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Effects of Aldicarb and Neonicotinoid Seed Treatments on Twospotted Spider Mite on Cotton

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ABSTRACT Twelve field experiments and one laboratory experiment were conducted to determine the effects of furrow applied aldicarb and seed treatments of thiamethoxam, imidacloprid, Avicta (thiamethoxam + abamectin), Aeris (imidacloprid + thiodicarb), and acephate on twospotted spider mite, *Tetranychus urticae* Koch, on cotton, *Gossypium hirsutum* L. For the field experiments, data were pooled across all experiments for analysis. Aeris, thiamethoxam, and imidacloprid treatments resulted in twospotted spider mite densities greater than those in the untreated check, aldicarb, and acephate treatments. However, cotton treated with Avicta (thiamethoxam + abamectin) had 34% fewer mites than other neonicotinoid seed treatments when infestations occurred near cotyledon stage. Untreated check and aldicarb treatments had the lowest mite densities. Only aldicarb reduced mite densities below that in the untreated check. In a laboratory trial, the fecundity of twospotted spider mite was measured. While neonicotinoid seed treatments increased mite densities in the field, they did not increase fecundity in the laboratory experiment. Foliar applied thiamethoxam slightly elevated average fecundity in the laboratory experiment. Increased use of neonicotinoid seed treatments instead of furrow applied aldicarb is likely at least partly responsible for recent increased twospotted spider mite infestations in seedling cotton across the mid-south.

KEY WORDS thiamethoxam, imidacloprid, acephate, aldicarb, soil-applied insecticides

Twospotted spider mite, *Tetranychus urticae* Koch, has traditionally been considered an occasional late season pest of cotton, *Gossypium hirsutum* L., across the mid-south. However, the percentage of hectares in Mississippi requiring acaricide applications targeting twospotted spider mite has increased by at least twofold in recent years (Williams 2009). Spider mites were the third most damaging pest of Mississippi Delta cotton in 2010, causing a loss of 15,240 bales and required treatment on 61,917 ha (Williams 2011). Before 2005, most acaricide applications were made during the late season, but from 2006 to 2009 a small percentage of seedling cotton in Mississippi has been heavily damaged by mites as well.

Several factors may have contributed to the increasing frequency of mites in seedling cotton, one of which is a shift in production practices from furrow applied aldicarb to neonicotinoid seed treatments for thrips control. Multiple reports have indicated that applications of neonicotinoid insecticides have resulted in population increases of *Tetranychus* species (Sclar et al. 1998, Van Duyn et al. 1998, James and Price 2002, Beers et al. 2005, Troxclair 2007). Soil applications of imidacloprid resulted in higher densities of twospotted spider mite and honeylocust spider mite, *Platytetranychus multidigituli* (Ewing), on field grown marigolds, *Tagetes erecta* L., and honeylocust, *Gleditsia triacanthos* L., respectively, compared with untreated plants (Sclar et al. 1998). Beers et al. (2005) observed that twospotted spider mite populations in Washington apple orchards were 4.6-fold higher when treated with acetamprid compared with organophosphate insecticides. In addition, seasonal mite density was positively correlated to total grams active ingredient (AI) of acetamiprid applied. Troxclair (2007) found that percentages of cotton plants infested with twospotted spider mite were significantly greater in plots treated with thiamethoxam and imidacloprid seed treatments than those treated with aldicarb or untreated.

Several theories have been proposed to explain the relationship between neonicotinoid insecticides and twospotted spider mite population increases. James and Price (2002) reported a hormoligant effect in twospotted spider mite females that were either directly sprayed with imidacloprid or fed leaf disks cut from systemically treated bean leaves. Hormoligosis is the phenomenon of reproductive stimulation or increased fecundity of arthropods from sublethal doses of insecticides (Luckey 1968). Sclar et al. (1998) determined that increased densities of twospotted spider mite were related to reductions of predators. In contrast, Ako et al. (2004) reported that four neonicotinoid insecticides caused lower fecundity. At sublethal

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Table 1. Planting date, infestation date, and treatments for each field exp from 2007 to 2009

	Planting date	Infestation date	Plt stage (infest)[a]	UTC	Aldicarb	Thiamethoxam	Imidacloprid	Avicta	Aeris	Acephate
Test 1	4/27/2007	5/23/2007	3	X	X	X				
Test 2	5/4/2007	5/29/2007	4	X	X			X	X	
Test 3	6/7/2007	6/27/2007	1	X	X			X		
Test 4	6/5/2007	7/6/2007	3	X	X			X		
Test 5	6/22/2007	7/12/2007	3	X	X	X	X			
Test 6	4/23/2008	5/20/2008	1	X	X			X	X	
Test 7	5/5/2009	5/29/2008	1	X	X	X		X		
Test 8[b]	6/5/2008	6/18/2008	1	X	X	X	X			X
Test 9	4/22/2009	5/12/2009	1	X	X	X	X	X	X	X
Test 10	4/27/2009	5/18/2009	1	X	X	X	X	X	X	X
Test 11	5/20/2009	6/8/2009	1	X	X	X	X	X	X	X
Test 12	5/21/2009	6/8/2009	1	X	X	X	X	X	X	X

