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ARTHROPOD MANAGEMENT AND APPLIED ECOLOGY

Potential Interaction of Pre-emergence Herbicides and the Efficacy of Insecticide and Fungicide Seed Treatments in Cotton

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ABSTRACT

The increasing presence of glyphosate-tolerant weeds has increased the use of pre-emergence herbicides in cotton, *Gossypium hirsutum* L., grown in Tennessee during the last 5 to 8 years. These herbicides could have negative effects on seedling growth and potentially affect thrips (Thysanoptera: Thripidae) management or seedling disease with at-planting insecticides or fungicides. Experiments were conducted in 2013 and 2014 to evaluate the potential interactions of pre-emergence herbicides on the efficacy of insecticide or fungicide seed treatments in cotton. Nontreated plots and plots treated with a thiamethoxam seed treatment generally exhibited higher number of thrips, more thrips injury, less vigor, delayed maturity, and lower yields compared to plots treated with an imidacloprid seed treatment or an in-furrow application of aldicarb. Similarly, fungicide seed treatments generally reduced the incidence of seedling disease (primarily *Rhizoctonia*), while improving seedling health and stand density. Negative effects of some pre-emergence herbicides on plant health also were observed. For example, combination treatments of fluometuron plus S-metolachlor or fluometuron plus fomesafen reduced plant vigor and seedling biomass and there was a tendency for higher thrips populations where pre-emergence herbicides were used. There were no substantial interactions between injury caused by pre-emergence herbicides and that caused by thrips or seedling disease suggesting that thrips, seedling disease, and herbicide injury acted independently and additively. Following herbicide label rates should reduce the risk of compounding the effects of thrips or seedling disease with herbicide injury.

Thrips are generally among the top three yield reducing insect pests of cotton (*Gossypium hirsutum* L.) production (e.g., Williams, 2016). Injury symptoms including delayed maturity, reduced yield, and plant death can occur when thrips feed on emerging leaves and terminal buds during the seedling stage (Layton and Reed, 2002). Because Tennessee is located on the northern edge of the Cotton Belt, maturity delays can be especially important. Several thrips species can injure seedling cotton, but the tobacco thrips (*Frankliniella fusca* (Hinds), Thysanoptera: Thripidae) is the most common species observed in the Mid-South (Stewart et al., 2013). At-planting treatments including in-furrow granular or liquid insecticides and seed treatments are recommended to manage thrips infestations in seedling cotton (Stewart et al., 2017). In the last decade, insecticide seed treatments such as Gaucho (imidacloprid; Bayer CropScience, Raleigh, NC) or Cruiser (thiamethoxam; Syngenta, Greensboro, NC) have been used almost exclusively in Tennessee for control of thrips.

Seed and seedling pathogens are the most important diseases affecting cotton in Tennessee (Kelly, 2016). Seedling diseases often reduce the general health and vigor of plants and potentially make the plants more susceptible to other biotic or abiotic stressors (Wrather and Sweets, 2009). Common seedling diseases include *Rhizoctonia solani* Kühn, *Pythium* spp., and *Thielaviopsis basicola* (Berk. & Broome) Ferraris (Kelly, 2016; Newman, 1996). Annual yield losses to these diseases in Tennessee ranged from 6 to 29% during 2000 to 2015 (Kelly, 2016). All commercial cotton seed planted in the U.S. is treated with base fungicides and additional fungicide treatments often are recommended to further mitigate seedling disease. These treatments might include in-furrow spray or granular applica-

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tions (Kelly, 2016), but fungicide seed treatments have all but replaced the use of in-furrow treatments in Tennessee.

The use of pre-emergence herbicides has increased dramatically in Tennessee and in many other areas of the Cotton Belt during the last 5 to 8 years in response to glyphosate (e.g., Roundup, Monsanto Co., St Louis, MO) resistant weeds, especially Palmer amaranth, *Amaranthus palmeri* S. Wats. (Merchant et al., 2014; Prince et al., 2012; Sosnoskie and Culpepper, 2014; Whitaker et al., 2011a, b). Pre-emergence herbicides can cause crop injury in some cases (e.g., if rainfall occurs during emergence) and this injury can reduce seedling vigor and extend the window of susceptibility to seedling disease or thrips (Main et al., 2012). Objectives of this study were to elucidate the effects and possible interactions of commonly used pre-emergence herbicides on the control of thrips and seedling diseases provided by seed treatments.

MATERIALS AND METHODS

Experimental Design. Protocols evaluating the interactions of herbicides with insecticides and fungicides were performed in 2013 and 2014 at the University of Tennessee-Milan Research and Education Center located in Gibson County. Individual trials were planted on 14 May 2013 and 13 May 2014 to evaluate insecticide treatments for control of thrips and potential interactions with pre-emergence herbicides (Table 1). In the second experimental protocol, trials were planted on 14 May 2013, 13 May 2014, and 28 May 2014 to evaluate fungicide seed treatments and interactions with these same herbicides (Table 1). All trials were conducted under dryland conditions and were planted no-till into cotton residue from the previous cropping season. Either Roundup WeatherMAX (glyphosate; Monsanto Company, St. Louis, MO) or Gramoxone (paraquat; Syngenta, Greensboro, NC) was used for a burndown application on all plots immediately after planting. Pre-emergence herbicide treatments were applied within 2 d after planting with a pressurized CO_2 backpack sprayer calibrated to deliver 151 L per hectare using XR 8002 flat fan nozzles at 275 kPa. Post-emergence herbicides applications were applied across a trial as needed to keep experiments free of weeds, but only Roundup WeatherMAX was applied prior to squaring to minimize potential for crop injury during this window.

