) Merrill (Yu et al. 1989; Wang et al. 1996; Wu et al. 1999; Sun et al. 2000; Hill et al. 2004; Ragsdale et al. 2004, 2007). Since its discovery in the United States in 2000, soybean aphid rapidly spread across the midwestern United States (Ragsdale et al. 2004). It is now recorded in 24 states in the United States and in three Canadian provinces (Ragsdale et al. 2004, Rutledge and O'Neil 2006).

Soybean aphid reduces yield directly by feeding on plants and indirectly by reducing seed protein content (Wang et al. 1994). Plants with heavy infestation show wrinkled and distorted foliage, early defoliation, stem and leaf stunting, reduction in number of pods and seed weight, and even plant death (Wang et al. 1962; Wang et al.1996; Lin et al. 1992, 1994; Wu et al. 1999; Wu et al., 2004; DiFonzo and Hines 2002; Diaz-Montano et al. 2006). Honeydew excreted by aphids builds up on foliage and supports the growth of sooty mold, affecting plant photosynthesis, yield, and seed quality (Chen and Yu 1988). In China, yield was reduced up to 52% when soybean in early vegetative stages was inoculated with 220 aphids per plant (Wang et al. 1994). In the United States, >40% yield loss can occur in untreated fields (Ragsdale et al. 2007). Song et al. (2006) estimated a total yield loss exceeding 350 million bushels in the north-central states if soybean was left untreated. In addition to yield loss from direct feeding, another threat posed by soybean aphid is its ability to transmit plant viruses to soybean and other crops (Iwaki et al. 1980, Hartman et al. 2001, Hill et al. 2001, DiFonzo and Agle 2008).

Soybean aphid management involves several distinct approaches, including biological control, host plant resistance, and chemical control (Wu et al. 2004). In North America, reports of native parasitism are generally <10% (Nielsen and Hajek 2005, Costamagna and Landis 2006, Noma and Brewer 2008). Generalist predators are common and often keep aphid populations in check. However, soybean aphid populations can increase rapidly, doubling in <2 d under optimal conditions (McCornack et al. 2004). Therefore, suppression of soybean aphid with biological control alone remains inconsistent. Soybean accessions with aphid resistance have been identified by

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several university breeding programs (Mensah et al. 2005, Diaz-Montano et al. 2006, Hesler et al. 2007, Hesler and Dashiell 2008, Mian et al. 2008), but resistant varieties are not yet widely available. In addition, host plant resistance may be overcome by certain aphid biotypes (Kim et al. 2008). Therefore, aphid control during outbreaks still relies primarily on insecticides.

In the United States, insecticide applications to soybean increased dramatically after the discovery of soybean aphid. In 1999, before the first aphid outbreak, <1% of the soybean acreage in Michigan was treated with insecticide (NASS 2000). In 2005, an outbreak year, 42% of Michigan soybean acres were treated (NASS 2006). Similar increases were observed in other north-central states (NASS 2000, 2006), and these increases have economic and environmental costs. For example, in 2004, Michigan soybean growers spent US$20–30/ha on insecticide applications for aphid control (Song et al. 2006). Increased insecticide use also raised the possibility of developing insecticide resistance in soybean aphids.

Organophosphates and pyrethroids were the first insecticides used to manage soybean aphid after its discovery in the United States (NASS 2001). These insecticides represent mode of action (MoA) groups 1B and 3, respectively (IRAC 2010). In 2005, an outbreak year, the top three insecticides used to control soybean aphid in the Midwest were the organophosphate chlorpyrifos, and the pyrethroids λ-cyhalothrin and esfenvalerate (NASS 2006). Dimethoate, another organophosphate, is one of the few general-use (i.e., not requiring applicator certification) products registered for soybean. However, it showed inconsistent efficacy against soybean aphid, for reasons unknown. Trimax Pro, a neonicotinoid (MoA group 4A), was registered on soybean in the late 2000s as a foliar spray. Its active ingredient imidacloprid also is registered as a seed treatment on soybean, raising concerns that multiple uses could lead to development of resistance.