[a] Plant Stage when infested (no. of true leaves).
[b] Three foliar insecticide treatments were included in experiment 8. They included thiamethoxam (Centric 40 WG, Syngenta Crop Protection), Imidacloprid (Trimax Pro 4.43 SC, Bayer Crop Science), and acephate (Orthene 90 S, Valent). Base treatments in all experiments included an untreated control (UTC), aldicarb (Temik 15 G, Bayer CropScience), and at least one neonicotinoid seed treatment. Five trials included an acephate seed treatment (Orthene 90 S, Valent). Neonicotinoid seed treatments included: thiamethoxam (Cruiser 5 FS, Syngenta Crop Protection), imidacloprid (Gaucho Grande 5 FS, Bayer CropScience), Avicta (28.1% thiamethoxam and 12.4% abamectin, Syngenta Crop Protection), and Aeris (24% imidacloprid and 24% thiodicarb, Bayer CropScience).

doses, there was no significant difference among the twospotted spider mites exposed to neonicotinoid insecticides and those that were not exposed to neonicotinoid insecticides. The impact of seed-applied and foliar-applied neonicotinoids on *Tetranychus* spp. has been documented in multiple plant species. The increased use of neonicotinoid seed treatments in cotton may be a factor in the recent early season outbreaks of twospotted spider mite that have occurred throughout the mid-south.

Most research studying the effects of neonicotinoid insecticides on twospotted spider mite has been conducted on plants or crops other than cotton. To this point there has not been a comprehensive experiment that examined the effects of neonicotinoid seed treatments on twospotted spider mite on cotton. In the current experiment, the effects of the six most widely used insecticide seed treatments on twospotted spider mite were examined. Multiple field experiments were conducted over 3 yr, allowing subtle differences and trends between treatments to be identified. The differences between these treatments with regard to mite densities and possible explanations are discussed.

Materials and Methods

From 2007 through 2009, a total of 12 field trials and one laboratory experiment were conducted. Twospotted spider mites were artificially infested onto cotton in each trial from a colony maintained on green bean, *Phaseolus vulgaris* L., at Mississippi State University, Mississippi State, MS. The colony was originally established in 2007 from field populations on cotton in the Mississippi Delta.

Field Experiments. Field experiments were conducted as a randomized complete block with 4–6 replications. Plot size was four rows wide (96.5 cm centers) by 6.1 or 9.1 m long. In most trials, Phytogen 485 WRF cotton seed (Dow AgroSciences, Indianapolis, IN), containing only a fungicide treatment (Maxim [fludioxinil] and Apron XL [mefenoxam]) was treated in the laboratory with appropriate insecticide seed treatments. Base treatments in all trials included thiamethoxam (Cruiser 5 FS, Syngenta Crop Protection) at 0.34 mg AI/seed, imidacloprid (Gaucho Grande 5 FS, Bayer CropScience) at 0.375 mg AI/seed, and acephate (Orthene 90 S, Valent) at 0.709 kg/45.4 kg seed, aldicarb (Temik 15 G, Bayer CropScience) at 0.841 kg ai/ha placed in furrow at planting, and untreated. Avicta (28.1% thiamethoxam and 12.4% abamectin, Syngenta Crop Protection) and/or Aeris (24% imidacloprid and 24% thiodicarb, Bayer CropScience) were included in two trials in 2007 and four trials in 2009. Rates of the insecticide seed treatments and aldicarb were the same in all trials. Planting dates, infestation dates, plant stages of cotton when infested with twospotted spider mite, and treatments are shown in Table 1. All experiments were conducted in Starkville, MS, except tests 2, 10, and 12, which were conducted in Stoneville, MS.

Seedling cotton plants were inoculated with twospotted spider mites by stapling one infested green bean leaf from the laboratory colony onto one cotyledon of each plant within a 1–2 m row foot section in the center two rows of each plot. Cotton was infested between the first and fifth true leaf stages. Twospotted spider mites were sampled by pulling the uppermost fully expanded leaf from five plants in 2007 and five or 10 leaves in 2008 and 2009, usually two nodes below the terminal. Leaves were placed in plastic bags and immediately transported to the laboratory where adult mites, immature mites, and/or mite eggs were counted with aid of a stereo microscope under 80 × magnification. Trials were generally sampled at ≈7 and 14 d after infestation (DAI).

