Cotton Aphid (Heteroptera: Aphididae) Susceptibility to Commercial and Experimental Insecticides in the Southern United States

J. GORE,[1,2] D. COOK,[1] A. CATCHOT,[3] B. R. LEONARD,[4] S. D. STEWART,[5] G. LORENZ,[6] AND D. KERNS[4]

J. Econ. Entomol. 106(3): 1430–1439 (2013); DOI: http://dx.doi.org/10.1603/EC13116

ABSTRACT Cotton aphid, *Aphis gossypii* Glover, has a history of developing resistance to novel insecticides. A program is needed to monitor cotton aphid susceptibility to new insecticides. Concentration-mortality bioassays were conducted from 2008 to 2011 to monitor the susceptibility of cotton aphids from fields across the midsouthern United States to thiamethoxam and sulfoxaflor. Flonicamid was included in 2010 and 2011. Bioassays followed the procedures described by the Insecticide Resistance Action Committee for testing neonicotinoids against cotton aphid. Mortality was rated at 48 and 72 h. These bioassays suggest that high levels of resistance to thiamethoxam occur in cotton aphid throughout the midsouthern United States. Resistance ratios ranged from 0.9 to 562.6 at 48 h, and from 0.9 to 29.1 at 72 h. Aphid colonies tested were considered susceptible to flonicamid and sulfoxaflor. The LC_{50} values ranged from 1.43 to 6.60 ppm for flonicamid. The LC_{50} values for sulfoxaflor ranged from 1.01 to 5.85 ppm and 0.92–4.13 ppm at 48 and 72 h, respectively. These values represent the baseline variability of the susceptibility of cotton aphid to flonicamid and sulfoxaflor. The moderate level of variability observed combined with the high level of efficacy at low rates and the high reproductive rate of cotton aphid suggests that an effective resistance management plan needs to be devised for these insecticides. Flonicamid and sulfoxaflor should provide effective control of cotton aphid in areas where thiamethoxam resistance occurs. However, these insecticides need to be incorporated into a rotation strategy to preserve their efficacy against cotton aphid.

KEY WORDS cotton aphid, neonicotinoid, flonicamid, sulfoxaflor, resistance

The cotton aphid (=melon aphid), *Aphis gossypii* Glover, is a pest of many crops worldwide (Blackmon and Eastop 1984). In the southern United States, the cotton aphid is an annual but sporadic pest of cotton, *Gossypium hirsutum* L. Insecticides are applied to a significant percentage of the cotton acreage every year for their control in the mid-South. Historically, cotton aphid has rapidly developed resistance to new insecticides soon after they are released for commercial use. In an article modeling the development of insecticide resistance in *Heliothis virescens* (F.), Mallet and Luttrell (1991) categorize pests into three groups depending on their reproductive potential and likelihood to develop resistance. They consider cotton aphid in the category with the potential to develop high levels of resistance in a relatively short period of time. This is based on the high reproductive potential of cotton aphid and the capacity for resurgence after an insecticide application.

Cotton aphid populations are generally maintained at low levels through the actions of natural enemies (Weathersbee and Hardee 1994). However, numerous applications of broad spectrum insecticides are often made during early to mid-June in cotton to control tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), in the midsouthern United States (Scott and Snodgrass 2000). Historically, pyrethroids, carbamates, and organophosphates were the insecticides of choice for those applications. Consequently, outbreaks of cotton aphid in mid- to late-June were usually the result of those applications because of the elimination of natural enemies (Slosser et al. 2001).

Recently, a new class of insecticides, the neonicotinoids, has been introduced that is relatively soft on natural enemies, and provides good control of both tarnished plant bug and cotton aphid (Tomizawa and Casida 2003). Imidacloprid (Provado 1.6 F, Bayer Crop Science, Research Triangle Park, NC) was the first neonicotinoid labeled for use in cotton in the United States, and there are now many different formulations of imidacloprid registered for use in cotton. Since the introduction of imidacloprid, other neonicotinoids have been introduced. They include thiamethoxam (Centric 40 WG, Syngenta Crop Protection, Greensboro, NC), and acetamiprid (Intruder 70WSP, Gowan Company, Yuma, AZ) (Tomizawa and Casida 2003). Currently, these insecticides are applied over large acreages during June because of their ac-

[1] Mississippi State University, DREC, P. O. Box 197, Stoneville, MS.
[2] Corresponding author, e-mail: jgore@drec.msstate.edu.
[3] Mississippi State University, EPP, Mississippi State, MS.
[4] LSU AgCenter, NERS, 212A Macon Ridge Road, Winnsboro, LA.
[5] University of Tennessee, WTREC, 605 Airways Blvd, Jackson, TN.
[6] University of Arkansas, 2001 Highway 70 E, Lonoke, AR.

tivity against both cotton aphid and tarnished plant bug. This combined with the historical ability of cotton aphid to rapidly develop resistance to new insecticides creates the need for a proactive program to monitor cotton aphid susceptibility to these compounds (Kerns and Gaylor 1993).

Flonicamid (Carbine 50 WG, FMC Corporation, Philadelphia, PA) is another insecticide that was recently granted a label for control of cotton aphid in cotton. It became available for use worldwide in 2005 and 2006 to control multiple aphid species on various crops (Morita et al. 2007). Flonicamid is a pyridinecarboxamide that has a novel mode of action, acting via the nervous system, and eventual death is a result of starvation from a cessation of feeding that occurs immediately after exposure (Morita et al. 2007). Field research in cotton demonstrated good control of cotton aphid with this product (Hancock 2003).

Currently, a new insecticide is being tested in the midsouthern United States that has shown good activity against both tarnished plant bug and cotton aphid (Siebert et al. 2012). The sulfoxamine, sulfoxaflor (Transform 50 WG, Dow AgroSciences, Indianapolis, IN), is a proposed new class of chemistry that acts on the nicotinic acetylcholine receptors in susceptible insects (Babcock et al. 2010, Watson et al. 2011, Zhu et al. 2011). It was recently classified as a nicotinic acetylcholine receptor agonist and was granted a 4C classification by the Insecticide Resistance Action Committee (IRAC, http://www.irac-online.org/eClassification/).

Recently, field control of cotton aphid with the neonicotinoid insecticides has been declining in the midsouthern United States. In field experiments conducted in Mississippi and Louisiana in 2002 and 2004, percent control of cotton aphid ranged from 81–89, 94–97, and 91–97% for imidacloprid, acetamiprid, and thiamethoxam, respectively (Layton et al. 2003, Bommireddy et al. 2005). In contrast, control ranged from 27–96% and 2–19% for acetamiprid and thiamethoxam, respectively, in each of those states during 2010 and 2011 (Adams et al. 2011, Emfinger et al. 2012). Imidacloprid was only included in one experiment, and control averaged 82% (Emfinger et al. 2012). Field experiments such as these confirmed reports from growers and consultants about the declining efficacy of neonicotinoids against cotton aphid. However, bioassays were needed to confirm resistance to this class of insecticides and to gain a better understanding about the distribution and spread of resistance in cotton aphid populations. Additionally, although flonicamid and sulfoxaflor are relatively new insecticides, baseline data are needed for these insecticides given the nature of cotton aphid and their history of resistance. Bioassays were conducted with thiamethoxam and sulfoxaflor from 2008 to 2011, and flonicamid from 2010 to 2011 to characterize the variability in the response of cotton aphid populations from across the midsouthern United States to these insecticides.

Materials and Methods

Bioassays were conducted to measure cotton aphid susceptibility to the currently labeled neonicotinoid insecticide, thiamethoxam (Centric 40 WG, Syngenta Crop Protection), and an experimental insecticide, sulfoxaflor (Transform 50 WG, Dow AgroSciences) from 2008 to 2011. Additional bioassays were conducted in 2010 and 2011 with flonicamid (Carbine 50 WG, FMC Corporation, Princeton, NJ). Methods followed those described by the IRAC (Method No. 019, http://www.irac-online.org/content/uploads/Method_019-_v3.2_May12_aphid.pdf). The bioassay arena consisted of individual 30 by 10 mm petri dishes with a 2 mm layer of a 1% agar solution in the bottom. A 5 mm diameter hole was cut into each lid and sealed with a piece of cotton cloth to allow excess moisture to escape. Commercial formulations of each insecticide were used for bioassays. Serial dilutions of each insecticide were made to obtain six or seven concentrations along with a water only control. Insecticides were diluted in water to obtain 500 ml of solution at the various concentrations. A nonionic surfactant (Scanner 80:20, Loveland Products, Inc., Greely, CO) was added to each solution at a rate of 0.5% vol:vol to ensure even distribution across the surface of the leaf disc.

