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ARTHROPOD MANAGEMENT AND APPLIED ECOLOGY

Evaluation of Seed Treatment, Herbicide, and Nematicide on Tobacco Thrips (Thysanoptera: Thripdae) and Reniform Nematode (Tylenchida: Hoplolaimidae) Control

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ABSTRACT

Numerous pests infest cotton early in the season. Some economically important are Palmer amaranth, *Amaranthus palmeri* (S. Wats); tobacco thrips, *Frankliniella fusca* (Hinds); and reniform nematode, *Rotylenchulus reniformis* (Linford and Oliveira). Thrips and weed management are essential to prevent delayed maturity and reduced crop yield. A field study was conducted during 2015 and 2016 to evaluate the influence of insecticide seed treatment, herbicide, and nematicide on tobacco thrips and reniform nematode control, as well as the impact on cotton growth, development, and yield. Treatments consisted of insecticide seed treatment (insecticide seed treatment and fungicide only), herbicide application (*S*-metolachlor, glufosinate, *S*-metolachlor plus glufosinate, and no herbicide), and nematicide application (1, 3-dichloropropene and no nematicide). There were no significant interactions between insecticide seed treatment, herbicide, and nematicide for any parameter. Nor were there any interactions in respect to nematode densities, thrips densities, thrips injury, herbicide injury, or plant biomass. Nematode densities were reduced with the use of 1,3-dichloropropene when sampled at first square and post-harvest. Thrips densities and injury were reduced at the 1- to 2-leaf stage sample timing with an insecticide seed treatment, but not at the 3- to 4-leaf stage sample timing. Herbicide injury was the greatest following *S*-metolachlor plus glufosinate applications (< 12%). A significant interaction between nematicide and insecticide seed treatment was observed for cotton yield, where the use of 1,3-dichloropropene and the insecticide seed treatment resulted in greater yields than all other treatments.

Cotton, *Gossypium hirsutum* L., producers face a number of early season pests that can impact crop growth and limit yields. These potentially compounding stress factors include tobacco thrips, *Frankliniella fusca* (Hinds), glyphosate-resistant (GR) weeds, and reniform nematode, *Rotylenchulus reniformis* (Linford and Oliveira). As the prevalence of GR weeds, predominantly Palmer amaranth, *Amaranthus palmeri* (S. Wats.), increases across the midsouthern region of the U.S. (Arkansas, Mississippi, Louisiana, Tennessee, and Missouri), there is an increased need for preemergent herbicides and early-postemergence herbicide applications during the thrips management window to minimize weed competition (Norsworthy et al., 2016; Steckel et al., 2012). Many of these herbicides have the potential to cause cotton injury and slow seedling development, which can intensify injury associated with other early season stresses including thrips and nematodes (Steckel et al., 2012; Stewart et al., 2013b).

Glufosinate is an important tool used in controlling GR Palmer amaranth; however, co-applications with residual herbicides often are required to provide effective control (Steckel et al., 1997). Co-application of glufosinate and *S*-metolachlor has not been observed to increase crop injury on GR cotton varieties and provides effective weed control (Culpepper et al., 2007, 2009; Everman et al., 2009; Whitaker et al., 2008). However, glufosinate applications to WideStrike® (Corteva Agriscience, Indianapolis, IN) cotton varieties can result in 15 to 25% crop injury with no yield reduction (Barnett et al., 2011; Culpepper et al., 2009; Dodds et al., 2011). WideStrike cotton varieties have conferred tolerance to glufos-

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inate similar to that of LibertyLink varieties; but this tolerance is incomplete (OECD 2002; Tan et al., 2006). Many cotton producers utilize combinations of glufosinate and residual herbicides despite the injury potential within their weed control programs to better manage troublesome weeds.

Tobacco thrips is one of the primary pests of seedling cotton annually throughout the midsouthern U.S. and can cause estimated yield losses between 10 to 304 kg ha^{-1} (Cook et al., 2003; Layton and Reed, 1996; North, 2016; Reed and Jackson, 2002; Stewart et al., 2013a). Thrips feeding on developing leaves and meristematic tissue of cotton seedlings can result in leaf malformation, poor growth and vigor, and/or loss of apical dominance (Cook et al., 2011; Stewart et al., 2013a; Watts, 1937). Insecticide seed treatments or at-planting in-furrow insecticide treatments are used to prevent thrips injury and reduce the associated yield losses (Stewart et al., 2013a).