Field experiment 8 also included three foliar treatments: thiamethoxam (Centric 40 WG, Syngenta Crop Protection) at 0.056 kg AI/ha, imidacloprid (Trimax Pro 4.43 SC, Bayer CropScience) at 0.071 kg AI/ha, and acephate (Orthene 90 S, Valent) at 0.22 kg AI/ha.

Foliar insecticides were applied with a tractor-mounted sprayer calibrated to deliver 93.5 liters/ha at 413.7 kpa through TX-6 hollow cone nozzles (two per row). Foliar treatments were applied directly after infestation. The number of adult, immature, and mite eggs were counted. Plots were also visually rated 14 d after infestation on a 1–10 scale with 10 being no damage and one equal to severe damage.

Field experiments 9–12 were conducted in Starkville and Stoneville, MS, during May and June 2009. All four experiments had identical designs and treatments. One trial was planted in each location during the early period of the optimum planting window (22 April in Starkville and 27 April in Stoneville) and during the later period of the optimum planting window (20 May in Starkville and 21 May in Stoneville). All seed treatments previously listed were included in the trials. In an effort to further explain treatment differences, estimates of thrips densities were determined by cutting and washing the three uppermost nodes at 21 d after infestation in the early planted tests and 14 d after infestation in the late planted tests.

Laboratory Fecundity Experiment. A laboratory experiment was conducted in the insect rearing facility in Clay Lyle Entomology at Mississippi State University to determine if seed treatments or applications of foliar insecticides impacted twospotted spider mite fecundity. Treatments were identical to those in field experiment 8, including: three seed treatments, three foliar treatments, aldicarb applied in-furrow, and an untreated check. Seed treatments were thiamethoxam, imidacloprid, and acephate. Foliar treatments were identical to those in field experiment 8. Cotton was grown in pots (14-cm diameter, 15-cm depth) in a greenhouse. The experiment contained six replications. Each replication included eight treatments, totaling 48 pots in the experiment. Soil was collected from a field on the R. R. Foil Plant Science Research Center where previous field trials had been conducted. Four cotton seeds were planted per pot. A furrow was dug across each pot, and seed were evenly spaced across it, and then covered. In the aldicarb treatment, Temik 15 G was placed into the furrow with the seed. Each pot was watered at 2–3 d intervals. Although water volume was not measured, the volume poured in each pot was limited to prevent it from running out the bottom of the pot. When plants reached the second true leaf stage, the first true leaf from one plant per pot was removed and placed directly in individual petri dishes. Leaves were positioned with the abaxial surface facing upward on saturated cotton wool. A small piece of saturated cotton wool was placed over the leaf petiole at the point it was severed to protect against desiccation.

To standardize mite age, mated females were collected from the colony described previously, and placed on untreated green bean leaves. Leaves were placed on top of saturated cotton wool in a plastic bowl covered with cheese cloth. Photoperiod was 16:8 (L:D) h and temperature was 27°C. Female mites were allowed to lay eggs for 24 h before being either removed or killed. Eggs began to hatch 2.5 d later. Mating in the offspring was observed 8 d after oviposition. Nine days after oviposition, all females had begun to oviposit. At this time, two adult female mites were placed on each cotton leaf with a fine hair brush. Foliar applications were made to individual leaves and mites of the foliar treatments in a closed spray chamber calibrated to deliver 46.75 liters/ha with a TeeJet 8015E nozzle at 220.6 kpa. Longevity and fecundity were measured daily for 14 d. Eggs were counted and removed daily by touching them with a fine hair brush that was lightly coated with Tangle Foot adhesive. Water was added to the cotton wool each day to maintain saturation of cotton wool. The experiment was completely randomized and analyzed with the mixed model (SAS Institute 1998) using replicate as a random factor. The containment method was used to compute degrees of freedom.

Field Experiment Analyses. Interactions between field experiments and treatments were first tested. Because interactions were not significant, a pooled analysis was then performed including all replicates of all trials. There was considerable variation in mite densities between different trials so each replicate was standardized as percent of the untreated control and a log base 10 transformation was taken of this percentage. Because some trials did not include all treatments, the design was unbalanced, so analysis was conducted with mixed model analysis of variance (ANOVA) using SAS (SAS Institute 1998). Degrees of freedom were computed using the containment method. Trial and replicate within trial were set as random variables. Data were analyzed by all observations combined, observations grouped within days after infestation when sampled, observations grouped by stage of cotton at time of infestation, neonicotinoid treatments grouped and compared against other treatments, and only experiments 9–12. Least square means were used to compare treatments within each analysis. Differences were considered significant when $P < 0.05$. Each trial was also analyzed individually with ANOVA using ARM (Gyling Data Management). In experiments 9–12, thrips counts were included as a covariant; however, thrips did not significantly affect mite treatment differences, so the covariant was removed in subsequent analyses.