Cotton leaves were removed from nontreated plants and washed with a mild solution of soap and water to remove naturally occurring aphids. The leaves were rinsed well and allowed to air dry. A 25 mm diameter disc was cut from each leaf with a sharpened steel tube. Individual leaf discs were dipped into individual solutions and swirled for 5 s. The leaf discs for the nontreated treatment were swirled in water with the nonionic surfactant only. The leaf discs were then placed on a wire rack with the adaxial surface (top) against the rack and allowed to dry completely. When completely dry, each leaf disc was placed in an individual petri dish with the adaxial surface against the agar. The edges of each leaf disc were gently pressed into the agar to minimize desiccation.

Cotton aphids used for bioassays were collected from commercial cotton fields across Mississippi, Louisiana, Arkansas, Tennessee, and Texas where less than adequate control was observed with foliar applications of neonicotinoid insecticides (Table 1). Additionally, a laboratory susceptible colony was tested each year. The susceptible colony was obtained from Dow AgroSciences. This colony was originally collected from cotton growing in a greenhouse in Indianapolis, IN, on 03 November 2006. It has been maintained in the laboratory and greenhouse on cotton and squash, *Cucurbita* spp. since that time and has no known exposure to any insecticides. Where it was possible, cotton aphids were also collected from nontreated cotton fields in the near vicinity on the same date as a comparison. For the Arkansas collections (A and B) in 2009, cotton aphids were collected from the same field on different dates. The first collection [Arkansas (A)], aphids were collected 1 wk before the first foliar application of a neonicotinoid insecticide. The second

Table 1. Locations, dates of collection, and field treatment history of each of the cotton aphid colonies tested from 2008 to 2011

Colony	Date	Treatment history[a]
Leland, MS	8 July 2008	Imidacloprid (0.048), Imidacloprid (0.059)
Stoneville, MS	8 July 2008	Not treated
Grenada, MS (A)	30 June 2008	Thiamethoxam (0.042), Thiamethoxam (0.056)
Grenada, MS (B)	30 June 2008	Thiamethoxam (0.056)
Grenada, MS (C)	30 June 2008	Not treated
Grenada, MS	12 June 2009	Not treated
Grenada, MS (A)	23 June 2009	Thiamethoxam (0.056)
Grenada, MS (B)	23 June 2009	Thiamethoxam (0.056)
Grenada, MS (C)	23 June 2009	Thiamethoxam (0.056)
Wayside, MS	11 July 2009	Thiamethoxam (0.056), Thiamethoxam (0.063)
Marks, MS	18 July 2009	Thiamethoxam (0.042), Imidacloprid 2X (0.059, 0.070)
Arkansas (A)	23 June 2009	Not treated
Arkansas (B)	13 July 2009	Thiamethoxam (0.056)
Grenada, MS	2 July 2010	Thiamethoxam (0.042)
Winnsboro, LA	23 June 2010	Not treated
Glendora, MS	13 July 2010	Thiamethoxam (0.056)
Alexandria, LA	12 July 2010	Thiamethoxam (0.056)
Stoneville, MS	19 July 2010	Thiamethoxam (0.056)
Tennessee	11 August 2010	Imidacloprid (0.059)
Belzoni, MS	5 July 2011	Imidacloprid 2X (0.059)
Wayside, MS	11 July 2011	Not Treated
Glendora, MS	11 July 2011	Thiamethoxam 2X (0.056)
St. Joseph, LA	25 July 2011	Thiamethoxam (0.056)
Winnsboro, LA	25 July 2011	Not Treated
Lubbock, TX	20 Sept. 2011	Not treated

[a] Insecticides (rate in kilograms of active ingredient per hectare) that cotton fields were sprayed with before collection of cotton aphids.

collection [Arkansas (B)] was made 2 wk after an application of thiamethoxam at 0.056 kg ai/ha (active ingredient per hectare). Aphids were collected by removing the terminals of heavily infested plants. Terminals included the upper four to five nodes of the plants and associated leaves. Infested terminals were placed in paper bags and transported or shipped overnight to the Mississippi State University Entomology laboratory at the Delta Research and Extension Center in Stoneville, MS. At the time of collection, field treatment history was recorded (Table 1). All bioassays were conducted within 48 h of aphid collection. In the laboratory, five late instar cotton aphid nymphs were transferred to each leaf disc with a small paint brush. In total, 10 leaf discs were used for each concentration of each insecticide. Each bioassay was replicated three to four times. The dishes were held in an environmentally controlled room at 26 ± 2°C, 75 ± 5% relative humidity (RH), and a photoperiod of 14:10 (L:D) h. Mortality of cotton aphids was rated after 48 and 72 h of exposure to the treated leaves. Mortality was scored based on the inability of cotton aphids to show coordinated movement after being lightly prodded with a small paint brush. Data were log transformed and analyzed with Probit analysis (PROC PROBIT, version 9.2, SAS Institute, Cary, NC). LC_{50} values along with 95% fiducial limits (FL) were obtained for each insecticide and are presented as untransformed values. The LC_{50} values of each field collection were compared with the LC_{50} value of the susceptible population within each year. Differences in LC_{50} values were considered significant if the 95% CL of the resistance ratio at LC_{50} did not include 1.0 (Robertson et al. 2007). Additionally, analysis of variance (ANOVA) was used to compare the effects of year and field treatment history on LC_{50} values of each insecticide (PROC MIXED, version 9.2, SAS Institute). Because LC_{50} values varied considerably, especially for thiamethoxam, LC_{50} values were square root transformed to normalize the variances. In the ANOVA model, year, field treatment history, and the year by field treatment history interaction were designated as fixed effects. Replication was designated as the random effect.

Results

In total, 36 field collected colonies of cotton aphid were tested from 2008 to 2011. Colonies with control mortality ≥10% were excluded from the final analysis. As a result, data were obtained from 25 colonies over the 4 yr period (Table 1).

Thiamethoxam. Cotton aphid response to thiamethoxam was highly variable at 48 h (Table 2). The LC_{50} values at 48 h ranged from 3.21 to 1,234 ppm for field collected cotton aphids. This represents a 384-fold range in LC_{50} values across the 4 yr. Across all years, resistance ratios of field collected cotton aphids ranged from 0.9 to 562.6 when compared with the susceptible colony within a year. All of the field collected colonies had significantly higher LC_{50} values than the susceptible colony, except cotton aphids collected from Tennessee in 2010.