Experimental design was a factorial arrangement of treatments in a randomized complete block design with four replications. Treatments included the four herbicide treatments in combination with either the four insecticide or four fungicide treatments, plus a completely nontreated check. The experimental unit was a plot of cotton measuring four rows wide (102-cm row spacing) by 10.7 m long. Phytogen 375 WRF and Phytogen 333 WRF (Dow AgroSciences, Indianapolis, IN) were the cotton varieties used for experiments in 2013 and 2014, respectively. A seeding rate of 13.2 seed per meter, planted at a depth of 1.9 cm, was used in all experiments. In the insecticide trials, all seed were treated with a fungicide seed treatment (Dynasty CST; Syngenta, Greensboro, NC), whereas Cruiser insecticide seed treatment (2013) or Gaucho (2014) was used in the fungicide trials at rates shown in Table 1. In the fungicide trials performed in 2013 and the first planting of 2014, a strain of *Rhizoctonia* AG2-2 IIIB was grown on millet seed and inoculated in-furrow at planting at 1 to 2 g/0.3 m of row.

Table 1. Pesticide rates used to evaluate the effect of pre-emergence herbicides on the performance of at-planting insecticides and fungicide seed treatments in cotton

Treatments	Trade Names [z]	Active Ingredients	Use Rates
Herbicides	Cotoran 4L Cotoran 4L+Reflex Cotoran 4L+Dual M.	fluometuron fluometuron+fomesafen fluometuron+S-metolachlor	2.84 l/ha 2.84+1.13 l/ha 2.84+1.42 l/ha
Insecticides	Gaucho 600 Cruiser 5F Temik 15G	imidacloprid thiamethoxam aldicarb	0.375 mg ai/seed 0.375 mg ai/seed 820 g ai/ha
Fungicides	Apron Maxx Dynasty CST Trilex Advanced	mefenoxam, fludioxonil mefenoxam, fludioxonil, azoxystrobin metalaxyl, triadimenol, trifloxystrobin	ml/45.4 kg seed 104 ml/45.4 kgseed 47. 3 ml/45.4 kg seed

[z] Cotoran 4L (DuPont, Eleutherian Mills, DE); Apron Maxx, Cruiser 5F, Dual Magnum, Dynasty CST, Reflex (Syngenta; Greensboro, NC); Gaucho, Temik 15G, Trilex Advanced (Bayer CropScience; Raleigh, NC).

Thrips Counts, Plant Biomass, Thrips Injury, and Vigor Ratings. Thrips were collected at the first and/or second true-leaf stage, depending upon the experiment, which occurred from18 to 22 days after planting (DAP). Either 5 or 8 plants per plot, depending upon the trial and sampling date, were collected by cutting seedlings at ground level and placing them in sealed plastic bags. The fresh weight of each sample was recorded, and the samples were stored in a refrigerator until thrips were enumerated. Plants were individually rinsed with ethyl alcohol over a glass container topped with a sieve to collect the thrips. The plastic bag was also rinsed with ethyl alcohol over the sieve to collect any remaining thrips left inside. Finally, the sieve was rinsed onto a gridded petri dish and the thrips were categorized as either adult or immature and enumerated underneath a microscope.

Visual ratings of thrips injury and plant vigor were obtained between 15 to 30 DAP. These relative, whole-plot ratings were based on a 0 to 5 scale. For thrips injury, a “0” rating represented no injury and a “5” was 100% plant death. Conversely, “0” vigor rating indicated 100% stand loss and a vigor rating of “5” represented complete stands of healthy plants.

Plant, Weather, and Yield Data. At least one stand count was taken in each trial by counting the total number of plants in the center two rows of each plot. Plant mapping techniques were conducted during bloom to measure the effects of treatments on crop maturity. Bloom counts (2014 only) of every white to pinkish bloom in the middle two rows of each plot were taken approximately 3 wks after first bloom. Later in the season, the nodes above the first position white flower (NAWF) were counted on 10 random plants per plot. Weather data were obtained from the NOAA weather station at the University of Tennessee Research and Education Center in Milan. Seed cotton yield was estimated by picking the center two rows of each plot.

Leaf Samples for Neonicotinoid Insecticide Concentration. In the 2013 experiment investigating possible interactions between insecticide and herbicides, 15 terminal leaves were collected from each Cruiser-treated plot. Expanded leaves were collected at the second-leaf stage and placed into zip-top plastic bags. In the laboratory, the leaves were rinsed and dried before being placed in a freezer (-20 ºC) until they were shipped for analysis of neonicotinoid residue levels. Samples were analyzed to determine the levels of neonicotinoid residues by the USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratories (Gastonia, NC) as previously described in Stewart et al. (2014).

Data Analyses. All data were analyzed with the GLIMMIX procedure in SAS (SAS Institute Inc., 2013). When significant differences were detected, Fisher’s protected LSD (LS Means, $\alpha = 0.05$) was used to separate individual treatment means. Analyses were conducted across trials for data collected within experiments during a similar time period. Main effects of insecticide or herbicide treatment were considered to be fixed model effects. Replicate (nested within year) was considered to be a random effect, as suggested by Carmer et al. (1989), and within-year mean separation was based on the least squares means for each main effect by year component of the across-year model.

RESULTS

There were many examples of year-by-main-effect interactions. Unless specifically indicated, interactions between the main effects of herbicide and insecticide treatments were not significant ($p > 0.05$), and thus, data for these main effects are presented separately.