Results

Field Experiments. Only three of nine individual field experiments resulted in significant differences for eggs, and two of six for immature mites (data not shown). Six of the 12 individual field experiments resulted in significant treatment differences of total mites (adult + immature) (Table 2). Interactions between experiments and treatments were not significant at 7 d after infestation for total mites ($F = 1.34$; df = 35,143; $P = 0.1185$) or 14 d after infestation ($F = 1.21$; df = 28,107; $P = 0.2385$)), so data were pooled to increase the statistical power of the experiments Three factors were considered in the pooled analysis: seed treatment, plant growth stage when infested, and

Table 2. Mean no. of twospotted spider mite adults and immatures, and statistical values for each exp and sample date

	Test 1	Test 6	Test 8	Test 9	Test 11	Test 12	
	7 DAI	8 DAI	14 DAI	15 DAI	15 DAI	7 DAI	14 DAI
Thiamethoxam	4.4a	—	41.0b	9.3b	24.7a	31.5a	30.2bcd
Imidacloprid	—	—	64.2a	10.1b	21.5ab	29.2a	60.6ab
Avicta	—	29.9b	—	9.0b	9.4c	12.3c	53.6abc
Aeris	—	71.1a	—	14.5a	24.9a	27.3a	68.4a
Acephate	—	—	39.5b	7.2bc	16.9b	25.3ab	23.1cd
Aldicarb	0.83b	48.5b	11.5c	4.5c	6.3c	10.7c	26.3cd
Untreated	2.9ab	41.8b	29.9bc	6.7bc	7.9c	16.9bc	18.5d
N	12	16	50	70	70	70	70
F-value	6.47	3.693	3.051	3.544	8.414	5.616	2.291
P	0.0318	0.0239	0.0223	0.0171	0.0002	0.002	0.0836

Only experiments with significant differences at the $P = 0.05$ level are shown. Statistics based on log transformation of the percentage of the untreated control.
Means followed by the same letter within a column are not significantly different (LSD with $\alpha = 0.05$).
N = total no. of observations (treatment × replications).

the number of days elapsed between infestation and sampling.

For adults and immatures, data were only collected from trials infested when cotton had one true leaf, so the impact of plant growth stage when infested could only be analyzed for eggs and total mites. No interactions were significant for egg densities (data not shown), but seed treatment (Table 3) and days after infestation (Table 4) were significant factors impacting spider mite egg densities. For total mites, the three-way interaction was not significant ($F = 1.53$; df = 7,381; $P = 0.1563$) so this interaction was deleted and a simpler model was evaluated. The treatment by days after infestation interaction was not significant ($F = 1.00$; df = 12,388; $P = 0.4509$) so it was also deleted from the model. The treatment by plant stage interaction ($F = 2.28$; df = 7,400; $P = 0.0275$) and days after infestation by plant stage interaction ($F = 2.52$; df = 3,400; $P = 0.0579$) were kept in the total mite model. To more clearly understand these interactions, the data were analyzed independently for each plant stage when infested and for each group of days after infestation.

To segment treatment differences over time, data were categorized based on the number of days after infestation that the experiments were sampled. The three sampling interval categories included 5–8 d after infestation (1 wk), 12–15 d after infestation (2 wk), and 16+ days after infestation (3 wk). Only total mite densities are presented because combining immature and adult mite densities was representative of both life stages independently. Treatment differences varied over these sampling intervals (Table 5). When cotton was sampled 1 wk after infestation, aldicarb had fewer mites than all other treatments. Mite densities in the untreated check and Avicta treatments were significantly less than acephate and other neonicotinoid treatments. Acephate ranked as the median treatment. There were no significant differences between thiamethoxam, imidacloprid, or Aeris. At 2 wk after infestation, mite densities in untreated check treatments were below all other treatments except aldicarb. Avicta, Aeris, imidacloprid, thiamethoxam, and acephate treatments were similar. By 3 wk after infestation, untreated check, acephate, and aldicarb treatments had lower mite densities than all four neonicotinoid treatments.