Overall, the LC_{50} values for thiamethoxam declined from 48 to 72 h (Table 3). At 72 h, LC_{50} values ranged from 2.56 to 122.42 ppm for field collected populations. This represents a 47.8-fold range in LC_{50} values across the 4 yr of the experiment. Resistance ratios compared with the susceptible colony within a year ranged from 0.9 to 29.1. The majority of field collected populations

Table 2. Leaf-dip bioassays with thiamethoxam (Centric 40WG) against cotton aphids in 2008–2012

Colony	Year	Ln slope[a]	LC_{50} (CI)	χ^2 (P)[b]	r	ll	ul
Leland, MS	2008	0.24*	1206 (165–33018230)	1.66 (0.80)	562.6	19.6	16,123.4
Stoneville, MS[c]	2008	0.95*	3.27 (2.70–3.92)	4.87 (0.43)	1.5	1.2	2.0
Grenada, MS (A)	2008	0.30*	33.41 (12.80–889.30)	9.68 (0.08)	15.6	5.4	44.9
Grenada, MS (B)	2008	0.20*	303.4 (71.3–30243)	5.60 (0.35)	141.6	15.5	1,269.5
Grenada, MS (C)[c]	2008	0.49*	5.54 (4.09–7.44)	7.24 (0.20)	2.6	1.8	3.7
Susceptible	2008	1.38*	2.14 (1.74–2.60)	1.77 (0.62)	NA	NA	NA
Grenada, MS[c]	2009	0.76*	3.48 (2.58–4.56)	7.96 (0.16)	1.6	1.1	2.2
Grenada, MS (A)	2009	0.26*	1234 (248–144539)	4.48 (0.61)	549.5	48.7	6,199.8
Grenada, MS (B)	2009	0.31*	476 (181–3598)	4.08 (0.77)	212.1	56.1	800.9
Grenada, MS (C)	2009	0.47*	108.6 (64.1–262.7)	4.10 (0.66)	48.4	24.3	96.3
Wayside, MS	2009	0.48*	220.6 (89.3–1993)	5.10 (0.40)	98.2	26.8	360.5
Marks, MS	2009	0.32*	1156 (182–3488672)	4.35 (0.50)	515.2	24.9	1,0645.5
Arkansas (A)[c]	2009	0.94*	6.53 (5.31–8.02)	2.72 (0.74)	2.9	2.2	3.8
Arkansas (B)	2009	0.64*	41.5 (28.9–71.9)	2.74 (0.74)	18.5	11.5	29.7
Susceptible	2009	1.52*	2.25 (1.85–2.71)	0.47 (0.92)	—	—	—
Grenada, MS	2010	0.68*	34.07 (23.88–59.95)	3.62 (0.46)	10.1	6.2	16.4
Winnsboro, LA[c]	2010	0.51*	16.81 (12.00–27.10)	1.15 (0.89)	5.0	3.2	7.8
Glendora, MS	2010	0.43*	74.65 (37.34–312.9)	1.84 (0.76)	22.0	8.4	57.8
Alexandria, LA	2010	0.47*	45.92 (30.47–85.50)	3.66 (0.60)	13.6	7.9	23.4
Stoneville, MS	2010	0.54*	119.3 (61.5–485.5)	3.37 (0.64)	35.2	13.9	89.2
Tennessee	2010	1.09*	3.21 (2.68–3.82)	6.09 (0.11)	0.9	0.7	1.3
Susceptible	2010	1.07*	3.39 (2.69–4.23)	1.17 (0.88)	—	—	—
Belzoni, MS	2011	0.57*	116.77 (65.09–363.71)	3.33 (0.65)	20.1	8.8	45.8
Wayside, MS[c]	2011	0.49*	27.94 (19.52–46.17)	3.57 (0.61)	4.8	2.9	7.9
Glendora, MS	2011	0.69*	96.99 (59.45–252.57)	2.71 (0.74)	16.7	8.3	33.9
St. Joseph, LA	2011	0.32*	108.59 (49.23–533.66)	4.10 (0.54)	18.7	6.3	56.0
Winnsboro, LA[c]	2011	0.52*	27.22 (19.48–49.93)	3.57 (0.61)	5.0	3.1	8.1
Lubbock, TX[c]	2011	0.60*	80.98 (50.52–183.81)	1.92 (0.86)	14.0	7.2	27.0
Susceptible	2011	0.81*	5.80 (4.44–7.73)	1.84 (0.77)	—	—	—

LC_{50}'s are reported as parts per million 48 h after treatment.
[a] Slopes with an asterisk have a significant χ^2 value ($\alpha = 0.05$).
[b] Goodness of fit χ^2 and P value.
[c] Colonies were collected from cotton fields that were not sprayed with a foliar neonicotinoid.

had significantly higher LC_{50} values than the susceptible colony. The colonies that did not have a significantly higher LC_{50} than the susceptible colony included Grenada (C) in 2008, Grenada in 2009, and Tennessee in 2010.

Sulfoxaflor. Cotton aphid response to sulfoxaflor showed little variability at 48 h (Table 4). LC_{50} values ranged from 0.37 to 5.85 ppm. This represents a 15.8-fold range in LC_{50} values across the 4 yr. Resistance ratios compared with the susceptible colony within a year ranged from 0.4 to 3.0. During 2008, all field collected populations had significantly lower LC_{50} values than the susceptible colony. In contrast, five of the eight populations tested in 2009 had higher LC_{50} values than the susceptible colony. The population Grenada (B) had a resistance ratio of 3.0, but lower and upper limits were not calculated. In 2010 and 2011, all field populations tested had LC_{50} values similar to the susceptible colony. Over the 4 yr, 13 populations had LC_{50} values similar to the susceptible colony, six populations had LC_{50} values lower than the susceptible colony, and five populations had LC_{50} values higher than the susceptible colony.

At 72 h, LC_{50} values ranged from 0.92 to 4.13 ppm (Table 5). This represents a 4.3-fold range in LC_{50} values across the 4 yr. Resistance ratios compared with the susceptible colony within a year ranged from 0.4 to 2.4. All field populations tested in 2008 had significantly lower LC_{50} values than the susceptible colony. During 2009, five of the populations had higher LC_{50} values than the susceptible colony. Similar to the data at 48 h, all field populations had LC_{50} values similar to the susceptible colony in 2010 and 2011 at 72 h. Over the 4 yr, 13 populations had LC_{50} values similar to the susceptible colony, six populations had LC_{50} values lower than the susceptible colony, and six populations had LC_{50} values higher than the susceptible colony.

Flonicamid. At 48 h, cotton aphid susceptibility to flonicamid was highly variable (Table 6). In these bioassays, LC_{50} values were well outside the range of concentrations tested for most of the populations. Because of that, no conclusions can be drawn from the flonicamid data at 48 h.

At 72 h, cotton aphid response to flonicamid was more consistent and the LC_{50} values fell within the range of concentrations tested (Table 7). LC_{50} values ranged from 2.07 to 5.22 ppm at 72 h. This represents a 2.5-fold range in LC_{50} values for flonicamid. Resistance ratios compared with the susceptible colony ranged from 1.1 to 2.5. All field populations tested in 2010 had higher LC_{50} values than the susceptible colony. In contrast, only one population out of the six tested in 2011 had a higher LC_{50} value than the susceptible colony. Over the 2 yr that flonicamid was tested, seven populations had LC_{50} values higher than the susceptible colony and five populations had similar LC_{50} values to the susceptible colony.

Impact of Year and Field Treatment History. There was a significant effect of field treatment history on cotton aphid susceptibility to thiamethoxam at 48 h

Table 3. Leaf-dip bioassays with thiamethoxam (Centric 40WG) against cotton aphids in 2008–2012