Although limited information is known about the interactions of nematodes in a system stressed from thrips and herbicide injury, the reniform nematode interaction results in restricted root development thereby limiting the plant's ability to effectively uptake water and nutrients (Koenning et al., 2004). When reniform nematodes are present cumulative stresses increase. The common above-ground symptomology in reniform nematode-infested fields includes stunted growth, interveinal chlorosis, and non-uniform plant stand (Lawrence and McLean, 2001; Monfort, 2005). Crop rotation and use of nematicides (including soil fumigants, nematicide seed treatments, or in-furrow at-planting pesticides that have both insecticidal and nematicidal activity) are common control options for nematode management (Robinson et al., 2008; Westphal and Smart, 2003). Nematicide seed treatments are the most widely used treatment for nematode control; however, such practices generally require supplemental foliar thrips application (Burris et al., 2010). The use of soil fumigants is an effective method for suppressing nematodes, but these products are generally expensive and require specialized application equipment. Substantial yield increases are necessary for this practice to be cost effective. The average estimated yield losses associated with the reniform nematode are generally between 7 and 8% (Birchfield and Jones, 1961; Blasingame et al., 2006, 2009; Davis et al., 2003).

Early season stress factors including tobacco thrips, reniform nematode, and herbicide injury all have the potential to cause chlorosis, reduced plant growth and vigor, delayed plant maturity, or reduced crop yield (Davidson et al., 1979; Gasaway Rush, and Edisten, 1992; Leonard et al., 1999; Monfort, 2005). Little is known about the impacts of multiple stresses, such as tobacco thrips, reniform nematode, and herbicide injury on cotton development and yield versus the individual stresses alone. To better understand the compounding stress of multiple early season factors, studies were conducted to evaluate the influence of insecticide seed treatment, herbicide application, and nematicide use on early season pest management, as well as cotton growth, development, and yield.

MATERIALS AND METHODS

A field experiment was conducted at the R.R. Foil Plant Science Research Center in Starkville, MS in 2015 and 2016, with two additional locations in 2016 in Hamilton, MS to evaluate the influence of herbicide injury on tobacco thrips and reniform nematode stressed verses non-stressed cotton. This experiment was conducted as a three-way factorial treatment structure that included nematicide treatment, herbicide treatment, and at-planting insecticide treatment. The experimental design was a split plot with nematicide at the main plot level and herbicide treatment and at-planting insecticide treatment randomized at the subplot level. Nematicide treatment consisted of two levels of a nematicide: 1, 3-dichloropropene (Telone II, Corteva Agriscience, Indianapolis, IN) at 28 L ha^{-1} using a four-row coulter injection system and no nematicide. Subplot factor A consisted of four levels of herbicide treatment: glufosinate (Liberty 280L, BASF, Florham Park, NJ) at 595 g ai ha^{-1}, *S*-metolachlor (Dual Magnum, Syngenta Crop Protection, Greensboro, NC) at 1068 g ai ha^{-1}, glufosinate at 595 g ai ha^{-1} plus *S*-metolachlor at 1068 g ai ha^{-1}, and an untreated control. Subplot factor B consisted of two levels of at-planting insecticide in the form of a seed treatment: imidacloprid (Gaucho 600, Bayer CropSciences, Research Triangle Park, NC) at 0.375 mg ai seed^{-1} and fungicide only. All seeds were treated with a base fungicide (ipconazole at 0.01 mg ai seed^{-1} + metalaxyl at 0.002 mg ai seed^{-1} + myclobutanil 0.06 mg ai seed^{-1} + penflufen at 0.02 mg ai seed^{-1}) to minimize the effects of seedling disease.

Individual plots consisted of four 3.7-m rows measuring 12.2 m (Starkville, MS location) or 15.2 m (Hamilton, MS location) in length. On 01 May 2015 and 12 and 22 Apr 2016, 1, 3- dichloropropene was applied to the designated plots using a four-row injection coulter system. Phytogen 499 WRF (Corteva Agriscience) cotton was planted at a depth of approximately 2 cm at a population of 135,850 seeds ha^{-1} on 12 May 2015 and Phytogen 333 WRF (Corteva Agriscience) on 09 and 10 May 2016. Herbicide applications were made to cotton between the 2- and 3-leaf stage using a tractor-mounted sprayer calibrated to deliver 93.5 L ha^{-1} using TX-6 hollow cone nozzle at 276 kPa.