Determining susceptibility of aphids to currently used insecticides and monitoring for insecticide resistance is essential to effectively manage soybean aphid in the future. To our knowledge, there is one published report of laboratory measurement of soybean aphid susceptibility to insecticides in the United States. Magalhaes et al. (2008) provided information on susceptibility of a field-collected colony from Nebraska to two neonicotinoid insecticides used as seed treatments (imidacloprid and thiamethoxam). Both active ingredients were toxic to soybean aphids, showing lethal and sublethal effects. Thus far, there are no documented reports on insecticide resistance of soybean aphids in North America. To proactively address the threat of aphid resistance, we developed an aphid-dip method by using a simple tea strainer, to bioassay susceptibility of soybean aphid to five foliar insecticides in three mode-of-action groups. This bioassay was used to evaluate the susceptibility of soybean aphids from a greenhouse colony and from several locations in Michigan with a history of insecticide use.

Materials and Methods

Greenhouse Strain. The greenhouse strain originated from aphids collected from several fields in Ingham Co., MI, in August 2000, the month that soybean aphid was confirmed in North America. The strain was first maintained at the former USDA–APHIS National Biological Control Laboratory in Niles, MI. In 2002, aphids from the Niles laboratory were used to establish a greenhouse colony at Michigan State University. This strain was maintained on 'Williams 82' soybean plants at vegetative stages V4–V8. New plants were provided weekly to the colony, and aphids were transferred to new foliage weekly by placing infested leaf pieces on uninfested plants. The colony was maintained at 27 ± 5°C, under photoperiod 16:8 (L:D) h. This strain originated from field collections made before insecticide use to control soybean aphids in Michigan and, since its establishment, the colony has never been exposed to insecticides.

Field Strains. In 2007, soybean aphids were collected in July and August from three fields in two Michigan counties. Each location had a history of insecticide use during aphid outbreaks in 2001, 2003, and 2005. The locations were a field at Michigan State University's Entomology Field Research Farm, East Lansing, MI (42° 41′ 29.38″ N, 84° 29′ 22.96″ W), a field at the Saginaw Valley Sugar Beet and Dry Bean Research Farm, Saginaw, MI (43° 22′ 41.38″ N, 84° 06′ 42.68″ W), and a commercial soybean field, Gera, MI (43° 23′ 13.78″ N, 83° 44′ 18.58″ W). These three strains were maintained in separate growth chambers at 22 ± 5°C and a photoperiod of 16:8 (L:D) h until experiments were completed. In 2008, soybean aphids were collected in August from a field at the Entomology Field Research Farm, East Lansing, MI, and maintained in growth chambers as described for 2007.

Voucher specimens of *A. glycines* from field and greenhouse populations were deposited in the A. J. Cook Arthropod Research Collection at Michigan State University, East Lansing, MI.

Developing a Standard Aphid-Dip Method. A protocol was standardized for dipping aphids in liquid solutions by using a metal mesh tea strainer (mesh size, 0.5 mm; Cost Plus Inc., Oakland, CA; Fig. 1). Five apterous adults from the greenhouse strain were placed inside the tea strainer. The closed tea strainer was then dipped in a 200-ml beaker of deionized water (a control treatment) for 10 s. After 10 s, the strainer was removed from the solution and soybean aphids were blotted on clean, dry filter paper to remove excess moisture. Finally, soybean aphids were transferred to a fresh-cut soybean leaf square (2 by 2 cm) on moist filter paper in a plastic petri dish (9 mm in depth, 50 mm in diameter) with a snap-on lid (351006, Falcon, BD Biosciences, Franklin Lakes, NJ). Petri dishes were placed on a layer of moist paper towels inside a disposable aluminum cake pan with a plastic lid to maintain high humidity. The pans were held in a growth chamber at 22°C and a photoperiod of 16:8 (L:D) h. After 48 h, soybean aphids were classified as dead or alive and then counted. Soybean aphids were

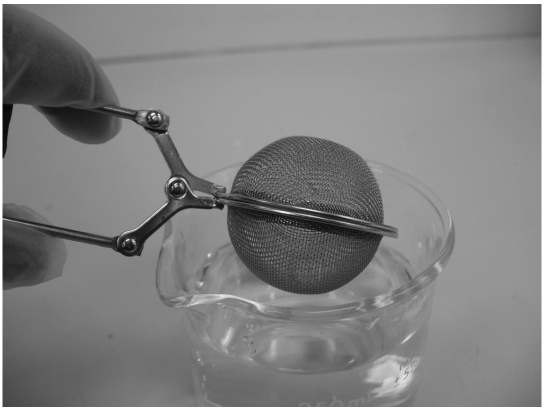

Fig. 1. Ball-type metal mesh tea strainer used in aphid-dip insecticide bioassays.

considered dead when they did not move after multiple proddings with a fine-haired paintbrush. All aphids that died also turned red, further assisting in classification. This protocol resulted in soybean aphid survival of ≥90% (≤10% mortality) after 48 h, indicating that the aphid-dip method was reliable for conducting insecticide bioassays.