Colony	Year	Ln slope[a]	LC_{50} (CI)	χ^2 (P)[b]	r	ll	ul
Leland, MS	2008	0.83*	12.95 (10.53–16.22)	4.45 (0.35)	6.8	5.1	9.3
Stoneville, MS[c]	2008	0.99*	3.05 (2.52–3.65)	4.98 (0.29)	1.6	1.2	2.1
Grenada, MS (A)	2008	0.73*	10.71 (6.44–18.81)	8.11 (0.09)	5.7	3.8	8.5
Grenada, MS (B)	2008	0.64*	15.56 (7.66–50.77)	12.97 (0.01)	8.2	4.7	14.4
Grenada, MS (C)[c]	2008	0.76*	2.93 (1.63–4.63)	12.90 (0.02)	1.5	1.0	2.4
Susceptible	2008	1.27*	1.89 (1.51–2.33)	2.66 (0.45)	—	—	—
Grenada, MS[c]	2009	0.66*	2.56 (1.76–3.47)	6.23 (0.28)	1.3	0.9	1.9
Grenada, MS (A)	2009	0.70*	14.50 (11.4–18.7)	10.76 (0.10)	7.2	5.3	9.8
Grenada, MS (B)	2009	0.78*	12.15 (9.8–15.1)	8.35 (0.30)	6.1	4.6	8.1
Grenada, MS (C)	2009	0.62*	5.93 (4.51–7.68)	8.89 (0.18)	3.0	2.1	4.1
Wayside, MS	2009	0.84*	10.05 (8.07–12.69)	2.01 (0.85)	5.0	3.8	6.7
Marks, MS	2009	0.64*	7.70 (5.91–10.15)	4.84 (0.44)	3.8	2.8	5.3
Arkansas (A)[c]	2009	0.92*	5.79 (4.69–7.12)	2.94 (0.71)	2.9	2.2	3.8
Arkansas (B)	2009	0.71*	10.61 (8.31–13.89)	5.98 (0.31)	5.3	3.9	7.3
Susceptible	2009	1.57*	2.00 (1.66–2.41)	0.28 (0.96)	—	—	—
Grenada, MS	2010	0.67*	20.90 (15.67–31.18)	3.79 (0.43)	7.1	4.8	10.5
Winnsboro, LA[c]	2010	0.55*	12.57 (9.35–18.37)	0.69 (0.95)	4.3	2.9	6.3
Glendora, MS	2010	0.94*	9.21 (7.64–11.27)	2.18 (0.70)	3.1	2.3	4.2
Alexandria, LA	2010	0.74*	13.35 (10.79–16.78)	3.53 (0.62)	4.5	3.3	6.2
Stoneville, MS	2010	0.73*	17.71 (13.73–23.98)	1.78 (0.88)	6.0	4.2	8.5
Tennessee	2010	1.18*	2.74 (2.31–3.24)	4.91 (0.18)	0.9	0.7	1.2
Susceptible	2010	1.18*	2.95 (2.38–3.67)	3.88 (0.28)	—	—	—
Belzoni, MS	2011	0.50*	122.42 (64.65–414.31)	0.93 (0.97)	29.1	12.2	69.5
Wayside, MS[c]	2011	0.51*	23.77 (17.11–36.96)	2.88 (0.72)	5.6	3.7	8.7
Glendora, MS	2011	0.65*	57.52 (39.26–107.64)	2.32 (0.68)	13.7	8.1	23.0
St. Joseph, LA	2011	0.33*	70.41 (36.15–243.07)	1.26 (0.94)	16.7	6.8	40.9
Winnsboro, LA[c]	2011	0.50*	26.19 (18.56–41.99)	2.54 (0.77)	6.2	3.9	9.8
Lubbock, TX[c]	2011	0.72*	17.89 (14.19–23.47)	1.76 (0.88)	4.3	3.0	5.9
Susceptible	2011	1.12*	4.21 (3.39–5.25)	4.75 (0.31)	—	—	—

LC_{50}'s are reported as parts per million 72 h after treatment.
[a] Slopes with an asterisk have a significant χ^2 value ($\alpha = 0.05$).
[b] Goodness of fit χ^2 and P value.
[c] Colonies were collected from cotton fields that were not sprayed with a foliar neonicotinoid.

($F = 7.55$; df = 1, 17; $P = 0.01$; Table 8). Cotton aphids collected from cotton fields that received at least one foliar application of a neonicotinoid insecticide had higher LC_{50} values than cotton aphids collected from cotton fields that were not previously treated with a foliar neonicotinoid. The mean (SEM) LC_{50} for cotton aphids collected from fields that did not have a previous foliar application of a neonicotinoid insecticide was 13.0 (4.15) ppm. In comparison, the mean (SEM) LC_{50} was 303.3 (100.71) ppm for cotton aphids collected from fields that received at least one foliar application of a neonicotinoid. Year ($F = 0.46$; df = 3, 17; $P = 0.71$) and the year by field treatment history interaction ($F = 1.10$; df = 3, 17; $P = 0.38$) were not significant (Table 9).

There was also a significant effect of field treatment history on cotton aphid susceptibility to thiamethoxam at 72 h ($F = 5.24$; df = 1, 17; $P = 0.04$; Table 8). The mean (SEM) LC_{50} was 11.0 (3.85) for cotton aphids collected from fields that did not receive a foliar application of a neonicotinoid insecticide. The mean (SEM) LC_{50} for thiamethoxam was 24.0 (7.09) ppm for cotton aphids collected from fields that received at least one foliar application of a neonicotinoid insecticide. Additionally, there was a significant effect of year ($F = 9.49$; df = 3, 17; $P < 0.01$) on cotton aphid susceptibility to thiamethoxam at 72 h (Table 9). The LC_{50} value in 2011 was significantly higher than the LC_{50} values from 2008 to 2010. Mean (SEM) LC_{50} values 9.0 (2.59), 8.7 (1.37), 12.7 (2.61), and 53.0 (16.28) in 2008 through 2011, respectively. The year by field treatment history interaction was not significant ($F = 0.89$; df = 3, 17; $P = 0.47$).

Field treatment history ($F = 0.43$; df = 1, 17; $P = 0.52$) and the year by field treatment history interaction ($F = 1.29$; df = 3, 17; $P = 0.31$) was not significant for cotton aphid susceptibility to sulfoxaflor at 48 h. Year had a significant effect on cotton aphid susceptibility to sulfoxaflor at 48 h ($F = 12.75$; df = 3, 17; $P < 0.01$). The LC_{50} in 2008 was significantly lower than the LC_{50} values from 2009 to 2011. The mean (SEM) LC_{50} values were 1.2 (0.23), 4.1 (0.46), 2.8 (0.10), and 3.5 (0.35) in 2008 through 2011, respectively.

Similar to 48 h, year had a significant effect on the susceptibility of cotton aphid to sulfoxaflor at 72 h ($F = 6.33$; df = 3, 17; $P < 0.01$). The LC_{50} in 2008 was significantly lower than the LC_{50} values in 2009 through 2011. The mean (SEM) LC_{50} values were 1.2 (0.09), 2.6 (0.32), 2.5 (0.15), and 2.6 (0.29) in 2008 through 2011, respectively.

For flonicamid, there was not a significant effect of year ($F = 3.53$; df = 1, 8; $P = 0.10$), field treatment history ($F = 2.30$; df = 1, 8; $P = 0.17$), or year by field treatment history interaction ($F = 0.01$; df = 1, 8; $P = 0.94$).

Insecticide Comparisons. For the comparison of cotton aphid susceptibility to the insecticides tested at 48 h, the data for flonicamid were not included in the analysis because the LC_{50} values were outside the range of concentrations tested. At 48 h, there was a

Table 4. Leaf-dip bioassays with sulfoxaflor (Transform 50WG) against cotton aphids in 2008–2012