Data were further partitioned by growth stage of cotton when mites were infested, including first, third, and fourth true leaf stages. Each day after infestation category within each growth stage at infestation was analyzed independently. When cotton was infested at first true leaf and sampled 1 wk after (≈3rd leaf stage), many of the trends apparent in the overall analysis

Table 3. Mean no. of adult, immature, total mites (adult + immature), and eggs averaged across all experiments

TRT	Adult			Immatures			Total mites			Eggs		
	N	Mean	SEM	*N*	Mean	SEM	*N*	Mean	SEM	*N*	Mean	SEM
Aeris	32	7.0ab	1.5	32	16.3ab	3.5	58	17.3a	3.2	40	31.0a	8.0
Avicta	36	4.2c	1.4	42	12.4b	3.3	67	11.2b	3.2	49	30.9a	7.5
Thiamethoxam	44	5.9a	1.4	44	13.9ab	3.3	66	14.9a	3.2	52	29.0a	7.5
Imidacloprid	39	6.6a	1.4	39	17.3a	3.4	53	18.6a	3.3	39	33.0a	8.0
Acephate	40	4.8bc	1.4	40	11.2ab	3.3	48	10.2bc	3.3	40	22.4ab	8.0
Aldicarb	44	2.9d	1.4	50	6.5c	3.2	89	7.2d	3.1	65	18.7c	7.1
Untreated	44	4.1c	1.4	50	7.7c	3.2	90	7.9c	3.1	66	17.0bc	7.1
df (num, den)	6, 249			6, 264			6, 411			6, 322		
F-value	8.68			9.54			16.23			3.31		
P	<0.0001			<0.0001			<0.0001			0.004		

Statistics based on log transformation of the percentage of the untreated control.
Means followed by the same letter within a column are not significantly different (LSD with $\alpha = 0.05$).

Table 4. Mean no. of spider mite eggs, by days after infestation (DAI) averaged across all experiments

Treatment	5–8 DAI			12–15 DAI			16+ DAI		
	N	Mean	SEM	*N*	Mean	SEM	*N*	Mean	SEM
Aeris	20	19.4abc	4.6	20	53.4a	14.0	0		
Avicta	29	11.2ab	4.3	20	67.1ab	14.0	0		
Thiamethoxam	28	20.8a	4.4	20	49.4ab	14.0	4	16.1a	4.9
Imidacloprid	20	17.0ab	4.6	19	62.1a	14.1	0		
Acephate	20	14.5abc	4.6	20	41.1ab	14.0	0		
Aldicarb	37	11.6c	4.2	24	38.3bc	13.5	4	2.9a	4.9
Untreated	38	11.6bc	4.2	24	31.2c	13.5	4	12.8a	4.9
df		6, 163			6, 117			2, 6	
F-value		2.12			4.19			0.75	
P value		0.0532			0.0007			0.5140	

Statistics based on log transformation of the percentage of the untreated control.
Treatments with the same letter within a column are not significantly different (LSD with α = 0.05).

were magnified (Table 6). Aldicarb, Avicta, and untreated check had significantly fewer mites than neonicotinoid treatments. However, when cotton was sampled 2 wk after infestation at first true leaf stage (5–6th leaf stage), mite densities in Avicta treatments were similar to those in the thiamethoxam treatment. Three weeks after infestation at first true leaf, acephate had mite densities similar to aldicarb and untreated check, and fewer mites than all other neonicotinoid treatments. Aldicarb and untreated check treatments contained statistically fewer mites than most treatments at all sampling intervals. Treatments were not statistically different when infested at third or fourth true leaf.

Because thiamethoxam, imidacloprid, and Aeris were statistically similar in overall analyses, those treatments were combined into a neonicotinoid category and compared against Avicta, Acephate, aldicarb, and untreated check. In this analysis, treatments ranked similar to other analyses (Table 7). Avicta contained 34% fewer mites than other neonicotinoids. Aldicarb and untreated check had 57 and 53% fewer mites than the neonicotinoid category, respectively.

Foliar Versus Seed Treatment. Experiment 8 contained treatments with seed applied and foliar applications of thiamethoxam, imidacloprid, and acephate in addition to aldicarb in-furrow and an untreated check. Overall, there were very few differences between seed applied and foliar applications of thiamethoxam, imidacloprid, and acephate (Table 8). Aldicarb contained fewer mites than all other treatments at 7 and 14 d after infestation. Visual injury ratings followed closely with mite densities. Cotton in aldicarb treatments was least injured and cotton treated with foliar applications of acephate was most injured.

Laboratory Fecundity Experiment. Foliar applied acephate, foliar applied imidacloprid, and aldicarb had significantly fewer mite days than the untreated check (Table 9). Only foliar applied acephate and aldicarb had fewer total eggs than the untreated check. After adjusting total oviposition for differences in mite survival, only foliar applied thiamethoxam significantly increased twospotted spider mite fecundity above the untreated check. All other foliar and systemic neonicotinoid treatments resulted in similar average fecundity to the untreated check.