Insecticide and Herbicide Experiments. *Thrips Counts.* The first thrips counts were taken 18 (2013) or 16 (2014) DAP as the second true leaf was emerging. Species identification of adult thrips from nontreated plots indicted that tobacco thrips composed more than 80% of the thrips species in all trials. For immature and total thrips, there was a significant effect for insecticide treatment and an interaction between years and insecticide for immature ($F = 3.21$; df = 3, 93; $p = 0.0265$) and total thrips ($F = 6.02$; df = 3, 93; $p = 0.0009$)(Table 2). In both years, insecticide treatments decreased immature and total thrips counts compared with the nontreated plots. In-furrow application of Temik (Bayer CropScience, Raleigh, NC) provided the greatest reduction in immature thrips numbers, and the Gaucho seed treatment significantly reduced immature thrips numbers more than Cruiser in 2013. Temik significantly reduced total thrips counts compared with Gaucho and Cruiser in 2013. In 2014, Temik and Gaucho reduced immature and adult thrips numbers compared with Cruiser. Herbicide did not affect numbers of immature thrips ($p = 0.5911$) or total thrips ($p = 0.3974$).

Table 2. The effects of insecticide and herbicide treatments on mean immature and total thrips per cotton plant. Means are shown by year when significant year*insecticide or year*herbicide interactions were found

Thrips	Year	DAP[z]	Insecticide				*F*-value	df	*p*-value
			Nontreated	Cruiser	Gaucho	Temik			
Immature	2013	16	17.9 a	10.4 b	6.7 c	1.1 d	3.21	3, 93	0.0265
	2014	18	19.4 a	9.0bc	1.2 d	1.2 d			
Total	2013	16	22.1 a	14.6 b	13.0 b	2.8 c	6.02	3, 93	0.0009
	2014	18	23.1 a	13.8 b	4.02 c	2.0 c			
Immature	2013-2014	22-23	14.5 a	13.3 a	6.5 b	2.3 c	61.8	3,93	< 0.0001
Total	2013-2014	22-23	19.7 a	20.4 a	11.6 b	6.0 c	55.17	3,93	< 0.001
Thrips	**Year**	**DAP**	**Herbicide**				***F-value***	**df**	***p*-value**
			Nontreated	**Cotoran**	**Cot. + Dual M.**	**Cot. + Reflex**			
Immature	2013-2014	22-23	7.2 b	9.9 a	9.0 ab	10.6 a	4.01	3, 93	0.0098
Total	2013	22	10.4 d	17.4 ab	14.1 bc	19.6 a	3.82	3, 93	0.0125
	2014	23	12.7 cd	13.7 cd	13.8 bcd	13.7 cd			

Means within rows not followed by a common letter are significantly different.

[z] DAP, days after planting.

Thrips were counted a second time at 22 (2013) or 23 (2014) DAP when plants were at the second true-leaf stage. Across both years, both insecticide and herbicide treatment affected numbers of immature thrips (Table 2). There were no treatment-by-year interactions ($F = 2.63$; $df = 3, 93$; $p = 0.0550$). Insecticide and herbicide treatment affected total thrips numbers across years, and there was a significant interaction of year and herbicide ($F = 3.82$; df= 3, 93; $p = 0.0125$). Temik and Gaucho provided better control than Cruiser, which did not significantly reduce thrips counts compared with nontreated plots. Herbicide treatments tended to increase the numbers of thrips, and plots treated with only Cotoran (DuPont, Eleutherian Mills, DE) or Cotoran plus Reflex (Syngenta, Greensboro, NC) had significantly more thrips than nontreated plots in 2013.

Biomass, Vigor, and Thrips Injury Ratings. Both insecticide and herbicide treatments affected plant biomass measured at 22 (2013) and 23 (2014) DAP (Table 3). There was a significant interaction between year and insecticide treatment ($F = 7.78$; df = 3, 93; p = < 0.0001), but not for year and herbicide treatment ($F = 0.22$; df = 3, 93; $p = 0.8825$). In 2013, there was a trend towards increased biomass in plots treated with insecticide, but only plots treated with Temik had more biomass than nontreated plots. In 2014, all insecticide treatments increased biomass compared with the nontreated plots; those treated with Temik accumulated more biomass followed by Gaucho and then Cruiser. Across years, biomass was greater in plots treated with Cotoran compared with treatments receiving Cotoran plus Dual Magnum (Syngenta, Greensboro, NC) or Cotoran plus Reflex but was not higher than plots not treated with a pre-emergence herbicide (Table 3).

Vigor ratings were taken at 27 (2013) or 26 (2014) DAP when plants were at the third true-leaf stage. There were significant effects attributed to both insecticide and herbicide treatment (Table 3), but no interactions between insecticide or herbicide and year. Across years, vigor was lowest in the nontreated plots compared with the insecticide treatments. Temik and Gaucho increased plant vigor compared to Cruiser. Herbicide treatments tended to reduce vigor, and plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex had less vigor than plots not treated with pre-emergence herbicides. Other vigor ratings showed similar results (data not shown).

Initial thrips injury ratings were taken at 22 (2013) or 20 (2014) DAP. There was an interaction ($F = 9.50$; df = 3, 93; $p < 0.0001$) between years and insecticide (Table 3). Regardless of year, thrips injury was numerically greater in plots not treated with insecticide. Temik provided the greatest reduction in thrips injury followed by Gaucho. Cruiser did not reduce thrips injury in 2014 compared with plots not treated with insecticide. There was a significant effect of herbicide treatment and an interaction ($F = 3.68$; df = 3, 93; $p = 0.0148$) between year and herbicide (Table 3). In

2013, plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex exhibited higher thrips injury scores. In 2014, there was no significant effect of herbicide application on thrips injury scores.

