

**BIOASSAYS AND MANAGEMENT OF COTTON APHIDS WITH NEONICOTINOIDS AND SULFOXAFLO****Jeff Gore****Don Cook****Mississippi State University****Stoneville, MS****Angus Catchot****Mississippi State University****Starkville, MS****Roger Leonard****LSU AgCenter****Winnsboro, LA****Gus Lorenz****University of Arkansas****Lonoke, AR****Scott Stewart****The University of Tennessee****Jackson, TN****Abstract**

A series of experiments were conducted in Mississippi to determine the susceptibility of cotton aphid, *Aphis gossypii* (Glover), to neonicotinoids and sulfoxaflor (GF-2032 2SC and GF-2372 50WG, Dow Agrosciences). Thiamethoxam (Centric 40WG, Syngenta Crop Protection) was used as a representative of the neonicotinoid class. Leaf dip bioassays were used for these experiments. Serial dilutions of each insecticide were prepared. Leaf discs (23 mm diam) were cut from non-treated cotton leaves, treated with insecticide, and placed into Petri dishes with a 1% agar solution. Aphids were collected from commercial cotton fields in Mississippi and Arkansas during 2008 and 2009. A total of 50 aphids were tested for each concentration and at a minimum of six concentrations were used for each bioassay. The susceptibility of cotton aphids to neonicotinoids appears to be declining in the mid-South. Bioassays with aphids collected from fields that had been treated with a neonicotinoid showed elevated LC50 values compared to aphids collected from fields that had not been treated with a neonicotinoid. Based on these experiments, sulfoxaflor susceptibility was not different among aphids collected from fields that were not treated and those that were treated with a neonicotinoid. These experiments show that cotton aphid tolerance to the neonicotinoids is increasing and that sulfoxaflor will be a valuable tool for cotton aphid management.

**Introduction**

Cotton aphids, *Aphis gossypii* (Glover), have been a secondary pest of cotton since the introduction of synthetic insecticides. They have a history of rapidly developing resistance to new classes of chemistry soon after their widespread use. The neonicotinoid class is the most recent group of insecticides released and they are widely used across the Cotton Belt. They include imidacloprid (Trimax Pro, Bayer CropScience), thiamethoxam (Centric, Syngenta Crop Protection), acetamiprid (Intruder, Nisso America), and clothianidin (Belay, Valent USA). All of these insecticides are labeled for foliar applications in cotton. Additionally, imidacloprid (Gaucho Grande and AERIS) and thiamethoxam (Cruiser and Avicta) are labeled as seed treatments. A large percentage of the cotton in the U.S. is planted with one of these seed treatments. Currently, insecticides in the neonicotinoid class are being over utilized in cotton and additional classes of insecticides are needed. Because of this over use, an independent monitoring program was initiated in 2006 to measure potential changes in the susceptibility of cotton aphids to neonicotinoids.

Flonicamid (Carbine, FMC Corporation) was recently registered and has provided good control of cotton aphids. Additionally, a new insecticide is currently being tested by University researchers. Sulfoxaflor (Dow Agrosciences) has been tested for the past two years as GF-2032 2SC and GF-2372 50WG against cotton aphids in the mid-South. Initial results suggest that this insecticide will be an important component of cotton aphid management in cotton. The current paper will summarize dose-mortality experiments conducted in 2008 and 2009 to measure the response of cotton aphids to thiamethoxam and sulfoxaflor.

### **Methods**

**Aphid Collections:** Bioassays were conducted on aphids collected from multiple fields in Mississippi and Arkansas in 2008 and 2009 (Table 1). Leaves with heavy infestations of aphids were removed from plants in each field, placed in paper bags, and transported to the laboratory in an ice chest.