Nematode samples were collected prior to the nematicide application, at first square, and post-harvest. Nematode populations were determined by collecting 10, 20-cm deep soil cores from the center two rows of each individual plot using a 2.5-cm diameter soil sampling probe. Cores were combined, and a subsample of 300 cm^3 was processed by the Mississippi State University Extension Plant Diagnostic Laboratory in Starkville, MS using a semi-automatic elutriator and sucrose extraction (Byrd et al., 1976; Jenkins, 1964).

Thrips injury ratings and thrips densities were evaluated at the 1-2 and 3-4 leaf stage of cotton growth. Injury ratings were recorded on a scale of 0 to 5 following the methods of North et al. (2019). Thrips densities were estimated by randomly cutting five plants from each plot at ground level and placing them into a 0.47-L glass jar with a 50% ethanol solution. Plants were rinsed with a 50% ethanol solution and the remaining solution was poured through a Buchner funnel. Thrips adults and nymphs were collected on filter paper and that paper was placed into a Petri dish for counting under a microscope. Dark-colored thrips adults were considered to be tobacco thrips based on the observations of Stewart et al. (2013a), where 98% of thrips species in Mississippi were determined to be tobacco thrips. Immature thrips were not identified to species and pooled.

Plant vigor was assessed at 1-2 and 3-4 leaf stages on a scale of 1 to 10 where 1 indicated that less than 10% of the plants within a plot had seedling establishment, 10 represented uniform emergence and plant growth, and 2 through 9 signified 10% increments in the varying levels of plant growth and development (North et al., 2019). Herbicide injury was evaluated 7 d after application (DAA) using a percentage scale of 0% (no injury) to 100% (plant death) based on visual estimates comparing the treated to the nontreated control. Total above- and below-ground biomass samples were evaluated by uprooting five random plants from the outer two rows at the 4-leaf stage. Above- and below-ground portions of the five uprooted plants were placed into paper bags and dried in a forced air dryer for 48 h at 38 °C. After drying, samples were weighed to estimate dry biomass. Cotton yield was estimated by harvesting the center two rows of each plot with a modified spindle-type cotton picker for small plot research.

Data were analyzed using analysis of variance (PROC GLIMMIX, SAS 9.4; SAS Institute; Cary, NC). Year and replication were considered random effects, and herbicide treatment, at-planting insecticide treatment and nematicide treatment were considered to be fixed effects. Means were separated using Fisher's Protected LSD procedure at the 0.05 level of significance.

RESULTS

Reniform Nematode Control. Prior to any treatment implementation, the number of nematodes ranged from 675 to 1300 per 500 cm^3 of soil. There were no significant interactions ($F > 0.51$; df = 3, 211; $p = 0.10$) among any factors, nor were there any significant main effects of herbicide ($F > 1.60$; df = 3, 209; $p > 0.19$) or at-planting insecticide treatment ($F > 7.22$; df = 1, 211; $p > 0.26$) at first square or post-harvest. However, significantly fewer nematodes per pint of soil were observed following application of 1, 3-dichloropropene treatments at first square ($F = 13.11$; df =1, 17; $p < 0.01$) and post-harvest ($F = 17.8$; df = 1, 18; $p < 0.01$ (Table 1).

Tobacco Thrips Densities and Injury. There were no significant interactions among factors with respect to tobacco thrips populations ($F > 0.47$; df = 3, 226; $p > 0.09$) or injury ($F > 1.28$; df = 3, 226; $p = 0.28$), nor were there any significant main effect treatments of herbicide ($F = 0.87$; df = 3, 210; $p = 0.87$), at-planting insecticide treatment ($F > 1.49$; df = 1, 212; $p = 0.22$), or nematicide ($F > 0.05$; df = 1, 17; $p = 0.82$) on immature thrips at the 1-2 or 3-4 leaf stage. Herbicide ($F > 0.54$; df = 3, 225; $p = 0.65$) and nematicide application ($F > 0.03$; df = 1, 226; $p > 0.86$) had no impact on thrips injury at either sample date. Use of at-planting treatments reduced the amount of thrips injury at the 1-2 and 3-4 leaf stage ($F > 17.6$; df = 1, 227; $p > 0.01$) (Table 2).