Colony	Year	Ln slope[a]	LC_{50} (CI)	χ^2 (P)[b]	r	ll	ul
Leland, MS	2008	0.67*	1.01 (0.75–1.34)	2.84 (0.58)	0.4	0.3	0.9
Stoneville, MS[c]	2008	0.70*	1.33 (1.04–1.73)	2.16 (0.71)	0.5	0.4	0.7
Grenada, MS (A)	2008	0.66*	1.37 (0.11–0.62)	8.65 (1.73)	0.5	0.4	0.8
Grenada, MS (B)	2008	0.63*	1.55 (0.85–2.79)	14.43 (0.01)	0.6	0.4	0.9
Grenada, MS (C)[c]	2008	0.53*	1.62 (1.19–2.25)	6.39 (0.17)	0.6	0.4	0.9
Susceptible	2008	1.33*	2.66 (2.17–3.25)	3.07 (0.38)	—	—	—
Grenada, MS[c]	2009	1.59*	2.49 (2.07–2.99)	2.83 (0.42)	1.3	0.9	1.6
Grenada, MS (A)	2009	0.72*	4.87 (3.79–6.20)	7.26 (0.20)	2.5	1.8	3.4
Grenada, MS (B)	2009	0.90*	5.85 (4.74–7.21)	7.53 (0.18)	3.0	—	—
Grenada, MS (C)	2009	1.10*	3.00 (2.47–3.63)	2.17 (0.70)	1.5	1.2	2.0
Wayside, MS	2009	0.90*	2.63 (2.08–3.26)	4.85 (0.30)	1.3	1.0	1.8
Marks, MS	2009	0.63*	5.46 (4.14–7.15)	4.47 (0.48)	2.8	2.0	3.9
Arkansas (A)[c]	2009	1.03*	4.46 (3.66–5.44)	4.35 (0.36)	2.3	1.7	3.0
Arkansas (B)	2009	1.29*	3.77 (3.17–4.52)	0.80 (0.85)	1.9	1.5	2.5
Susceptible	2009	1.67*	1.96 (1.63–2.34)	0.22 (0.97)	—	—	—
Grenada, MS	2010	1.32*	3.16 (2.70–3.68)	3.32 (0.34)	1.0	0.8	1.3
Winnsboro, LA[c]	2010	1.24*	2.63 (2.22–3.09)	1.55 (0.67)	0.8	0.7	1.1
Glendora, MS	2010	1.20*	2.63 (2.28–3.05)	4.09 (0.54)	0.8	0.7	1.1
Alexandria, LA	2010	1.39*	2.80 (2.41–3.26)	4.34 (0.23)	0.9	0.7	1.2
Stoneville, MS	2010	1.15*	2.64 (2.29–3.08)	4.27 (0.51)	0.9	0.7	1.1
Tennessee	2010	1.64*	3.08 (2.68–3.53)	2.26 (0.52)	1.0	0.8	1.3
Susceptible	2010	1.26*	3.10 (2.52–3.82)	3.85 (0.28)	—	—	—
Belzoni, MS	2011	1.23*	3.54 (3.01–4.14)	1.57 (0.81)	1.0	0.8	1.3
Wayside, MS[c]	2011	1.38*	4.62 (3.99–5.37)	2.01 (0.73)	1.3	1.0	1.7
Glendora, MS	2011	1.40*	3.76 (3.24–4.36)	0.93 (0.92)	1.1	0.8	1.4
St. Joseph, LA	2011	1.39*	2.07 (1.78–2.40)	3.62 (0.46)	0.6	0.5	0.8
Winnsboro, LA[c]	2011	1.42*	3.98 (3.44–4.61)	1.49 (0.83)	1.1	0.9	1.5
Lubbock, TX[c]	2011	1.30*	3.16 (2.71–3.69)	2.06 (0.72)	0.9	0.7	1.2
Susceptible	2011	1.42*	3.48 (2.87–4.24)	5.28 (0.15)	—	—	—

LC_{50}'s are reported as parts per million 48 h after treatment.
[a] Slopes with an asterisk have a significant χ^2 value ($\alpha = 0.05$).
[b] Goodness of fit χ^2 and P value.
[c] Colonies were collected from cotton fields that were not sprayed with a foliar neonicotinoid.

significant difference in cotton aphid susceptibility to sulfoxaflor and thiamethoxam ($F = 40.14$; df = 1, 48; $P < 0.01$). Cotton aphids were more susceptible to sulfoxaflor than thiamethoxam. The mean (SEM) LC_{50} values were 3.1 (0.3) ppm and 222.0 (76.7) ppm for sulfoxaflor and thiamethoxam, respectively.

At 72 h, flonicamid was included in the analysis. There were significant differences in the susceptibility of cotton aphid between the insecticides tested ($F = 31.44$; df = 2, 59; $P < 0.01$). Cotton aphids were more susceptible to sulfoxaflor and flonicamid than thiamethoxam. Cotton aphid susceptibility to sulfoxaflor and flonicamid at 72 h was similar among the populations tested. The mean (SEM) LC_{50} values were 2.3 (0.2), 3.9 (0.2), and 20.4 (5.3) ppm for sulfoxaflor, flonicamid, and thiamethoxam, respectively.

Discussion

Cotton aphid has a long history of rapidly developing resistance to multiple insecticides (Gong et al. 1964, O'Brien and Graves 1992, O'Brien et al. 1992). Until recently, the neonicotinoid class of chemistry has provided effective control of this insect on cotton in the southern United States. In the current experiment, bioassays conducted on field populations demonstrated a significant level of resistance to thiamethoxam in cotton aphid. Populations collected from nontreated fields and fields that had previously been treated with at least one foliar application of a neonicotinoid had LC_{50} values significantly higher than the susceptible colony. Additionally, populations collected from treated fields were generally more resistant than those collected from nontreated fields. Previously, colonies of cotton aphid with high levels of resistance to imidacloprid were artificially selected in the laboratory (Wang et al. 2002, Shi et al. 2011). After 45 generations, the resistance ratio of a cotton aphid colony selected for resistance to imidacloprid was 41.7 (Shi et al. 2011). The first cases of field evolved resistance to neonicotinoids in cotton aphid were documented from Australia (Herron and Wilson 2011). Field evolved resistance to acetamiprid, clothianidin, and thiamethoxam in Australia was 6.4-, 10-, and 22-fold, respectively, at 24 h. In the current experiment, field evolved resistant strains of cotton aphid had resistance ratios as high as 562.6-fold at 48 h, and 29.1-fold at 72 h.

Multiple insects around the world have developed resistance to neonicotinoids. Resistance ratios of field collected green peach aphid, *Myzus persicae* (Sulzer), ranged from 1.9 to 63.8 for imidacloprid in the eastern United States with 20 of the 45 populations tested having resistance ratios of 10.0 or higher (Srigiriraju et al. 2010). Similar results were observed for housefly, *Musca domestica* L., in Denmark (Kristensen and Jespersen 2008) and Florida (Kaufman et al. 2010). Brown planthopper, *Nilaparvata lugens* Stål, has developed resistance to imidacloprid in several Asian countries (Gorman et al. 2008, Wen et al. 2009). Other

Table 5 Leaf-dip bioassays with sulfoxaflor (Transform 50WG) against cotton aphids in 2008–2012

Colony	Year	Ln slope[a]	LC_{50} (CI)	χ^2 $(P)^b$	r	ll	ul
Leland, MS	2008	0.74*	0.92 (0.70–1.20)	6.46 (0.17)	0.4	0.3	0.5
Stoneville, MS[c]	2008	0.70*	1.25 (0.97–1.62)	1.92 (0.75)	0.5	0.4	0.7
Grenada, MS (A)	2008	0.66*	1.23 (0.95–1.58)	6.82 (0.23)	0.5	0.4	0.7
Grenada, MS (B)	2008	0.79*	1.23 (0.98–1.54)	8.83 (0.12)	0.5	0.4	0.7
Grenada, MS (C)[c]	2008	0.53*	1.51 (1.11–2.09)	5.66 (0.23)	0.6	0.4	0.9
Susceptible	2008	1.36*	2.41 (1.96–2.94)	1.29 (0.73)	—	—	—
Grenada, MS[c]	2009	1.05*	1.6 (1.20–2.03)	4.56 (0.24)	0.9	0.7	1.3
Grenada, MS (A)	2009	0.54*	2.86 (1.97–3.88)	8.20 (0.15)	1.7	1.2	2.4
Grenada, MS (B)	2009	0.43*	1.79 (1.00–2.69)	6.72 (0.24)	1.1	0.6	1.7
Grenada, MS (C)	2009	0.80*	1.60 (1.16–2.06)	2.73 (0.60)	0.9	0.7	1.3
Wayside, MS	2009	0.92*	2.40 (1.90–2.97)	5.01 (0.29)	1.4	1.1	1.9
Marks, MS	2009	0.54*	4.13 (2.97–5.59)	1.53 (0.91)	2.4	1.7	3.5
Arkansas (A)[c]	2009	0.75*	2.90 (2.22–3.71)	6.50 (0.16)	1.7	1.3	2.3
Arkansas (B)	2009	1.16*	3.40 (2.82–4.12)	0.51 (0.92)	2.0	1.7	2.4
Susceptible	2009	2.07*	1.70 (1.44–2.00)	0.42 (0.81)	—	—	—
Grenada, MS	2010	1.34*	2.96 (2.53–3.45)	3.45 (0.33)	1.4	1.1	1.7
Winnsboro, LA[c]	2010	1.21*	2.55 (2.14–3.00)	1.57 (0.67)	1.2	0.9	1.5
Glendora, MS	2010	1.49*	2.14 (1.89–2.44)	5.09 (0.28)	1.0	0.8	1.2
Alexandria, LA	2010	1.30*	2.78 (2.37–3.25)	2.49 (0.48)	1.3	1.0	1.6
Stoneville, MS	2010	1.29*	2.09 (1.83–2.40)	4.53 (0.48)	1.0	0.8	1.2
Tennessee	2010	1.47*	2.75 (2.37–3.18)	2.08 (0.56)	1.3	1.0	1.6
Susceptible	2010	1.38*	2.18 (1.78–2.65)	0.83 (0.84)	—	—	—
Belzoni, MS	2011	1.32*	2.77 (2.37–3.23)	3.11 (0.37)	0.9	0.7	1.2
Wayside, MS[c]	2011	1.73*	3.56 (3.11–4.07)	3.18 (0.53)	1.2	0.9	1.5
Glendora, MS	2011	1.72*	2.71 (2.37–3.10)	4.03 (0.26)	0.7	0.6	1.0
St. Joseph, LA	2011	1.50*	1.46 (1.27–1.69)	0.66 (0.96)	0.5	0.4	0.6
Winnsboro, LA[c]	2011	1.71*	2.88 (2.52–3.30)	1.56 (0.67)	1.0	0.8	1.2
Lubbock, TX[c]	2011	1.16*	2.24 (1.88–2.64)	4.39 (0.22)	0.7	0.6	0.9
Susceptible	2011	1.38*	3.01 (2.48–3.67)	2.94 (0.40)	—	—	—