Discussion

Aeris, thiamethoxam, and imidacloprid treatments resulted in twospotted spider mite densities greater than those in the untreated check, aldicarb, and acephate treatments. However, cotton treated with Avicta (thiamethoxam + abamectin) had 34% fewer mites than other neonicotinoid seed treatments. Efficacy of Avicta against mites was only apparent when

Table 5. Mean no. of total mites (adult + immature), by days after infestation (DAI) averaged across all experiments

TRT	5–8 DAI			12–15 DAI			16+ DAI		
	N	Mean	SEM	*N*	Mean	SEM	*N*	Mean	SEM
Aeris	30	12.6a	2.4	20	26.5a	6.4	8	18.3a	4.1
Avicta	39	8.0b	2.3	20	18.9ab	6.4	8	12.6ab	4.1
Thiamethoxam	28	14.8a	2.4	26	18.3a	6.2	12	14.9a	3.8
Imidacloprid	20	15.4a	2.6	25	25.1a	6.3	8	19.3a	4.1
Acephate	20	11.8ab	2.6	20	15.0a	6.4	8	3.5c	4.1
Aldicarb	47	6.5c	2.3	30	11.3c	6.1	12	6.1bc	3.8
Untreated	48	8.3b	2.3	30	10.8bc	6.1	12	5.4bc	3.8
No. experiments	11	7	3						
df (num, den)	6, 178			6, 135			6, 50		
F-value	7.83			6.98			5.25		
P	<0.0001			<0.0001			0.0003		

Statistics based on log transformation of the percentage of the untreated control.
Treatments with the same letter within a column are not significantly different (LSD with α = 0.05).

Table 6. Mean no. of total mites (adult + immature), when infested at first true leaf and sampled at 5–9, 12–14, and 16+ days after infestation (DAI)

	First true leaf								
	5–9 DAI (3)[a]			12–14 DAI (5)			16+ DAI (7)		
	N	Mean	SEM	*N*	Mean	SEM	*N*	Mean	SEM
Aeris	26	15.17a	2.93	16	32.84ab	7.88	8	21.35a	2.77
Avicta	30	9.5b	2.9	16	24.5c	7.9	8	15.7a	2.77
Thiamethoxam	24	17.5a	2.9	20	23.3abc	7.6	8	21.6a	2.77
Imidacloprid	20	17.5a	3.0	19	32.5a	7.7	8	22.4a	2.77
Acephate	20	13.8ab	3.0	20	19.4bc	7.7	8	6.6b	2.77
Aldicarb	34	8.3c	2.8	20	12.9d	7.7	8	8.1b	2.77
Untreated	34	10.6bc	2.8	20	13.4d	7.7	8	6.8b	2.77
No. experiments		8			5			2	
df (num, den)		6, 148			6, 105			6, 42	
F-value		7.3			7.46			10.84	
P		<0.0001			<0.0001			<0.0001	

Data from infestations initiated at third and fourth true leaf are not shown. Statistics based on log transformation of the percentage of the untreated control.

Treatments with the same letter within a column are not significantly different (LSD with $\alpha = 0.05$).

[a] (3) - approximate growth g stage or no. of nodes at time of sampling.

mites were infested close to cotyledon stage and the effect became less apparent as cotton grew larger. Foliar formulations of abamectin are highly effective against twospotted spider mite (Price et al. 2009, Smith and Catchot 2009). Abamectin is not systemic within plant tissues, but may be deposited on cotyledons during germination and plant emergence from the soil (C. Crimm, Syngenta Crop Protection AG, personal communication). This may explain why Avicta reduced mite densities for only a brief period after cotyledon stage. As a seed treatment, acephate consistently ranked as the median treatment, and this may be related to less activity against thrips than the other seed treatments. Thrips are a known predator of twospotted spider mites (Agrawal et al. 1999, Colfer et al. 2000). Lindquist and Wolgamott (1980) reported that neither foliar nor systemic applications of acephate caused significant twospotted spider mite mortality. It is generally believed that in-furrow applications of acephate begin to diminish just after cotyledon stage. Predator populations may have rebounded quicker in acephate treatments than in neonicotinoid treatments. As a result, increased predation may partly explain why twospotted spider mite densities were lower in the acephate and untreated treatments. Untreated and aldicarb treatments had the lowest mite densities of twospotted spider mite. Aldicarb was the only treatment that reduced twospotted spider mite densities below the untreated check. Van Duyn et al. (1998) and Troxclair (2007) reported that on cotton with neonicotinoid seed treatments twospotted spider mite populations were significantly higher compared with untreated or aldicarb treated cotton, and that densities in untreated and aldicarb treated cotton were similar.