Table 1. Impact of nematicide application on nematode populations in cotton at first square and post-harvest in Starkville, MS and Hamilton, MS during 2015 and 2016

Treatment	Density per 500 cm^{3z}	
	1st square (±SE)	Post harvest (±SE)
No 1,3-dichloropropene	**1072a (178.6)**	**3573a (727.7)**
1,3-dichloropropene	**409b (171.6)**	**2,488b (706.9)**

[z] Means within the column that are followed by the same letter are not different according to Fisher's Protected LSD with an alpha of 0.05.

Table 2. Impact of insecticide seed treatment on thrips injury and plant vigor at the 1-2 leaf and the 3-4 leaf stage in Starkville, MS and Hamilton, MS during 2015 and 2016

Treatment	1-2 Leaf^{z}		3-4 Leaf^{z}	
	Injury (±SE)^{y}	Vigor (±SE)^{x}	Injury (±SE)^{y}	Vigor (±SE)^{x}
Insecticide Seed Treatment	**3.1b (0.05)**	**6.6a (0.25)**	**3.1a (0.06)**	**5.6a (0.13)**
Untreated Control	**3.3a (0.06)**	**6.3b (0.26)**	**3.4a (0.05)**	**5.3a (0.12)**

[z] Means within the column that are followed by the same letter are not different according to Fisher's Protected LSD with an alpha of 0.05.

[y] Injury ratings are based on a 0 (no injury) to 5 (plant death) scale.

[x] Plant vigor ratings are based on a 1 (poor, uniform stand) to 10 (excellent, uniform stand) scale.

Herbicide Injury. No significant interactions were observed among factors for herbicide injury ($F > 0.05$; df = 3,226; $p > 0.10$), nor was there a significant difference for nematicide ($F > 0.15$; df = 1,226; $p = 0.69$); however, herbicide injury was significant for the main effects of herbicide ($F = 16.50$; df = 3, 225; $p < 0.01$) and at-planting insecticide treatment ($F = 5.30$; df = 1, 228; $p = 0.02$). Applications of *S*-metolachlor plus glufosinate resulted in more injury than other herbicide treatments. Also, glufosinate alone resulted in more injury than *S*-metolachlor (Table 3). Additionally, plants in plots that received an at-planting insecticide treatment had significantly less herbicide injury compared to those in plots that did not receive an insecticide seed treatment (Table 3). By reducing early season stress from thrips, plants with increased vigor had the ability to tolerate herbicide injury better than those with additional plant stresses.

Effect on Plant Vigor, Biomass, and Cotton Yield. No significant interactions were observed between nematicide, insecticide seed treatment, and/or herbicide for plant vigor ($F > 0.32$; df = 3, 205; $p > 0.24$), biomass ($F > 0.42$; df = 3, 213; $p > 0.73$), or yield ($F > 0.81$; df = 3, 220; $p > 0.49$). At the 1-2 leaf ($F = 6.41$; df = 1, 213; $p < 0.01$) and 3-4 leaf ($F = 947$; df = 1, 205; $p < 0.01$) stages, the use of an insecticide seed treatment resulted in greater plant vigor compared to the untreated control (Table 2). There was no impact of herbicide treatment ($F > 1.54$; df = 3, 211; $p > 0.20$), at-planting insecticide treatment ($F > 2.07$; df = 1, 214; $p > 0.15$), or nematicide treatment ($F > 3.83$; df = 1, 19; $p > 0.10$) on above- or below-ground biomass at the 4-leaf stage. There was a significant interaction between nematicide treatment and insecticide seed treatment for yield where applications of 1, 3-dichlorpropene along with an at-planting insecticide treatment resulted in higher yields compared to all other treatment combinations ($F = 3.98$; df = 1, 227; $p = 0.05$) (Table 4). Further, there was a significant interaction between herbicide treatment and insecticide seed treatment ($F = 3.57$; df = 3, 202; $p = 0.01$) (Table 5). Generally, yield increases were observed in plots where early season stresses were minimized suggesting that managing stresses is important for maximizing yield.