LC_{50}'s are reported as parts per million 72 h after treatment.
[a] Slopes with an asterisk have a significant χ^2 value ($\alpha = 0.05$).
[b] Goodness of fit χ^2 and P value.
[c] Colonies were collected from cotton fields that were not sprayed with a foliar neonicotinoid.

species with documented resistance in field collected populations include Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Olson et al. 2000, Zhao et al. 2000), greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Karatolos et al. 2010), and sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Schuster et al. 2010, Wang et al. 2010).

Mechanisms of resistance to the neonicotinoids have been well studied in several of these species. In general, the mechanism of resistance to the neonicotinoids is related to increased activity of detoxification enzymes (Philippou et al. 2010). Specifically, resistance in brown plant hopper (Karunker et al. 2008, Wen et al. 2009) and sweetpotato whitefly (Feng et al. 2010) appears to be related to increased levels of monooxygenase enzymes resulting from over expression of the P450 *CYP6CM1* gene. Mechanisms of resistance were not quantified in the current experiment. Because all current cases of field evolved resistance have been metabolic in nature, it is reason-

Table 6. Leaf-dip bioassays with flonicamid (Carbine 50WG) against cotton aphids in 2010–2012

Colony	Year	Ln slope[a]	LC_{50} (CI)	χ^2 $(P)^b$	r	ll	ul
Grenada, MS	2010	0.07	2525	11.13 (0.03)	191.6	—	—
Winnsboro, LA[c]	2010	0.34*	24.15 (14.2–64.9)	1.92 (0.75)	1.8	0.8	4.4
Glendora, MS	2010	1.02*	7.57 (6.27–9.42)	2.59 (0.46)	0.6	0.3	1.0
Alexandria, LA	2010	0.60*	20.87 (15.22–32.79)	6.18 (0.19)	1.6	0.8	3.1
Stoneville, MS	2010	0.36*	16.91 (9.52–55.30)	11.26 (0.02)	1.3	0.6	2.7
Tennessee	2010	0.87*	9.83 (5.08–83.77)	9.90 (0.02)	0.7	0.4	1.6
Susceptible	2010	0.71*	13.16 (8.60–31.03)	2.32 (0.51)	—	—	—
Belzoni, MS	2011	0.16*	115899*	0.87 (0.97)	—	—	—
Wayside, MS[c]	2011	0.34*	301.98 (61.36–13949241)	10.02 (0.07)	27.2	3.2	229.2
Glendora, MS	2011	0.44*	169.95 (77.62–881.62)	5.33 (0.38)	15.3	4.8	49.2
St. Joseph, LA	2011	0.51*	64.88 (40.38–141.79)	6.49 (0.26)	5.9	2.8	12.4
Winnsboro, LA[c]	2011	0.38*	173.05 (74.36–1020)	6.37 (0.27)	15.6	4.5	54.1
Lubbock, TX[c]	2011	0.24*	2769.00 (293.70–33803506)	1.51 (0.91)	250.0	6.2	10018.7
Susceptible	2011	0.76*	11.08 (7.66–21.63)	2.73 (0.43)	—	—	—

LC_{50}'s are reported as parts per million 48 h after treatment.
[a] Slopes with an asterisk have a significant χ^2 value ($\alpha = 0.05$).
[b] Goodness of fit χ^2 and P value.
[c] Colonies were collected from cotton fields that were not sprayed with a foliar neonicotinoid.

Table 7. Leaf-dip bioassays with flonicamid (Carbine 50WG) against cotton aphids in 2010–2012

Colony	Year	Ln slope[a]	LC_{50} (CI)	χ^2 (P)[b]	r	ll	ul
Grenada, MS	2010	0.81*	3.76 (3.01–4.62)	6.08 (0.19)	1.8	1.4	2.4
Winnsboro, LA[c]	2010	0.72*	4.82 (3.82–6.03)	5.87 (0.21)	2.3	1.7	3.2
Glendora, MS	2010	1.14*	3.01 (2.54–3.62)	3.59 (0.31)	1.5	1.1	1.9
Alexandria, LA	2010	1.28*	4.31 (3.69–5.04)	0.89 (0.93)	2.1	1.6	2.7
Stoneville, MS	2010	1.37*	5.22 (4.50–6.07)	6.60 (0.16)	2.5	2.0	3.2
Tennessee	2010	1.11*	4.37 (3.68–5.20)	5.10 (0.16)	2.1	1.6	2.8
Susceptible	2010	1.33*	2.07 (1.67–2.53)	1.31 (0.73)	—	—	—
Belzoni, MS	2011	1.61*	3.09 (2.69–3.55)	2.56 (0.47)	1.1	0.8	1.3
Wayside, MS[c]	2011	1.41*	4.20 (3.63–4.87)	3.53 (0.47)	1.4	1.1	1.8
Glendora, MS	2011	1.45*	3.23 (2.79–3.73)	1.43 (0.84)	1.1	0.9	1.4
St. Joe, LA	2011	1.89*	3.66 (3.22–4.16)	2.19 (0.53)	1.2	1.0	1.6
Winnsboro, LA[c]	2011	1.79*	3.70 (2.57–5.41)	4.34 (0.23)	1.3	1.0	1.7
Lubbock, TX[c]	2011	1.85*	3.46 (3.05–3.94)	2.20 (0.53)	1.2	0.9	1.5
Susceptible	2011	1.32*	2.93 (2.40–3.59)	2.70 (0.44)	—	—	—

LC_{50}'s are reported as parts per million 72 h after treatment.
[a] Slopes with an asterisk have a significant χ^2 value ($\alpha = 0.05$).
[b] Goodness of fit χ^2 and P value.
[c] Colonies were collected from cotton fields that were not sprayed with a foliar neonicotinoid.

able to assume that increased metabolism is at least partially responsible for the resistance detected in these bioassays. However, more research is needed on cotton aphids from the midsouthern United States to characterize the resistance mechanisms in individual populations.

Cross-resistance among the neonicotinoids has been documented in several insects. A thiamethoxam resistant strain of B-biotype *B. tabaci* showed high levels of cross-resistance to imidacloprid, acetamiprid, and nitenpyram (Feng et al. 2010). A significant correlation was observed for the LC_{50} values of thiamethoxam and imidacloprid in housefly (Kristensen and Jesperson 2008) and Colorado potato beetle (Alyokhin et al. 2007). In those experiments, the LC_{50} for imidacloprid increased as the LC_{50} for thiamethoxam increased indicating a high level of cross-resistance. In a laboratory selected strain of cotton aphid, no cross-resistance was observed to other neonicotinoids (Shi et al. 2011). Although other neonicotinoids were not tested for cross-resistance in the current experiment, field results have shown declining levels of cotton aphid control in the southern United States (Layton et al. 2003, Bommireddy et al. 2005, Adams et al. 2011, Emfinger et al. 2012). Based on field control, it appears that the populations resistant to thiamethoxam in the current experiments likely express cross-resistance to other neonicotinoids and further research needs to be conducted to confirm this.