Twospotted spider mite densities on cotton treated with neonicotinoid seed treatments were consistently greater than those on untreated cotton or cotton treated with aldicarb or acephate. These results corroborate reports from Van Duyn et al. (1998), Sclar et al. (1998), James and Price (2002), Raupp et al. (2004), and Beers et al. (2005). Authors have postulated several theories explaining why mite populations increase after neonicotinoid applications. First, neonicotinoids have a hormoligant effect on mites, causing increased fecundity (James and Price 2002). Second, neonicotinoid insecticides reduce interspecific competition and promote mite population growth (Ako et al. 2004). Third, neonicotinoid insecticides reduce predation by killing predatory insects and mites (Sclar et al. 1998).

Table 7. Mean no. of total mites (adult + immature), averaged across all experiments sampled at 5–8, 12–15, and 16+ days after infestation (DAI)

TRT	All timing intervals			5–8 DAI			12–15 DAI			16+ DAI		
	N	Mean	SEM	*N*	Mean	SEM	*N*	Mean	SEM	*N*	Mean	SEM
Avicta	67	11.1b	3.2	39	8.1b	2.4	20	18.7bc	6.4	8	12.2ab	4.3
Untreated	90	7.8c	3.1	48	8.4b	2.3	30	10.8cd	6.1	12	5.4bc	4.1
Neonicotinoid	177	16.7a	3.0	78	14.1a	2.2	71	23.1a	5.8	28	16.9a	3.8
Acephate	48	10.1bc	3.3	20	11.6ab	2.6	20	15.0ab	6.4	8	3.1c	4.3
Aldicarb	89	7.1d	3.1	47	6.6c	2.3	30	11.3d	6.1	12	6.1c	4.1
df (num, den)		4,413			4,180			4,137			4,52	
F-value		24.4			11.83			10.62			7.56	
P		<0.0001			<0.0001			<0.0001			<0.0001	

Aeris, Gaucho, and Cruiser categorized into neonicotinoid class for analysis. Statistics based on log transformation of the percentage of the untreated control.

Means followed by the same letter within a column are not significantly different (LSD with $\alpha = 0.05$).

Table 8. Mean no. of immatures, eggs, and total mites per leaf on cotton treated with foliar and systemically applied insecticides when sampled at 7 and 14 d after infestation (DAI) in experiment 8

	7 DAI			14 DAI			Visual rating[a]
	Immatures	Eggs	Total mites	Immatures	Eggs	Total mites	
Thiamethoxam (SD trt)[b]	25.65a	29.25a	29.85a	33.23b	45.53cd	41.03ab	4.5b
Imidacloprid (SD trt)	25.95a	25.73a	31.25a	55.55a	105.58a	64.18a	4.0bc
Acephate (SD trt)	19.83a	18.63a	24.18a	24.68bc	86.5abc	39.53ab	4.0bc
Thiamethoxam (Foliar)	19.93a	31.63a	24.9a	33.78ab	65.23a–d	39.55ab	4.8b
Imidacloprid (Foliar)	26.45a	23.78a	32a	37.98ab	87.13ab	49.13ab	4.3b
Acephate (Foliar)	33.1a	38.83a	40.18a	36.48ab	63.93bcd	46.73ab	3.3c
Aldicarb	1.88b	3.43a	2.75b	8.28c	38.65d	11.5c	8.3a
Untreated	17.98ab	31.95a	22.55a	17.45bc	50.18bcd	29.88bc	4.3b
df (num, den)	7, 24	7, 24	7, 24	7, 24	7, 24	7, 24	7, 24
LSD ($\alpha = 0.05$)	171.21	241.51	189.31	221.47	415.77	256.91	0.81
F-value	2.525	1.713	2.884	3.614	2.737	3.051	30.19
P	0.0473	0.1602	0.0282	0.0103	0.0348	0.0223	0.0001

Means followed by the same letter within a column are not significantly different (LSD with $\alpha = 0.05$).
[a] Visual plant injury rating taken 14 DAI on a 1–10 scale, 10 = no damage.
[b] SD trt = seed treatment.