Table 3. Impacts of herbicide application on cotton injury seven days after application in Starkville, MS and Hamilton, MS during 2015 and 2016

Treatment	Herbicide Injury 7 DAA (±SE)^{zy}
Insecticide Seed Treatment	**4.4b (0.96)**
Untreated Seed	**7.1a (0.85)**
***S*-metolachlor**	**2.1c (1.108)**
Glufosinate	**6.9b (1.109)**
***S*-metolachlor plus glufosinate**	**11.26a (1.108)**

[z] Means within the column that are followed by the same letter are not different according to Fisher's Protected LSD with an alpha of 0.05.

[y] Injury ratings of visual herbicide injury estimate on scale 0 (no injury) to 100 (plant death).

Table 4. Interaction between nematicide application and at-planting insecticide treatment on cotton yields in Starkville, MS and Hamilton, MS during 2015 and 2016

Treatment		Lint ^{zy} kg ha (±SE)
No 1,3-dichloropropene	Untreated Seed	929b (64.3)
	Insecticide Seed Treatment	898b (67.2)
1,3-dichloropropene	Untreated Seed	938b (64.7)
	Insecticide Seed Treatment	1012a (64.6)

[z] Means within the column that are followed by the same letter are not different according to Fisher's Protected LSD with an alpha of 0.05.

[y] Cotton yield was taken from the center two rows of each plot.

Table 5. Interaction between herbicide application and at-planting insecticide treatment on cotton yields in Starkville, MS and Hamilton, MS during 2015 and 2016

Treatment		Lint ^{zy} kg ha (±SE)
S-metolachlor	Untreated Seed	901ab (65.6)
Glufosinate		896b (66.1)
S-metolachlor plus glufosinate		944ab (65.6)
Untreated Control		993a (64.4)
S-metolachlor	Insecticide Seed Treatment	981a (65.8)
Glufosinate		991a (65.4)
S-metolachlor plus glufosinate		945ab (65.8)
Untreated Control		897b (75.3)

[z] Means within the column that are followed by the same letter are not different according to Fisher's Protected LSD with an alpha of 0.05.

[y] Cotton yield was taken from the center two rows of each plot.

DISCUSSION

With the continued spread of GR Palmer amaranth across the U.S. cotton belt, early season applications of glufosinate and residual herbicides are becoming common (Norsworthy et al., 2016; Steckel et al., 2012). Barnett et al. (2011) found that WikeStrike cotton varieties can withstand 15 to 25% glufosinate injury from one to two applications with no maturity delay or yield losses. However, delayed maturity and yield losses have resulted from co-applications of herbicide and insecticide applications to WideStike cotton already injured by thrips (Stewart et al., 2013b). Depending on environmental conditions, the impact of early season co-applications is likely to be variable. Although herbicide injury was minor with no adverse effects on yield, the amount of herbicide injury decreased with the use of an insecticide seed treatment.

Injury from herbicide applications, nematodes, or thrips injury alone did not seem to be limiting factors, although all have the potential to delay crop maturity and/or reduce yield. Thrips are an annual pest in Mississippi cotton production systems and with the spread of herbicide-resistant Palmer amaranth, reducing early season stress from these factors can be beneficial later in the season. Historically, yield responses to thrips management have been variable and dependent on the severity of infestation and the environmental conditions during the remainder of the growing season. Numerous studies have shown yield increases when thrips were effectively controlled in seedling cotton (Burris et al., 1989; Carter et al., 1989; Davis et al., 1966; Lentz and Van Tol, 2000; Watts, 1937).

Prior to documented resistance, thiamethoxam and imidacloprid have been shown to increase cotton yield 15 to 20% compared to untreated cotton (Reed and Jackson, 2000). Injury to seedling cotton has the potential to cause delayed crop maturity, which could result in increased exposure to late season pests that could require additional management costs. In addition to the increase in management costs, there is also the likelihood for exposure to adverse environmental conditions that can reduce lint quality and yield (Barker et al., 1976; Stewart et al., 2013a; Williford et al., 1995). The use of at-planting pesticides and preemergence herbicides can reduce the need for co-applications of herbicides and insecticides to cotton seedlings, thus reducing the potential for crop injury. Phototoxicity as a result of early season postemergence herbicide applications seemed to be decreased when stress from pests such as, thrips and/or nematodes were reduced, suggesting the importance of reducing early season plant stresses were applicable.

Additional, research is needed to better understand the relationship between early season herbicide applications on cotton injured by thrips and/or nematodes.

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