Thrips injury was rated a second time at 27 (2013) or 26 (2014) DAP. There was a significant effect of insecticide treatment and an interaction (F = 3.54; df = 3, 93; p = 0.0178) between year and insecticide (Table 3). In 2013, Temik provided the greatest reduction in thrips injury and Gaucho reduced thrips injury more than Cruiser. Cruiser did not reduce thrips injury compared with the nontreated plots. In 2014, thrips injury was highest in the nontreated plots compared with those receiving any insecticide. Again, Temik provided the greatest reduction in thrips injury, and Gaucho reduced thrips injury more than Cruiser (Table 3). There was no interaction between herbicide treatment and year (F = 2.39; df = 3, 93; p = 0.0739). When analyzed across years, plots treated with pre-emergence herbicides tended to have higher thrips injury scores (F = 4.84; df = 3, 93; p = 0.0036). Specifically, plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex had higher thrips injury scores than nontreated plots or plots only treated with Cotoran.

Plant, Plant Mapping, and Yield Data. There was no significant effect of herbicide application on plant stands when analyzed across years (F = 0.43; df = 3, 93; p = 0.7339). Also, insecticide treatment caused no significant differences in stand counts in 2013. Plant stands increased by approximately 20% in Gaucho- and Temik-treated plots compared with the Cruiser treatment or plots not treated with insecticide (F = 15.13; df = 3, 45; p < 0.0001; data not shown).

Insecticide and herbicide treatments affected bloom counts at 72 DAP in 2014 (Table 4). Temik and Gaucho increased the number of blooms compared with Cruiser, which did not increase the number of blooms compared with the nontreated plots. Treatment with Cotoran resulted in a similar number of blooms compared with plots not receiving a pre-emergence herbicide, but more blooms than plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex. In 2013, insecticide treatment did not significantly affect the number of nodes above white flower for data collected 74 DAP (Table 4). In 2014, Temik and Gaucho significantly decreased the number of nodes above white flower relative to the Cruiser treatment and plots not receiving an at-planting insecticide. Herbicide treatment had no significant effect either year.

Table 3. The effects of insecticide and herbicide treatments on mean plant biomass, vigor, and thrips injury for seedling cotton plants. Means are shown by year when significant year*insecticide or year*herbicide interactions were found[z]

Rating	Year	DAP[y]	Insecticide				F-value	df	p-value
			Nontreated	Cruiser	Gaucho	Temik			
Biomass	2013	22	1.35 f	1.41 ef	1.48 ef	1.63 e	7.78	3, 93	< 0.0001
	2014	23	2.01 d	2.28 c	2.58 b	3.02 a			
Vigor[x]	2013-2014	26-27	2.2 d	2.5 c	3.1 b	3.4 a	75.24	3, 93	< 0.0001
Thrips injury	2013	22	3.9 a	3.3 b	2.1 d	1.3 f	9.5	3, 93	< 0.0001
	2014	20	3.4 b	3.1 c	1.9 de	1.7 e			
Thrips injury	2013	27	4.1 b	3.9 b	2.6 c	1.6 e	3.54	3, 93	0.0178
	2014	26	4.4 a	4.1 b	2.6 c	2.2 d			

Rating	Year	DAP	Herbicide				F-value	df	p-value
			Nontreated	Cotoran	Cot. + Dual M.	Cot. + Reflex			
Biomass	2013-2014	22-23	1.99 ab	2.11 a	1.86 b	1.91 b	3.67	3, 93	0.0151
Vigor	2013-2014	26-27	2.9 a	2.8 ab	2.7 c	2.7 bc	3.68	3, 93	0.0149
Thrips injury	2013	22	2.4 c	2.5 bc	2.9 a	2.9 a	3.68	3, 93	0.0148
	2014	20	2.5bc	2.6bc	2.5bc	2.7 ab			
Thrips injury	2013-2014	26-27	3.1 b	3.1 b	3.3 a	3.3 a	4.84	3, 93	0.0036

[z] Means within rows not followed by a common letter are significantly different.

[y] DAP, days after planting.

[x] Vigor per plot rated on a (0-5) scale with 5 being most vigorous; thrips injury on a (0-5) scale with 5 being maximum injury.

Table 4. The effects of insecticide and herbicide treatments on the mean blooms per hectare, mean number of nodes above a first position white flower (NAWF), and final seed cotton yield in kg/ha. Means are shown by year when significant year*insecticide or year*herbicide interactions occurred[z]

Rating	Year	DAP[y]	Insecticide				*F*-value	df	*p*-value
			Nontreated	Cruiser	Gaucho	Temik			
Blooms	2014	72	13,377 b	17,903 b	26,494 a	26,609 a	14.49	3, 45	< 0.0001
NAWF	2013	74	7.5 a	7.5 a	7.4 a	7.4 a	3.73	3, 93	0.0139
	2014	86	6.4 b	6.2 b	5.7 c	5.7 c			
Yield	2013	187	3,263 b	3,296 b	3,409 ab	3,556 a	5.51	3, 93	0.0016
	2014	178	2,651 c	2,641 c	3,290 b	3,381 ab			

Rating	Year	DAP	Herbicide				*F*-value	df	*p*-value
			Nontreated	Cotoran	Cot. + Dual M.	Cot. + Reflex			
Blooms	2013-2014	72	21,189 ab	25,543 a	19,315 b	18,335 b	3.43	3, 45	0.0249
NAWF	2013-2014	74, 86	6.8	6.6	6.8	6.7	1.02	3, 93	0.3872
Yield	2013-2014	187, 178	3,267	3,261	3,077	3,139	2.42	3, 93	0.0713

[z] Means within rows not followed by a common letter are significantly different.