Aphid-Dip Insecticidal Bioassay. Five commercial insecticides in three groups were used in the bioassay (Table 1). The stock concentration for each insecticide was 25% of the rate recommended for soybean aphid control in Michigan (DiFonzo and Warner 2010). The recommended application rates for all five insecticides were calculated using a spray volume of 243.2 liters/ha.

Stock solutions were prepared by mixing deionized water with commercial formulations of each insecticide measured using a precision micro liter pipette (PIPETMAN, Rainin Instruments LLC, Oakland, CA). Stock solutions were stored in a refrigerator and used within a week from the date of preparation. A series of dilutions (see discussion) were then prepared from stock solutions by using deionized water. Fresh dilutions were made on the day of each trial, and each fresh dilution series was used to test soybean aphids from field and greenhouse strains in a single trial.

Using the dip method, individual groups of five adult apterous aphids were dipped for 10 s in deionized water (control), in an insecticide stock solution, or in an insecticide dilution. Each group of aphids was then transferred to a fresh-cut soybean leaf square (2 by 2 cm) on moist filter paper in a petri dish (9 mm in depth, 50 mm in diameter) for 48 h. In a preliminary test, some aphids were poisoned and alive at 24 h, but dead after 48 h; thus, mortality was assessed at 48 h. Multiple tea strainers were used, each assigned to a particular treatment, to prevent cross-contamination. Petri dishes from the same soybean aphid strain exposed to a given insecticide were placed in individual pans on a layer of moist paper towels to maintain high humidity. The exception was chlorpyrifos, which in preliminary tests volatilized easily under laboratory conditions. Therefore, aphids dipped in different doses of chlorpyrifos were separated in the laboratory to avoid the fuming effect. The pans were held in growth chambers at 22°C and a photoperiod of 16:8 (L:D) h. Control aphids were maintained separately under the same conditions, in a different growth chamber, to prevent exposure to insecticides. After 48 h, dead soybean aphids were counted.

Four replicates (trials) were conducted in our study, each replicate a series of dilutions from a stock solution. Within each replicate, 15 aphids were dipped per dilution in groups of five and mortality in a total of 15 aphids was recorded per trial. The total number of aphids tested for each insecticide ranged from 346 to 600, depending on the number of dilutions tested. Uniform responses were observed within replicates, thus data from the four replicates were pooled before Probit analysis (60 aphids per dilution per insecticide). In 2007, field-collected aphids from all three locations (East Lansing, Gera, and Saginaw) were compared with the greenhouse strain for chlorpyrifos and esfenvalerate. A single field strain from East Lansing was compared with the greenhouse strain for λ-cyhalothrin. In 2008, field-collected aphids from East Lansing were compared with the greenhouse strain for dimethoate and imidacloprid.

Data Analyses. Mortality data analyses were conducted by probit analysis (PROC PROBIT, SAS Institute 2003). If control survival was <100%, mortality was corrected using Abbott's formula (Abbott 1925). LC_{50}, LC_{90}, and 95% fiducial limits (FLs) were calculated using PROC PROBIT. PROC PROBIT also calculated the slope of the logarithmic dose–response relationship for each combination of insecticide by location. A high slope value indicates less heterogeneity in population sensitivity to a particular insecticide (Georghiou and Metcalf 1961). In contrast, a shallow slope indicates high heterogeneity in sensitivity among individuals of a population.