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

<b>Colony</b>	<b>Date</b>	<b>Treatment History</b>
Leland (2X)	7/8/2008	Trimax Pro (1.25 oz./A) and Trimax Pro (1.5 oz./A)
Stoneville (NT)	7/8/2008	Not treated
Grenada (2X)	6/30/2008	Centric (1.5 oz./A) and Centric (2 oz./A)
Grenada (1X)	6/30/2008	Centric (2 oz./A)
Grenada (NT08)	6/30/2008	Not treated
Grenada (NT09)	6/12/2009	Not treated
GrenadaA (1X)	6/23/2009	Centric (2 oz./A)
GrenadaB (1X)	6/23/2009	Centric (2 oz./A)
GrenadaC (1X)	6/23/2009	Centric (2 oz./A)
Wayside (2X)	7/11/2009	Centric (2 oz./A) and Centric (2 oz./A)
Marks (3X)	7/18/2009	Centric (1.5 oz./A) and Trimax Pro 2X (1.5 oz./A and 1.8 oz./A)
Arkansas (NT)	6/23/2009	Not treated
Arkansas (1X)	7/13/2009	Centric (2 oz./A)

**Leaf Dip Procedures:** Cotton leaves were removed from non-treated 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 23 mm disc was cut from each leaf with a no. 18 cork borer. Serial dilutions of sulfoxaflor (GF-2032 2SC, 2 lb ai/Gal) and thiamethoxam (Centric 40WG, 40% w/w) were prepared in 500 ml beakers. Leaf discs were dipped into each dilution and swirled for 5 seconds. The leaf discs were then placed on a wire rack with the adaxial surface (top) against the rack and allowed to dry completely.

A 1% agar solution was prepared and a thin layer (approximately 2 mm deep) was dispensed into 55mm diameter Petri dishes. Once completely dry, the treated leaf discs were placed into the Petri dishes with the adaxial (top) surface against the agar. The leaf was gently pressed into the agar with a pair of forceps to create a solid seal with the edge of the leaf disc. A total of ten dishes were used for each dose and a minimum of 6 doses (plus non-treated) were used for each bioassay. Five cotton aphids were transferred from the leaves collected in the fields onto each leaf disc with a very fine camel hair brush. A small piece of cotton cloth was placed over each dish and the lid was placed over the cotton cloth. The dishes were held closed with a rubber band. Mortality was rated at 48 and 72 hours after treatment. Aphids were considered dead if they could not take a coordinated step after being gently stroked several times with a fine paint brush. Data were subjected to Probit analysis in SAS. The Probit results for 2008 at 48 hours and 72 hours are reported in tables 2 and 3, respectively, and for 2009 at 48 and 72 hours in tables 4 and 5, respectively.

### **Results**

Five aphid colonies were tested in 2008. Three of the colonies were collected from cotton fields that had been previously treated with at least one foliar application of a neonicotinoid insecticide. Two of the colonies were collected from cotton fields that had not been treated with any foliar applications of a neonicotinoid. All five colonies were collected from cotton fields that were planted with a neonicotinoid seed treatment. In 2008, LC50 values at 48 hours ranged from 0.37 ppm to 1.62 ppm for sulfoxaflor and from 3.37 ppm to 1206 ppm for thiamethoxam (Table 2). At 72 hours, LC50 values ranged from 0.92 ppm to 1.51 ppm for sulfoxaflor and from 2.93 ppm to 15.56 ppm for thiamethoxam (Table 3).

Eight aphid colonies were tested in 2009. Six of the colonies were collected from cotton fields that had been previously treated with at least one foliar application of a neonicotinoid insecticide. Two of the colonies were collected from cotton fields that did not receive a foliar application of a neonicotinoid. All eight of the colonies were collected from cotton fields that were planted with a neonicotinoid seed treatment. In 2009, LC50 values at 48

hours ranged from 2.49 ppm to 5.85 ppm for sulfoxaflor and from 3.48 ppm to 1234.00 ppm for thiamethoxam (Table 4). At 72 hours, LC50 values ranged from 1.60 ppm to 4.13 ppm for sulfoxaflor and from 2.56 ppm to 14.50 ppm for thiamethoxam (Table 5).