[y] DAP, days after planting.

Insecticide treatment affected seed cotton yields, and there was a year-by-insecticide interaction (F = 5.51; df = 3, 93; p = 0.0016). In both years, Temik increased seed cotton yields compared with plots not treated with insecticide (Table 4). Gaucho significantly increased yield in 2014 only, whereas Cruiser did not significantly increase yield compared with nontreated plots in either year. There was not a year-by-herbicide interaction for seed cotton yields (F = 0.29; df = 3, 93; p = 0.8316). Further, there were no differences in yield attributed to herbicide treatment (F = 2.42; df = 3, 93; p = 0.0713).

Neonicotinoid Insecticide Concentrations in Leaves. In 2013, insecticide residue detected in second true leaves that were collected 20 DAP included thiamethoxam and its metabolites including clothianidin, clothianidin TMG [N-methyl-N'-nitroguanidine], clothianidin TZMU [N-(2-chlorothiazol-5-ylmethyl)-N′-methylurea], and clothianidin TZNG [N-(2-chlorothiazol-5ylmethyl)-N'-nitroguanidin]. Depending upon the treatment, the total concentration of thiamethoxam plus its metabolites ranged from 4,026 to 7,142 PPB in the leaf tissue (Table 5).

Thiamethoxam and its primary metabolite, clothianidin, composed 75.8% and 19.6% of the total neonicotinoid concentration found in the leaf tissue, respectively. Plots treated with Cotoran plus Dual Magnum had statically higher concentrations of thiamethoxam and clothianidin compared with those not treated with Dual Magnum, including those treated with Reflex or Cotoran (Table 5).

Fungicide and Herbicide Experiments. *Thrips Counts.* Across years, there was no significant effect from either fungicide treatment (F = 0.71; df = 3, 45; p = 0.5504) or herbicide treatment (F = 0.64; df = 3, 45; p = 0.5920) on the number of thrips observed on seedling cotton. However, there was a significant interaction between fungicide and herbicide treatments in the 2014 early planting (Table 6). Numbers ranged from 1.2 to 4.2 immature thrips per plant, depending upon the treatment. Because a base insecticide seed treatment was used in these trials, thrips numbers were not particularly high, and the pattern of this interaction could not be readily explained (Table 6).

Biomass, Thrips Injury, Herbicide Injury, and Vigor Ratings. There was no significant effect of fungicide seed treatment on plant biomass across years (Table 7), but herbicide treatment had a significant effect where Cotoran plus Dual Magnum reduced biomass compared with all other treatments. When analyzed across years, fungicide seed treatments did not have significant effect on visual estimates of thrips injury for data collected at the second true-leaf stage (21-24 DAP, Table 7). However, an additional rating made 30 DAP in the 2014 early planting showed that thrips injury scores were significantly lower for plots treated with Dynasty CST compared with other treatments. Thrips injury scores were generally higher in plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex, and these differences were significant in the 2014 late planting. Plots treated with Cotoran plus Reflex exhibited noticeable herbicide injury in a visual estimate of chlorosis (% leaf burn) in the 2013 trial (Table 7).

Table 5. The effects of pre-emergence herbicide treatments on the concentration (ppb) of thiamethoxam and its metabolites including clothianidin, clothianidin TMG, clothianidin TZMU, and clothianidin TZNG in the second true leaf of cotton, 2013

Herbicide Treatment	Thiam.	Cloth.	Cloth. TMG	Cloth. TZMU	Cloth. TZNG	Total
Nontreated	2,700 b[z]	891.3 b	75.8	60.9 b	297.5 a	4,026 b
Cotoran	3,920 b	921.8 b	28.3	85.9 ab	0.0 b	4,956 b
Cotoran + Dual M.	5,470 a	1,370 a	69.2	108.6 a	125.0 ab	7,143 a
Cotoran + Reflex	3,550 b	847.0 b	32.8	65.8 b	0.0 b	4,496 b
F-value	5.81	6.38	1.69	4.30	5.54	5.14
df	3, 9	3, 9	3, 9	3, 9	3, 9	3, 9
p-value	0.0172	0.0131	0.2380	0.0384	0.0197	0.0243

[z] Means within columns not followed by a common letter are significantly different.

Table 6. Effects of fungicide seed treatments and pre-emergence herbicides on immature thrips in the 2014 early planting at 20 DAP

Treatment	Immature thrips per plant			
Fungicide	Herbicide			
	Nontreated	Cotoran	Cotoran + Dual M.	Cotoran + Reflex
Nontreated	1.90 bc[z]	4.15 a	1.10 c	2.50 abc
Apron Maxx	2.15 bc	2.55 abc	2.15 bc	2.70 abc
Dynasty CST	1.20 c	1.65 c	2.85 abc	1.45 c
Trilex Advanced	3.75 ab	2.00 bc	1.80 bc	1.50 c
F-value	2.11			
df	9, 45			
p-value	0.0489			

[z] Means not followed by a common letter are significantly different.