Table 1. Insecticides used in soybean aphid-dip bioassays, 2007–2008

Common name	Brand name	Class (IRAC group[a])	Manufacturer
Chlorpyrifos	Lorsban 4E	Organophosphate (group 1B)	Dow AgroSciences LLC, Indianapolis, IN
Dimethoate	Dimethoate	Organophosphate (group 1B)	Bayer CropScience Research, Triangle Park, NC
Esfenvalerate	Asana XL	Pyrethroid (group 3)	DuPont, Wilmington, DE
λ-Cyhalothrin	Warrior with Zeon Tech.	Pyrethroid (group 3)	Syngenta Crop Protection Wilmington, DE
Imidacloprid	Trimax Pro	Neonicotinoid (group 4A)	Bayer CropScience

[a] Mode of action group, as given by IRAC.

Table 2. Susceptibility of greenhouse colony and field-collected soybean aphids to five insecticides in aphid-dip bioassays

Insecticide	Aphid strain	n	Slope ± SE	LC_{50} ppm (95% FL)	LC_{90} ppm (95% FL)	χ^2 (df)	P
Chlorpyrifos	Greenhouse	597	5.55 ± 0.68	0.0073 (0.0065–0.0081)	0.0124 (0.0108–0.0151)	1.7 (4)	0.7966
	E. Lansing	600	1.47 ± 0.36	0.0108 (0.0041–0.0233)	0.08056 (0.0336–1.2262)	36.7[a] (7)	<0.0001
	Saginaw	597	3.57 ± 0.38	0.0065 (0.0057–0.0075)	0.0150 (0.0125–0.0191)	2.0 (7)	0.9562
	Gera	594	3.58 ± 0.44	0.0063 (0.0055–0.0073)	0.0145 (0.0117–0.0197)	4.5 (6)	0.6074
Dimethoate	Greenhouse	353	0.70 ± 0.07	4.05 (2.22–7.11)	263 (116–815)	1.8 (3)	0.6050
	E. Lansing	350	0.54 ± 0.06	0.71 (0.39–1.01)	3.01 (2.61–3.83)	6.0 (3)	0.1139
Esfenvalerate	Greenhouse	535	0.76 ± 0.22	0.0093[b]	0.4590[b]	76.8[a] (6)	<0.0001
	E. Lansing	528	4.41 ± 0.53	0.0043 (0.0038–0.0049)	0.0086 (0.0072–0.0108)	0.0 (6)	1.0000
	Saginaw	528	4.40 ± 0.55	0.0049 (0.0044–0.0055)	0.0095 (0.0084–0.0124)	5.7 (6)	0.4585
	Gera	531	3.59 ± 5.64	0.0034[b]	0.0076[b]	915.5[a] (6)	<0.0001
λ-Cyhalothrin	Greenhouse	600	0.77 ± 0.18	0.054 (0.000–0.307)	2.443 (0.417–4.456)	10.1 (3)	0.0978
	E. Lansing	591	2.19 ± 0.26	0.004 (0.003–0.006)	0.016 (0.011–0.035)	12.1 (7)	0.0965
Imidacloprid	Greenhouse	346	0.33 ± 0.06	2.55 (0.18–162)	5178 (105–1.24×10^{13})	6.8 (3)	0.0781
	E. Lansing	353	0.45 ± 0.14	0.45[b]	362.96[b]	16.6[a] (3)	0.0008

[a] High χ^2 value indicated a significant deviation from probit model $P < 0.05$.
[b] 95% FLs not generated by Probit analysis (PROC PROBIT, SAS Institute 2003).

Results and Discussion

Aphid-Dip Insecticidal Bioassay. Aphids from both greenhouse and field strains were extremely susceptible to chlorpyrifos, tested in nine serial dilutions ranging from 1,302 ppm to 0.0026 ppm (1,302, 130.2, 13.02, 1.302, 0.13, 0.026, 0.013, 0.0065, and 0.00260). The LC_{50}s of the greenhouse strain and the three field populations (East Lansing, Gera, and Saginaw) were not significantly different, based on overlapping FLs (Table 2). The LC_{90} of the East Lansing strain was significantly higher (0.08056 ppm) than the LC_{90} values of the other strains (0.0124–0.0150 ppm) based on nonoverlapping FLs. However, the East Lansing strain had a high χ^2 value (Table 2), indicating a significant deviation from the Probit model. The value for the slope for the East Lansing strain was low (1.47 ± 0.36) compared with Saginaw (3.57) and Gera (3.58) populations. The highest value for slope was found for the greenhouse strain (5.55), which indicated the least heterogeneity in population sensitivity to chlorpyrifos. Despite these differences among populations, all populations were very sensitive to chlorpyrifos at much lower concentration than the recommended application rate (≈5,248 ppm).