Table 2. Leaf-dip bioassays with sulfoxaflor and thiamethoxam against cotton aphids in 2008. LC50's are reported as parts per million 48 hours after treatment.

Colony	Sulfoxaflor		Thiamethoxam	
	LC50 (CI)	X <sup>2</sup> (P)	LC50 (CI)	X <sup>2</sup> (P)
Leland (2X)	1.01 (0.75-1.34)	2.84 (0.58)	1206 (165-33018230)	1.66 (0.80)
Stoneville (NT)	1.33(1.04-1.73)	2.16 (0.71)	3.27 (2.70-3.92)	4.87 (0.43)
Grenada (2X)	0.37 (0.11-0.62)	8.65 (1.73)	33.41 (12.80-889.30)	9.68 (0.08)
Grenada (1X)	1.55 (0.85-2.79)	14.43 (0.01)	303.4 (71.3-30243)	5.60 (0.35)
Grenada (NT)	1.62 (1.19-2.25)	6.39 (0.17)	5.54 (4.09-7.44)	7.24 (0.20)

Table 3. Leaf-dip bioassays with sulfoxaflor and thiamethoxam against cotton aphids in 2008. LC50's are reported as parts per million 72 hours after treatment.

Colony	Sulfoxaflor		Thiamethoxam	
	LC50 (CI)	X <sup>2</sup> (P)	LC50 (CI)	X <sup>2</sup> (P)
Leland (2X)	0.92 (0.70-1.20)	6.46 (0.17)	12.95 (10.53-16.22)	4.45 (0.35)
Stoneville (NT)	1.25 (0.97-1.62)	1.92 (0.75)	3.05 (2.52-3.65)	4.98 (0.29)
Grenada (2X)	1.23 (0.95-1.58)	6.82 (0.23)	10.71 (6.44-18.81)	8.11 (0.09)
Grenada (1X)	1.23 (0.98-1.54)	8.83 (0.12)	15.56 (7.66-50.77)	12.97 (0.01)
Grenada (NT)	1.51 (1.11-2.09)	5.66 (0.23)	2.93 (1.63-4.63)	12.90 (0.02)

Table 4. Leaf-dip bioassays with sulfoxaflor and thiamethoxam against cotton aphids in 2009. LC50's are reported as parts per million 48 hours after treatment.

Colony	Sulfoxaflor		Thiamethoxam	
	LC50 (CI)	X <sup>2</sup> (P)	LC50 (CI)	X <sup>2</sup> (P)
Grenada (NT)	2.49 (2.07-2.99)	2.83 (0.42)	3.48 (2.58-4.56)	7.96 (0.16)
GrenadaA (1X)	4.87 (3.79-6.20)	7.26 (0.20)	1234 (248-144539)	4.48 (0.61)
GrenadaB (1X)	5.85 (4.74-7.21)	7.53 (0.18)	476 (181-3598)	4.08 (0.77)
GrenadaC (1X)	3.00 (2.47-3.63)	2.17 (0.70)	108.6 (64.1-262.7)	4.10 (0.66)
Wayside (2X)	2.63 (2.08-3.26)	4.85 (0.30)	220.6 (89.3-1993)	5.10 (0.40)
Marks (3X)	5.46 (4.14-7.15)	4.47 (0.48)	1156 (182-3488672)	4.35 (0.50)
Arkansas (NT)	4.46 (3.66-5.44)	4.35 (0.36)	6.53 (5.31-8.02)	2.72 (0.74)
Arkansas (1X)	3.77 (3.17-4.52)	0.80 (0.85)	41.5 (28.9-71.9)	2.74 (0.74)

Table 5. Leaf-dip bioassays with sulfoxaflor and thiamethoxam against cotton aphids in 2009. LC50's are reported as parts per million 72 hours after treatment.