Table 7. The effects of fungicide seed treatments and pre-emergence herbicides on above ground biomass per cotton plant, thrips injury, and herbicide injury (% leaf burn)

Rating[z]	Year[y]	DAP[x]	Fungicide				*F*-value	df	*p*-value
			Nontreated	Apron M.	Dynasty CST	Trilex Adv.			
Biomass (g)	2013-2014	20-21	1.20[w]	1.21	1.27	1.27	1.55	3, 141	0.2055
Thrips injury	2013-2014	21-24	2.6	2.6	2.5	2.6	1.47	3, 141	0.2245
Thrips injury	2014 E	30	2.5 a	2.4 a	2.2 b	2.5 a	5.55	3, 45	0.0025
Thrips injury	2014 L	15	1.5 a	1.5 a	1.5 a	1.4 a	1.92	3, 45	0.1398
Herbicide injury	2013	9	7.1	5.9	5.8	8.8	1.34	3, 45	0.2726

Rating	Year	DAP	Herbicide				*F*-value	df	*p*-value
			Nontreated	Cotoran	Cot. + Dual M.	Cot. + Reflex			
Biomass (g)	2013-2014	20-21	1.25 a	1.29 a	1.14 b	1.27 a	5.36	3, 141	0.0016
Thrips injury	2013-2014	21-24	2.5b	2.6 ab	2.6 a	2.6 a	3.37	3, 141	0.0204
Thrips injury	2014 E	30	2.4	2.4	2.4	2.5	0.61	3, 45	0.6107
Thrips injury	2014 L	15	1.4 c	1.5 bc	1.5 a	1.5 ab	4.8	3, 45	0.0055
Herbicide injury	2013	9	2.6 b	2.5 b	5.6 b	16.8 a	32.54	3, 45	< 0.0001

[z] Thrips injury rated per plot on a (0-5) scale with 5 being the most injury.

[y] Early, E, and late, L, plantings within 2014.

[x] DAP, days after planting.

[w] Means within rows not followed by a common letter are significantly different.

Across years, fungicide seed treatments and pre-emergence herbicides effected vigor ratings taken 21 to 24 DAP (Table 8). There also were significant interactions between year and fungicide treatment and year and herbicide treatment. In 2013, there was no difference in vigor scores between fungicide treatments, but plots treated with Cotoran plus Dual Magnum had less vigor than other herbicide treatments. Vigor scores were generally low in the 2014 early planting with relatively large differences among fungicide seed treatments. In the 2014 early planting, the highest vigor scores were in plots treated with Dynasty CST, followed by Trilex Advanced (Bayer CropScience, Raleigh, NC) and Apron Maxx (Syngenta, Greensboro, NC). In 2014, early vigor scores were significantly higher in all plots that received a fungicide treatment compared with the nontreated plots (Table 8), and plots treated with Cotoran plus Reflex had higher vigor scores than other treatments. Vigor scores of all treatment combinations were relatively high in the 2014 late planting. There was no difference among fungicide treatments, but plots treated with Cotoran plus Dual Magnum had less vigor than those treated with Cotoran or those not treated with pre-emergence herbicides.

Vigor ratings were taken again at the fourth true-leaf stage (29 DAP) in 2013, and fungicide and herbicide treatments effected vigor (Table 8). Differences in vigor ratings among fungicide seed treatments were relatively small, but nontreated plots and those treated with Trilex Advanced had lower vigor than those treated with Apron Maxx. Fungicides had a significant effect on vigor ratings taken at the fourth true-leaf stage (30 DAP) in the 2014 early planting where all fungicide treatments increased vigor scores compared with the nontreated plots. Vigor ratings were highest in plots treated with Dynasty CST, followed by Trilex Advanced and Apron Maxx (Table 8). There was no effect from herbicide treatment on vigor at this time. Similar to the subsequent rating, fungicide treatment did not affect vigor scores taken at the first true-leaf stage in the 2014 late planting (15 DAP, Table 8). Similar to vigor ratings taken a week later, there was a significant decrease in vigor scores in the plots treated with Cotoran plus Dual Magnum compared with other herbicide treatments.

Weather, Plant Mapping, and Yield Data. The 5 d immediately following planting were considerably wetter and cooler for the 2014 early planting compared with the other fungicide trials. For example, more than 50 mm of rain fell during the first 48 h after planting and temperatures were cool. Based on a cotton developmental threshold of 15.6 °C, fewer than seven growing degree days accumulated in the 5 DAP. This contrasted with the other trials where less rainfall (6 and 18 mm) occurred within 5 DAP and degree day accumulation was 5 to 7 times higher.

Counts taken either 15 or 16 DAP indicated that fungicide seed treatment affected plant stand, but herbicide treatment had no effect (Table 9). There was also a significant year-by-fungicide interaction. In 2013, plots treated with Dynasty CST and Trilex Advanced exhibited more plants per hectare than plots not treated with a fungicide. The Trilex Advanced treatment also had more plants per hectare than plots treated with Apron Maxx. Seed and seedling diseases were prevalent in the 2014 early planting. All fungicide seed treatments increased plant stands. The highest stand counts were observed in plots treated with Dynasty CST followed by Trilex Advanced and Apron Maxx (Table 9). Fungicide seed treatments also tended to increase plant stands in the 2014 late planting, and stand counts in plots treated with Trilex Advanced were greater than plots not treated with fungicide.

A final stand count was taken 30 DAP in the 2014 early planting because treatment effects were evident. As with the previous count, all fungicide treatments increased the number of plants per hectare (Table 9). Dynasty CST treatment was superior to Trilex Advanced and Apron Maxx. Again, herbicide treatments had no effect on final plant populations.