In the current experiment, systemically applied imidacloprid and thiamethoxam did not increase twospotted spider mite fecundity above that in the untreated check, but foliar applied thiamethoxam did increase fecundity. The current experiment did not show hormoligosis when mated females were exposed to treated leaves, but the results may be different if mites were allowed to develop on treated leaves. The second theory, that neonicotinoid seed treatments increase mite densities by reducing interspecific competition and plant injury, cannot be ruled out. Several thrips species are pests of seedling cotton, and in the absence of insecticides, can injure plants and reduce leaf area. It is possible that untreated cotton sustained more thrips injury and as a result was a less suitable host than cotton treated with insecticides. It is important to point out that 3 of the 12 experiments did receive enough thrips injury to reduce leaf area in untreated cotton, but, although some injury was evident, large differences in leaf area were not visually apparent among treatments in other experiments. The theory that neonicotinoid seed treatments caused mortality in predators, thus flaring twospotted spider mite populations is also plausible. Studies by Steinkraus et al. (1999) showed that immature thrips are often found in the center of mite colonies underneath leaves. *Orius insidiosus* (Say), *Geocoris punctipes* (Say), and *Frankliniella occidentalis* (Pergande), are other omnivorous insects that are frequently found on seedling cotton (Colfer et al. 2000, Studebaker and Kring 2003, Tillman and Mullinix 2003). All three species are reported to be important predators of twospotted spider mite (Lincoln et al. 1953, Wilson et al. 1991, Agrawal et al. 1999, Rondon et al. 2004), and have been documented to reduce populations by 76–99% (Colfer et al. 2000). Neonicotinoid insecticides have been reported to be deleterious to these and many other omnivorous predators (Sclar et al. 1998, Van Duyn et al. 1998, Elzen 2001, Studebaker and Kring 2003). Sclar et al. (1998) suggested that a reduction in minute pirate bug, *Orius tristicolor* (White), densities on plants treated with imidacloprid resulted in higher spider mite populations. Systemic soil-drench applications of thiamethoxam and imidacloprid also have been reported to be highly toxic to the omnivorous Brazilian spined soldier bug, *Podisus nigrispinus* (Dallas) (Torres and Ruberson 2004, Torres et al. 2003). Smith and Krischik (1999) found that the coccinellid predator *Coleomegilla maculata* (DeGeer) expressed higher mortality, and decreased mobility and oviposition when exposed to flowers after systemic applications of imidacloprid. Neonicotinoid insecticides used as seed treatments in cotton may have similar effects on omnivorous insects. Although other factors cannot be completely ruled out, predation was likely an important factor impacting the results of the current experiment.

Table 9. The impact of seed treatments and foliar insecticides on twospotted spider mite longevity and fecundity in a laboratory study

Treatment	Mite days	Total eggs	Eggs/mite/d
Imidacloprid (SD trt)[a]	22.8a	137.6a	6.0b
Thiamethoxam (SD trt)	21.5a	149.6a	7.2b
Acephate (SD trt)	20.2ab	118.1ab	5.7b
Untreated	20.0ab	152.6a	7.3b
Thiamethoxam (foliar)	14.3bc	150.9a	11.1a
Acephate (foliar)	11.8cd	72.6bc	5.7b
Imidacloprid (foliar)	11.5cd	109.2ab	7.5b
Aldicarb	7.3d	38.6c	5.6b
df	7, 35	7, 35	7, 35
LSD ($\alpha = 0.05$)	6.3	58.6	3.5
F-value	6.66	4.13	2.30
P value	<0.0001	0.0021	0.0489

Means followed by the same letter within a column are not significantly different (LSD with $\alpha = 0.05$).
[a] SD trt = seed treatment.

Aldicarb generally suppressed mite densities across all field experiments, but did not eliminate populations. Although control was variable among experiments, most aldicarb treatments resulted in mite populations that would have required further management. Knowles et al. (1988) determined that aldicarb was highly toxic to twospotted spider mite, and that a LC_{50} of 21 ppm was less (>four-fold) than for other carbamates, organophosphates, pyrethroids, and organochlorines tested, except for bifenthrin. Aldicarb was also found to be effective at reducing twospotted spider mite populations in large-seeded Virginia-type peanuts (Smith and Mozingo 1983). It is important to emphasize that a higher density of mites was placed on the seedling cotton plants using artificial infestations than would usually occur under natural conditions. Aldicarb may provide adequate control of twospotted spider mite under natural infestations where densities are low. The difference in the effects of neonicotinoid seed treatments and aldicarb on twospotted spider mite is explained by the fact that both are deleterious to predators; however, only aldicarb is effective against twospotted spider mite.

Overall, these trials indicated that twospotted spider mite densities are higher on cotton with a neonicotinoid seed treatment than on untreated cotton, or cotton treated with acephate as a seed treatment or aldicarb. All treatments except aldicarb resulted in higher mite densities than those on untreated cotton. Thrips are a major pest of cotton across the United States and require at-planting insecticides in nearly all situations. Despite widespread use of neonicotinoid seed treatments on most cotton acres in Mississippi, mite outbreaks in seedling cotton occur on only a small percentage of acres. Another factor that may impact twospotted spider mite densities in seedling cotton is weed management. Outbreaks on seedling cotton are often the result of alternate hosts remaining within or next to field borders. In situations where mite populations exist along field borders or on vegetation within fields, using a neonicotinoid seed treatment could further promote mite population growth. If there is a known risk of mite infestation, aldicarb would likely reduce the risk of economic infestations occurring.

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