Colony	Sulfoxaflor		Thiamethoxam	
	LC50 (CI)	X <sup>2</sup> (P)	LC50 (CI)	X <sup>2</sup> (P)
Grenada (NT)	1.60 (1.20-2.03)	4.56 (0.24)	2.56 (1.76-3.47)	6.23 (0.28)
GrenadaA (1X)	2.86 (1.97-3.88)	8.20 (0.15)	14.50 (11.4-18.7)	10.76 (0.10)
GrenadaB (1X)	1.79 (1.00-2.69)	6.72 (0.24)	12.15 (9.8-15.1)	8.35 (0.30)
GrenadaC (1X)	1.60 (1.16-2.06)	2.73 (0.60)	5.93 (4.51-7.68)	8.89 (0.18)
Wayside (2X)	2.40 (1.90-2.97)	5.01 (0.29)	10.05 (8.07-12.69)	2.01 (0.85)
Marks (3X)	4.13 (2.97-5.59)	1.53 (0.91)	7.70 (5.91-10.15)	4.84 (0.44)
Arkansas (NT)	2.90 (2.22-3.71)	6.50 (0.16)	5.79 (4.69-7.12)	2.94 (0.71)
Arkansas (1X)	3.40 (2.82-4.12)	0.51 (0.92)	10.61 (8.31-13.89)	5.98 (0.31)

### **Discussion**

Neonicotinoid insecticides have become an important component of IPM in cotton. In the mid-South, most foliar applications in cotton occur between pin-head square and first flower. These applications primarily target tarnished plant bugs, but are used at this time of the season because they also have cotton aphid activity. University Extension Specialists recommend the neonicotinoids for tarnished plant bug during pre-flower as a rotation strategy to minimize selection pressure on pyrethroids and organophosphates. Several consultants across the mid-South have expressed concerns about higher than expected survival rates of cotton aphids following foliar applications of neonicotinoids. Based on results of the current experiment, susceptibility of cotton aphids to the neonicotinoids appears to be declining. LC50 values of thiamethoxam against a susceptible cotton aphid population are generally around 3 ppm at 72 hours. In the current experiment, several populations of cotton aphids significantly exceeded this value. All of the populations showing elevated levels tolerance to the neonicotinoids were collected from cotton fields that received at least one foliar application with a neonicotinoid. Collections were made 5 to 14 days after the foliar applications.

Results at 48 hours were highly variable in these experiments. Dose-mortality curves could not be obtained for several populations at 48 hours within the range of doses tested. Estimated LC50 values were significantly elevated. All of the populations showing extremely high LC50 values were collected from fields following a foliar neonicotinoid application. At the 72 hour rating, all of the LC50 values were within a normal range although several values were elevated. This time delay in mortality suggests a metabolic change in those populations. Cotton aphids collected from fields prior to treatment with a foliar neonicotinoid had LC50 values within a susceptible range.

Acetamiprid (Intruder) has continued to provide acceptable control of cotton aphids compared to the other neonicotinoids. However, the use rates for this insecticide have nearly doubled in the last five years. Although it was not tested in the bioassays, flonicamid (Carbine, FMC Corporation) has provided good control of cotton aphids in fields following control concerns with neonicotinoids. Therefore, Carbine will remain an important insecticide for cotton aphid control.

Based on bioassays in the current experiment, the experimental insecticide sulfoxaflor will be an important component of IPM for cotton aphids. LC50 values for sulfoxaflor were similar for all populations regardless of field treatment history. LC50 values range from 0.92 ppm to 4.13 ppm at 72 hours, representing a 4.5-fold range in the susceptibility of cotton aphid to sulfoxaflor. This is most likely natural variability because sulfoxaflor has not been registered, and therefore, not used commercially.

The results of these experiments indicate that cotton aphid susceptibility to the neonicotinoids needs to be closely monitored in the mid-South. Additionally, monitoring cotton aphid susceptibility to flonicamid should be initiated because the frequency of applications will likely increase if the neonicotinoids become less consistent in their level of control. These data also serve as baseline values for sulfoxaflor against cotton aphid. Monitoring cotton aphid susceptibility to sulfoxaflor should continue to detect changes that may occur after registration and subsequent field use.