Fungicide treatment affected the number of blooms present at 76 DAP in the 2014 early planting, but there was no significant effect from herbicide treatment (Table 9). The numbers of blooms per hectare were greater in plots treated with Dynasty CST, followed by Trilex Advanced and Apron Maxx. Fungicide seed treatment also affected the number of nodes above first position white flowers for counts made 90 DAP in the 2014 early planting ($F = 8.90$; df = 3, 24; $p = 0.0004$). There was a significant decrease in the number of nodes above first position white flowers in plots treated with Dynasty CST (5.6 ± 0.1) compared with Trilex Advanced (6.1 ± 0.2) and Apron Maxx (6.1 ± 0.2). Conversely, herbicide treatment had no effect ($F = 1.43$; df = 3, 24; $p = 0.2577$). However, number of nodes above first position white flowers could not be estimated in plots not treated with a fungicide seed treatment or plots that received an application of Cotoran plus Dual Magnum because those plants were so delayed that white flowers were not present at the time of rating (data not shown).

Table 8. The effects of fungicide seed treatments and pre-emergence herbicides on the mean vigor score of seedling cotton plants. Means are shown by year when significant year*insecticide or year*herbicide interactions were found[z]

Year[x]	DAP[w]	Fungicide—Vigor[y]				*F*-value	df	*p*-value
		Nontreated	Apron M.	Dynasty CST	Trilex Adv.			
2013	23	2.5 b	2.6 b	2.6 b	2.6 b			
2014 E	24	0.6 f	1.0 e	2.2 c	1.3 d	25.65	6, 141	< 0.0001
2014 L	21	4.1 a	4.1 a	4.1 a	4.1 a			
2013	29	2.4 b	2.6 a	2.5 ab	2.4 b	3.74	3, 45	0.0175
2014 E	30	0.7 c	1.3 b	2.6 a	1.6 b	61.57	3, 45	< 0.0001
2014 L	15	2.6	2.6	2.8	2.7	0.73	3, 45	0.5407

Year	DAP	Herbicide—Vigor[y]				*F*-value	df	*p*-value
		Nontreated	Cotoran	Cot. + Dual M.	Cot. + Reflex			
2013	23	2.7 c	2.6 c	2.3 d	2.6 c			
2014 E	24	1.2 f	1.2 f	1.2 f	1.5 e	2.43	6, 141	0.0287
2014 L	21	4.2 a	4.2 a	3.9 b	4.1 ab			
2013	29	2.6 a	2.5 a	2.4 b	2.5 a	6.37	3, 45	0.0011
2014 E	30	1.6	1.4	1.6	1.6	0.63	3, 45	0.5974
2014 L	15	2.8 a	2.8 a	2.4 b	2.7 a	5.66	3, 45	0.0022

[z] Means within rows not followed by a common letter are significantly different.

[y] Vigor per plot rated on a (0-5) scale with 5 being the most vigorous.

[x] Early, E, and late, L, plantings within 2014.

[w] DAP, days after planting.

Table 9. The effects of fungicide and herbicide treatments on the number of plants per hectare, blooms per hectare in the 2014 early planting, and seed cotton yields (kg/ha). Means are shown by year when significant year*insecticide or year*herbicide interactions were found[z]

Rating	Year[y]	DAP[x]	Fungicide				*F*-value	df	*p*-value
			Nontreated	Apron M.	Dynasty CST	Trilex Adv.			
Plants	2013	15	103,525 d	106,956 bcd	111,424 ab	113,039 a			
	2014 E	16	12,598 h	24,505 g	53,651 e	32,317 f	16.06	6, 141	< 0.0001
	2014 L	16	102,833 d	104,938 cd	108,628 abcd	110,214 abc			
Plants	2014 E	30	9,456 c	19,460 b	49,500 a	24,966 b	66.36	3, 45	< 0.0001
Blooms	2014 E	76	4,440 d	8,678 c	22,746 a	13,117 b	29.01	3, 45	< 0.0001
Yield	2013	189	3,523 a	3,561 a	3,678 a	3,622 a			
	2014 E	182	1,378 f	2,001 e	3,008 b	2,673 c	13.13	6, 135	< 0.0001
	2014 L	166	2,237 de	2,267 de	2,227 de	2,382 cd			

Rating	Year	DAP	Herbicide				*F*-value	df	*p*-value
			Non-treated	Cotoran	Cot. + Dual M.	Cot. + Reflex			
Plants	2013-2014	15-16	83,662	80,231	82,259	82,057	1.35	3, 141	0.2608
Plants	2014 E	30	26,696	24,793	25,687	26,206	0.15	3, 45	0.9282
Blooms	2014 E	76	12,368	10,465	12,541	13,607	0.81	3, 45	0.4975
Yield	2013-2014	166-189	2,722	2,726	2,668	2,736	0.23	3, 135	0.8744

[z] Means within rows not followed by a common letter are significantly different.

[y] Early, E, and late, L, plantings within 2014.

[x] DAP, days after planting.

Fungicide treatments significantly increased seed cotton yields (Table 9). However, there was a significant interaction between fungicide treatment and year. In 2013 and the 2014 late planting, there was no significant effect of fungicide seed treatment on yield. In contrast, fungicide seed treatments dramatically increased yields in the 2014 early planting; the highest yield was in plots that were treated with Dynasty CST (3,008 kg/ha), followed by Trilex Advanced (2,673 kg/ha), and Apron Maxx (2,001 kg/ha).There were no significant effects of herbicides on yield (Table 9). When analyzed across all five trials, herbicides did not affect final yield ($p < 0.4452$; df = 3, 270; $F = 0.89$).