Although cross-resistance was not tested among other neonicotinoids, all of the populations in the current experiment were also tested against flonicamid and sulfoxaflor. No cross-resistance was evident for thiamethoxam resistant populations to either one of these insecticides. Over the 4 yr of testing, LC_{50} values remained relatively low. Resistance ratios for flonicamid ranged from 1.1 to 2.5 at 72 h. Resistance ratios were much higher for flonicamid at 48 h, but the LC_{50} values fell well outside the range of concentrations tested. Flonicamid inhibits feeding of susceptible insects and is included as a group 9 subgroup C homopteran feeding blocker (http://www.irac-online.org/eClassification/) and death occurs from

Table 8. Impact of field treatment history on cotton aphid susceptibility to thiamethoxam, sulfoxaflor, and flonicamid

	Mean LC50 (SEM)		
	Thiamethoxam	Sulfoxaflor	Flonicamid
48 h			
Treated[a]	303.3 (100.71)A	3.1 (0.33)	13,498.1 (12,805.63)
Untreated[b]	13.0 (4.15)B	3.0 (0.51)	166.4 (80.27)
$P > F$	0.01	0.52	0.50
72 h			
Treated[a]	24.0 (7.09)A	2.3 (0.20)	3.8 (0.24)
Untreated[b]	11.0 (3.85)B	2.3 (0.33)	4.2 (0.32)
$P > F$	0.04	0.50	0.17

LC_{50}'s are reported as parts per million 72 h after treatment. Means within a column and rating interval with a common letter are not significantly different ($\alpha = 0.05$).
[a] Colonies were collected from commercial cotton fields that were sprayed at least one time with a foliar neonicotinoid insecticide.
[b] Colonies were collected from commercial cotton fields that were not sprayed with a foliar neonicotinoid insecticide, but may have had a neonicotinoid seed treatment.

Table 9. Impact of year on cotton aphid susceptibility to thiamethoxam, sulfoxaflor, and flonicamid

	Mean LC50 (SEM)		
	Thiamethoxam	Sulfoxaflor	Flonicamid
48 h			
2008	310.3 (230.88)	1.2 (0.23)B	—
2009	405.8 (180.79)	4.1 (0.46)A	—
2010	49.0 (17.29)	2.8 (0.10)A	434.1 (418.20)
2011	76.4 (16.20)	3.5 (0.35)A	19,896.3 (19,205.23)
$P > F$	0.71	<0.01	0.48
72 h			
2008	9.0 (2.59)B	1.2 (0.09)B	—
2009	8.7 (1.37)B	2.6 (0.32)A	—
2010	12.7 (2.61)B	2.5 (0.15)A	4.2 (0.32)
2011	53.0 (16.28)A	2.6 (0.29)A	3.6 (0.16)
$P > F$	<0.01	<0.01	0.10

LC_{50}'s are reported as parts per million 72 h after treatment. Means within a column and rating interval with a common letter are not significantly different ($\alpha = 0.05$).

starvation (Morita et al. 2007). As a result, flonicamid takes longer than other insecticides to reach maximum levels of mortality. In general, all of the populations with resistance to thiamethoxam remained susceptible to flonicamid. As a result, flonicamid will remain an important component of integrated pest management (IPM) programs for cotton aphid in cotton and other crops.

Resistance ratios for sulfoxaflor ranged from 0.4–3.0 and 0.4–2.4 at 48 and 72 h, respectively. Although sulfoxaflor acts on the nicotinic acetylcholine receptors in susceptible insects (Zhu et al. 2011), populations of cotton aphid resistant to thiamethoxam remained susceptible to sulfoxaflor. Previous research showed that sulfoxaflor has low binding affinity for the [^{3}H]Imidacloprid binding site (Zhu et al. 2011). Additionally, studies showed that sulfoxaflor is not metabolized by cytochrome P450 monooxygenases that are important in neonicotinoid resistance in several insects (Sparks et al. 2012). This is likely because of the fact that the sulfoximines lack the amine nitrogen associated with N-alkyl-hydroxylation/N-dealkylation that are present in neonicotinoids. Sulfoxaflor is classified as a group 4 nicotinic acetylcholine receptor agonist, but because of the differences in binding and metabolism, it is included in subgroup C of the IRAC mode of action classification (http://www.irac-online.org/eClassification/). Similar to flonicamid, sulfoxaflor will be an important component of cotton aphid IPM programs in multiple crops.

The results of these bioassays demonstrate the high levels of thiamethoxam resistance in cotton aphid from the midsouthern United States. In general, cotton aphids were more resistant to thiamethoxam when they were collected from fields that were previously treated with a foliar neonicotinoid, but resistant populations were also collected from nontreated fields. It is important to note that the majority of cotton fields in the midsouthern United States are planted with a neonicotinoid seed treatment; therefore, all of the populations tested were likely exposed to a neonicotinoid before testing. Field results suggest that cross-resistance is likely with other neonicotinoids, but more research is needed to elucidate this. These results also establish cotton aphid baseline susceptibility levels to flonicamid and sulfoxaflor. The LC_{50} values for flonicamid ranged from 2.07 to 5.22 ppm at 72 h. Flonicamid has been labeled for use in the United States since 2005 and these figures may not represent a true baseline for flonicamid. However, these values represent a range in the susceptibility of cotton aphid before field control has been compromised and can be used for future comparisons. The LC_{50} values for sulfoxaflor ranged from 1.01 to 5.85 ppm and from 0.92 to 4.13 ppm at 48 and 72 h, respectively. Sulfoxaflor had not been labeled or used commercially at the time of these experiments, but some populations had significantly higher LC_{50} values than the susceptible colony. These values most likely represent natural variability in the populations and not resistance events. Therefore, these values represent the baseline variability in the susceptibility of cotton aphid to sulfoxaflor.

Results from these bioassays demonstrate the high level of efficacy of flonicamid and sulfoxaflor against cotton aphid at relatively low concentrations. Given the high level of efficacy at low rates and high reproductive capacity of cotton aphid, these compounds are likely to provide a high selection pressure for cotton aphids to develop resistance in the near future (Palumbo et al. 2001). Caution should be used with these insecticides in cotton aphid IPM programs to minimize the future risk of resistance. Both of these insecticides will be important for cotton aphid management in areas where resistance to neonicotinoids occurs. Additionally, they will be an important rotation partner with the neonicotinoids in areas where cotton aphids remain susceptible to the neonicotinoids.

References Cited

Adams, B. P., A. L. Catchot, L. N. Owen, J. W. McPherson, W. S. Scott, B. Von Kanel, D. Bao, and J. L. Jones. 2011. Efficacy of selected insecticides against cotton aphid on cotton, 2010. Arthropod Management Tests 36: (F29).

Alyokhin, A., G. Dively, M. Patterson, C. Casaldo, D. Rogers, M. Mahoney, and J. Wollam. 2007. Resistance and cross-resistance to imidacloprid and thiamethoxam in the Colorado potato beetle, *Leptinotarsa decemlineata.* Pest Manag. Sci. 63: 32–41.

Babcock, J. M., C. B. Gerwick, J. X. Huang, M. R. Loso, G. Nakamura, S. P. Nolting, R. B. Rogers, T. C. Sparks, J. Thomas, G. B. Watson, and Y. Zhu. 2010. Biological classification of sulfoxaflor, a novel insecticide. Pest Manag. Sci. 67: 328–334.

Blackmon, R. L., and V. F. Eastop. 1984. Aphids on the world's crops: an identification guide. Wiley, New York, NY.

Bommireddy, P. L., R. Gable, K. Tindall, and B. R. Leonard. 2005. Evaluation of neonicotinoids against cotton aphid, 2004. Arthropod Management Tests 30: (F37).

Emfinger, K. D., S. Williams, J. Chapman, and B. R. Leonard. 2012. Evaluation of insecticides against cotton aphids on cotton, 2011. Arthropod Management Tests 37: (F37).

Feng, Y., Q. Wu, S. Wang, X. Chang, W. Xie, B. Xu, and Y. Zhang. 2010. Cross-resistance study and biochemical mechanisms of thiamethoxam resistance in B-biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 66: 313–318.