Soybean aphid strains from the greenhouse and East Lansing responded variably to dimethoate. Susceptibility to dimethoate was tested in five dilutions ranging from 1,302 ppm to 0.13 ppm (1,302, 130.2, 13.02, 1.302, and 0.13). A higher LC_{50} value was obtained for the greenhouse strain (4.05 ppm) compared with the field strain (0.71 ppm), and the FLs did not overlap (Table 2). The LC_{90} of the greenhouse strain was 263 ppm, but only three ppm for the East Lansing strain, with nonoverlapping FLs. This indicated that the greenhouse strain was more tolerant to dimethoate than the field strain. Relatively shallow slopes of both strains may be associated with high population heterogeneity in susceptibility to dimethoate. This was in contrast to the response to chlorpyrifos, where the same two strains had much higher slopes (less population heterogeneity) and extremely low LC_{50} and LC_{90} values. This finding supports observations from the efficacy trials where chlorpyrifos was very effective against soybean aphid, whereas dimethoate showed inconsistent and unsatisfactory control (Ragsdale et al. 2001). However, in the bioassay, both strains were susceptible to dimethoate at lower concentrations than its recommended application rate (≈5,248 ppm).

Aphids from all strains were highly susceptible to esfenvalerate, tested in eight dilutions ranging from 470 ppm to 0.00235 ppm (470, 47, 4.7, 0.47, 0.047, 0.0094, 0.0047, and 0.00235). Fiducial limits for LC_{50} and LC_{90} of East Lansing and Saginaw strains overlapped (Table 2). Due to departure from the model, PROC PROBIT did not calculate FLs for the greenhouse and Gera strains. A lower slope was calculated for the greenhouse strain compared with the other strains, indicating more heterogeneity in susceptibility to esfenvalerate. High heterogeneity in data is a possible cause for poor fit to a Probit model (Robertson et al. 1980, Mostert et al. 2002). However, even when data show high heterogeneity, the LC_{50} value is still often calculated, allowing comparison of toxicity levels among insecticides (Mostert et al. 2002). LC_{50} and LC_{90} values for all populations (although FLs were not obtained for some) were low compared with the recommended application rate (≈1,888 ppm).

The second pyrethroid, λ-cyhalothrin was tested in nine dilutions ranging from 260 ppm to 0.0013 ppm. All dilutions (260, 26, 2.6, 0.26, 0.026, 0.013, 0.022, 0.00163, and 0.0013) were highly toxic to soybean aphid. The LC_{50} for the East Lansing strain (0.004 ppm) was not significantly different from the LC_{50} for the greenhouse strain (0.054 ppm). However, the LC_{90} values were significantly different and did not overlap. Both strains were highly susceptible when compared with the recommended application rate for λ-cyhalothrin (≈1,040 ppm). Wider FLs and a relatively flat slope (Table 2) suggested that although susceptible, the greenhouse strain had more heterogeneity in sensitivity to λ-cyhalothrin than the East Lansing field population.

The neonicotinoid, imidacloprid was tested in five dilutions ranging from 121 ppm to 0.0012 ppm (121, 12.1, 1.21, 0.121, and 0.012), which behaved differently from the other insecticides in the study. The LC_{50} value for the greenhouse strain was 2.55 ppm, with FLs of 0.18–162.0. Due to possible deviation from the model ($P = 0.0008$), FLs were not calculated for the East Lansing strain, but the LC_{50} (0.45 ppm) fell within the FLs of the greenhouse strain. Again, FLs for the LC_{90} could not be calculated for the East Lansing strain but were relatively large for both the greenhouse strain (5,178 ppm) and the field strain (363 ppm). These concentrations were above or near the recommended application rate (484 ppm). Both strains had shallow slopes indicating high heterogeneity to susceptibility. This was observed directly in the bioassay, as active survivors were observed even at the stock concentrations. Overall, soybean aphids showed less susceptibility to imidacloprid after 48 h than to other insecticides tested in the assay.