DISCUSSION

This research was initiated to investigate an apparent reduction in thrips suppression provided by insecticide seed treatments. Local observations in experimental plots and production fields during the proceeding few years suggested diminished performance of neonicotinoid seed treatments, especially Cruiser. One hypothesis for this decreased performance was negative interactions between insecticide seed treatments and pre-emergence herbicides, which have been used more widely and at relatively high rates in recent years in response to glyphosate-resistant Palmer amaranth and other herbicide resistant weeds. We used near maximum labeled rates of pre-emergence herbicides to increase the likelihood of inflicting crop injury and observing interactions. Also, Reflex was used post-planting, which is not recommended in Tennessee because of the potential for crop injury. Fungicide seed treatments were tested because their efficacy also might be affected by use of herbicides. Apron Maxx is not labeled or recommended for use on cotton, in part because it lacks a quinone outside inhibitor (i.e., strobilurin) component; it was included to provide an intermediate level of seedling protection. Trilex Advanced and Dynasty CST are common, additional treatments made to cotton seed, but all commercial cotton seed comes with a base fungicide seed treatment. Base fungicide treatments were not used in these trials to increase the likelihood of seeing variable treatment responses.

The efficacy of insecticide or fungicide seed treatments was the primary factor affecting plant health and yield in these experiments. There were minimal interactions between the use of pre-emergence herbicides and the performance of at-planting treatments for thrips and seedling disease. Thus, the negative effects of thrips or seedling disease and injury caused by pre-emergence herbicides acted independently and additively. The herbicides used in these experiments did not affect substantially the efficacy of insecticide or fungicide treatments.

Data indicated that Temik provided a greater reduction in thrips numbers and injury compared with Gaucho or Cruiser neonicotinoid seed treatments. This is consistent with most previous research (Burris et al., 1989). Temik has been the standard preventative thrips treatment in years past, but is no longer commercially available (EPA, 2010). AgLogic 15G (AgLogic Chemical LLC, Chapel Hill, NC), an alternative aldicarb product, is now available in some states for use on cotton. However, most growers in the Mid-South have transitioned to insecticide seed treatments because of convenience, generally satisfactory protection against thrips, and safety considerations. In our trials, Gaucho and Cruiser reduced thrips numbers compared with seed not treated with an insecticide. The relative performance of these thrips treatments was reflected in other measures of plant health such as vigor, plant biomass, maturity, and yield. Cruiser did not provide commercially acceptable protection against thrips in this study. Plots not treated with insecticide and those treated with Cruiser had generally less vigor, delayed maturity, and lower yields.

Beneficial effects of fungicide seed treatment were observed in all three fungicide trials, and these data indicated at least some fungicide seed treatments increased vigor and biomass of seedling plants. Generally speaking, Trilex Advanced and Dynasty CST treatments provided a similar level of protection against seedling disease, primarily *Rhizoctonia solani*. In the 2014 early planting, when a high incidence of seedling disease was observed, Dynasty CST provided better protection than Trilex Advanced. The benefits of fungicide seed treatments were much more pronounced in the 2014 early planting. Improved performance was observed in stand counts, vigor ratings, bloom counts, and yield. Indeed, the late planting was made over concerns that the first planting would fail to establish. The variable response of fungicide treatments across years was likely due to environmental conditions that occurred after planting. Cold and wet conditions occurred after planting the 2014 early planting, which resulted in poor conditions for seedling emergence. Our data strongly indicate that fungicide seed treatments are necessary to produce a viable plant stand. An increased response to fungicide seed treatments under conditions of poor seedling emergence is consistent with most previous research (Rothrock et al., 2012).

Although there were minimal effects of herbicide treatments on plant stands, it was apparent that some herbicide treatments negatively affected plants. Cotoran plus Dual Magnum or Cotoran plus Reflex reduced plant health as evidenced by vigor ratings, plant biomass measurements, and bloom counts. The treatment containing Reflex caused chlorosis of seedling leaf tissue in the 2013 fungicide trial, but this was not apparent in the other trials. Copeland et al. (2017) reported that cotton treated with Dual Magnum plus Cotoran yielded less than cotton not treated with a pre-emergence herbicide. Here, there were no significant effects attributed to pre-emergence herbicides on yield.

In the 2014 late-planted fungicide trial, less thrips injury was observed in plots treated with Dynasty CST. This probably resulted from these plants growing more vigorously, and thus, being less susceptible to thrips injury. Across both insecticide trials, there was a tendency for higher thrips populations where pre-emergence herbicides were used, but it is unclear why this occurred. Increased thrips injury occurred in plots treated with Cotoran plus Dual Magnum or Cotoran plus Reflex. One explanation is that thrips injury ratings were confounded with injury caused by herbicides. The use of pre-emergence herbicides is needed in Tennessee. Using pre-emergence herbicides at recommended rates and according to label restrictions should help minimize the compounding of injury caused by thrips or seedling disease with herbicide injury. Leaf assays suggested that neonicotinoid concentrations were higher in seedling plants where pre-emergence herbicides were used, and a similar but nonsignificant trend was also observed in a similar greenhouse study (Vineyard, 2015). This might be because the herbicides slowed growth and reduced plant biomass, and thus, insecticide concentrations were less diluted within the plants. Regardless, it does not support that pre-emergence herbicides were somehow impeding the uptake of insecticides.

Decreased performance of neonicotinoid seed treatments is a serious concern. Vineyard and Stewart (2017) eliminated microbial degradation of neonicotinoid insecticides in the soil as a likely cause of their diminished efficacy on thrips, and subsequent research shows that tobacco thrips have developed resistance to neonicotinoid insecticides in much of the Mid-South and Southeast (Darnell et al., 2015, 2016; Huseth et al., 2016). The data strongly suggest that insecticide resistance explains the poor efficacy of the Cruiser seed treatments in our trials. Currently, there are no labeled alternative treatments for thrips control in Tennessee that provide the level of protection previously observed with neonicotinoid seed treatments. Additional work is urgently needed to identify and demonstrate efficacious thrips suppression practices.

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