Gong, G. Y., G. L. Zhang, and G. R. Zhou. 1964. Detecting and measuring the resistance of cotton aphids to Systox. Acta Entomol. Sin. 13: 1–9.

Gorman, K., Z. Liu, I. Denholm, K. U. Brüggen, and R. Nauen. 2008. Neonicotinoid resistance in rice brown planthopper, *Nilaparvata lugens.* Pest Manag. Sci. 64: 1122–1125.

Hancock, H. G. 2003. Field performance of flonicamid (F1785, IKI-220) in cotton, pp. 1629–1636. *In* Proceedings of the Beltwide Cotton Conference, National Cotton Council of America, Memphis, TN.

Herron, G. A., and L. J. Wilson. 2011. Neonicotinoid resistance in *Aphis gossypii* Glover (Aphididae: Hemiptera) from Australian cotton. Aust. J. Entomol. 50: 93–98.

Karatolos, N., I. Denholm, M. Williamson, R. Nauen, and K. Gorman. 2010. Incidence and characterization of resistance to neonicotinoid insecticides and pymetrozine in the greenhouse whitefly, *Trialeurodes vaporariorum*

Westwood (Hemiptera: Aleyrodidae). Pest Manag. Sci. 66: 1304–1307.

Karunker, I., J. Benting, B. Leuke, T. Ponge, R. Nauen, and E. Roditakis. 2008. Over-expression of cytochrome P450 *CYP6CM1* is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Insect Biochem. Mol. Biol. 38: 634–644.

Kaufman, P. E., S. C. Nunez, R. S. Mann, C. J. Geden, and M. E. Scharf. 2010. Neonicotinoid and pyrethroid insecticide resistance in houseflies (Diptera: Muscidae) collected from Florida dairies. Pest Manag. Sci. 66: 290–294.

Kerns, D. L., and M. J. Gaylor. 1993. Biotic control of cotton aphids (Homoptera: Aphididae) in cotton influenced by two insecticides. J. Econ. Entomol. 86: 1824–1834.

Kristensen, M., and J. B. Jespersen. 2008. Susceptibility to thiamethoxam of *Musca domestica* from Danish livestock farms. Pest Manag. Sci. 64: 126–132.

Layton, M. B., J. L. Long, L. M. Green, and S. Winters. 2003. Cotton aphid insecticide efficacy evaluation, 2002. Arthropod Management Tests 28: (F68).

Mallet, J., and R. Luttrell. 1991. A model of insecticidal control failure: the example of *Heliothis virescens* on cotton. Southwest. Entomol. Suppl. 15: 201–212.

Morita, M., T. Ueda, T. Yoneda, T. Koyanagi, and T. Haga. 2007. Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding. Pest Manag. Sci. 63: 969–973.

O'Brien, P. J., and J. B. Graves. 1992. Insecticide resistance and reproductive biology of *Aphis gossypii* Glover. Southwest. Entomol. 17: 115–122.

O'Brien, P. J., Y. A. Abdel-Aal, J. A. Ottea, and J. B. Graves. 1992. Relationship of insecticide resistance to carboxylesterases in *Aphis gossypii* (Homoptera: Aphididae) from midsouth cotton. J. Econ. Entomol. 85: 651–657.

Olson, E. R., G. P. Dively, and J. O. Nelson. 2000. Baseline susceptibility to imidacloprid and cross resistance patterns in Colorado potato beetle (Coleoptera: Chrysomelidae) populations. J. Econ. Entomol. 93: 1508–1514.

Philippou, D., L. Field, and G. Moores. 2010. Metabolic enzymes confer imidacloprid resistance in a clone of *Myzus persicae* (Sulzer) (Hemiptera: Ahididae) from Greece. Pest Manag. Sci. 66: 390–395.

Palumbo, J. C., A. R. Horowitz, and N. Prabhaker. 2001. Insecicidal control and resistance management for *Bemisia tabaci.* Crop Prot. 20: 739–765.

Robertson, J. L., R. M. Russell, H. K. Preisler, and N. E. Savin. 2007. Bioassays with arthropods, 2nd ed. CRC, Boca Raton, FL.

Schuster, D. J., R. S. Mann, M. Toapanta, R. Cordero, S. Thompson, S. Cyman, A. Shurtleff, and R. F. Morris, I. I. 2010. Monitoring neonicotinoid resistance in biotype B of *Bemisia tabaci* in Florida. Pest Manag. Sci. 66: 186–195.

Scott, W. P., and G. L. Snodgrass. 2000. A review of chemical control of the tarnished plant bug in cotton. Southwest. Entomol. Suppl. 23: 67–81.

Shi, X. B., L. L. Jiang, H. Y. Wang, K. Qiao, D. Wang, and K. Y. Wang. 2011. Toxocities and sublethal effects of seven neonicotinoids on survival, growth and reproduction of imidacloprid-resistant cotton aphid, *Aphis gossypii.* Pest Manag. Sci. 67: 1528–1533.

Siebert, M. W., J. D. Thomas, S. P. Nolting, B. R. Leonard, J. Gore, A. Catchot, G. M. Lorenz, S. D. Stewart, D. R. Cook, L. C. Walton, R. B. Lassiter, R. A. Haygood, and J. D. Siebert. 2012. Field evaluations of sulfoxaflor, a novel insecticide, against tarnished plant bug (Hemiptera: Miridae) in cotton. J. Cotton Sci. 16: 129–143.

Slosser, J. E., M. N. Parujulee, G. B. Idol, and D. R. Rummel. 2001. Cotton aphid response to irrigation and crop chemicals. Southwest. Entomol. 26: 1–14.

Sparks, T. C., G. J. DeBoer, N. X. Wang, J. M. Hasler, M. R. Loso, and G. B. Watson. 2012. Differential metabolism of sulfoximine and neonicotinoid insecticides by *Drosophila melanogaster* monooxygenase CYP6G1. Pesticide Biochem. Physiol. 103: 159–165.

Srigiriraju, L., P. J. Semtner, and J. R. Bloomquist. 2010. Monitoring for imidacloprid resistance in the tobacco-adapted form of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), in the eastern United States. Pest Manag. Sci. 66: 676–685.

Tomizawa, M., and J. E. Cassida. 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol. 48: 339–364.

Watson, G. B., M. R. Loso, J. M. Babcock, J. M. Hasler, T. J. Letherer, C. D. Young, Y. Zhu, J. E. Casida, and T. C. Sparks. 2011. Novel nicotinic action of the sulfoximine insecticide sulfoxaflor. Insect Biochem. Mol. Biol. 41: 432–439.

Wang, K. Y., T. X. Liu, C. H. Yu, X. Y. Jiang, and M. Q. Yi. 2002. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to fenvalerate and imidacloprid and activities of detoxification enzymes on cotton and cucumber. J. Econ. Entomol. 95: 407–413.

Wang, Z., H. Yan, Y. Yang, and Y. Wu. 2010. Biotype and insecticide resistance status of the whitefly, *Bemisia tabaci* from China. Pest Manag. Sci. 66: 1360–1366.

Weathersbee III, A. A., and D. D. Hardee. 1994. Abundance of cotton aphids (Homoptera: Aphididae) and associated biological control agents on six cotton cultivars. J. Econ. Entomol. 87: 258–265.

Wen, Y., Z. Liu, H. Bao, and Z. Han. 2009. Inidacloprid resistance and its mechanisms in field populations of brown planthopper, *Nilaparvata lugens* Stål, in China. Pesticide Biochem. Physiol. 94: 36–42.

Zhao, J. Z., B. A. Bishop, and E. J. Grafius. 2000. Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 93: 1508–1514.

Zhu, Y., M. R. Loso, G. B. Watson, T. C. Sparks, R. B. Rogers, J. X. Huang, B. C. Gerwick, J. M. Babcock, D. Kelly, V. B. Hegde, et al. 2011. Discovery and characterization of sulfoxaflor, a novel insecticide targeting sap-feeding insects. J. Agric. Food Chem. 59: 2950–2957.

Received 7 March 2013; accepted 1 April 2013.