Insecticide toxicity symptoms observed for all insecticides were similar in all aphid strains. All insecticide-affected aphids had excessive secretions from their cornicles, perhaps due to elevated secretion of alarm pheromone. Alarm pheromone is released with the cornicle secretions exuded by many aphid species when they are disturbed, particularly aphids in the subfamily Aphidinae (Hardie et al. 1999). The pheromone signals neighboring aphids to withdraw their stylets from the plant and move away from the pheromone source. van Toor et al. (2008) demonstrated that insecticide-resistant *Myzus persicae* (Sulzer) had a reduced alarm response compared with susceptible *M. persicae.* Excessive cornicle secretions occurred only from aphids exposed to insecticides in this bioassay. Cornicle secretions were not observed from dead control aphids, which were dipped only in water.

Although currently there are no reports of development of soybean aphid resistance to the five insecticides tested in this study, there are numerous reports of insecticide resistance in a closely related species, the cotton/melon aphid, *Aphis gossypii* Glover. In Australia, Herron and Powis (2005) documented chlorpyrifos resistance in 40% of *A. gossypii* field populations in a bioassay. *A. gossypii* collected from cotton fields in Pakistan had resistance ratios ranging from 1 to 41 for dimethoate (Ahmad and Arif 2008). Fenvalerate was extensively used to control aphids on cotton and other crops for decades, particularly in Asian countries (Wang et al. 2002), and *A. gossypii* is resistant to fenvalerate, with survival at extremely high concentrations (Zil'bermints and Zhuravleva 1984, Thayumanavan et al. 1993, Sun et al. 1994). Although not identical, fenvalerate and esfenvalerate are conformational isomers. Populations of *A. gossypii* from Hawaii showed a 390-fold resistance to esfenvalerate (Hollingsworth et al. 1994). *A. gossypii* populations collected in central Pakistan from 1997 to 2000 were resistant to seven pyrethroid insecticides, with resistance ratios ranging from 205 to 723 for λ-cyhalothrin (Ahmad et al. 2003). For imidacloprid, Wang et al. (2002) reported an eight-fold increase in the resistance ratio after 13 generations of selection in the laboratory. Thus far, *A. gossypii* resistance to imidacloprid in the field has not been confirmed.

In this study, all the field aphid strains were highly susceptible to the four organophosphates and pyrethroids tested. However, both the greenhouse and East Lansing strains were fairly tolerant to imidacloprid (Table 2). In particular, the risk of resistance development seems greatest for the neonicotinoids, which are used both as seed and foliar treatments on soybeans, potentially exposing aphids multiple times in the same field season. To date, there are no published reports of field failures with imidacloprid for soybean aphid. Recently, Magalhaes et al. (2008) studied baseline susceptibility for imidacloprid delivered systemically, as in a seed treatment. They found imidacloprid was very toxic to soybean aphid and had both lethal and sublethal effects on reproductive capacity and survivorship. They attributed these effects not only to direct insecticide toxicity but also possible anti-feedant behavior (Magalhaes et al. 2008).

In this study, aphids were directly dipped in imidacloprid solution and evaluated for survivorship only at 48 h. Sublethal antifeedant effects or impacts on reproduction occurring after 48 h were not measured. Many seed treatments take longer time to act on the insect, because they are required to remain in high concentrations in the growing plant for a longer period (Magalhaes et al. 2009). Magalhaes et al. (2008) reported that when aphids were fed on imidacloprid-immersed leaflets, lethal effects were observed after 7 d. In contrast, our assay involved a 10-s aphid-dip and a 48-h observation window, probably too short a time to observe the full lethal effects of imidacloprid. We believe that further or different bioassays are necessary to confirm low soybean aphid susceptibility to imidacloprid and perhaps other neonicotinoids.

The 10-s aphid-dip bioassay produced results within 48 h for most insecticides and was sufficiently sensitive to detect toxicity at very low insecticide concentrations. This method produced consistent results in repeated trials for the same product and allowed rapid assessment of mortality through visual observation. The method was repeatable and used only simple laboratory equipment. The ball-type mesh tea strainer provided an easy-to-clean and inexpensive tool to dip aphids. In conclusion, this study provided a method for testing susceptibility of soybean aphid to insecticides and enabled comparison of aphid susceptibility for five insecticides. In the event of a field failure, the aphid populations involved can be tested quickly to determine the extent of resistance development.

Acknowledgments

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