Article

Evaluation of Cotton Cultivar and At-Plant Nematicide Application on Seasonal Populations of Reniform Nematode

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Abstract: The reniform nematode, *Rotylenchulus reniformis* (Linford and Oliveira), remains a common, widespread nematode pest of cotton across the southern United States. Trials were conducted during 2017 at three non-irrigated locations: one location in Hamilton, MS, and two locations in Tchula, MS, in field settings with a history of cotton production and documented economically-damaging reniform nematode populations. Trials were designed to evaluate the response of two cotton cultivars to in-furrow nematicides consisting of aldicarb, 1,3-dichloropropene, and a non-treated control applied for nematode suppression. No significant interactions between cotton cultivar and nematicide were observed. However, treatment with 1,3-dichloropropene produced greater plant biomass, and plant height compared to aldicarb-treated cotton and the nontreated. Nematode densities were suppressed with the use of 1,3-dichloropropene compared to aldicarb and the non-treated control. The use of 1,3-dichloropropene resulted in positive early-season plant growth responses; however, these responses did not translate into greater yield.

Keywords: cotton; nematodes; at-planting nematicide; plant system interactions

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1. Introduction

The reniform nematode, *Rotylenchulus reniformis* (Linford and Oliveira), is a primary semi-endoparasitic nematode pest of cotton (*Gossypium hirsutum* L.) in the southern United States [1]. Estimated yield losses associated with this plant parasitic nematode pest average between 7 and 8% annually; however, the potential for yield reductions of 40 to 60% under adverse environmental conditions exists [2–6]. In 2018, Mississippi averaged an approximate yield loss of 32,000 bales as a result of the reniform nematode [6]. Populations of the reniform nematode generally occur in a 1:1 ratio of males to females, yet only the females are parasitic [7]. The vermiform female penetrates the cortex of the root to establish a feeding site within the stele. As the female feeds, her reproductive system matures resulting in the familiar kidney-shaped body form characteristic of the reniform nematode. The generally warm climate in the southern U.S. has a great impact on the life cycle of the reniform nematode by providing optimal soil conditions for population densities to rapidly increase and the production of multiple generations in a single season in the presence of a suitable host [7]. Unlike other nematode species, the reniform nematode tends to be more uniformly distributed across fields that have established infestations with more irregular distributions in fields having become recently infested [8]. The symptomatology on reniform nematode-affected cotton plants includes reduced plant vigor which may manifest as irregular plant growth, generalized wilting, interveinal chlorosis, delayed plant maturity, and even reduced yield depending on the plant parasitic nematode population

density [9]. However, there are no measures of nematode yield losses associated with specific plant parasitic nematode population numbers and, in general, economic thresholds to guide management alternatives are based on estimated populations rather than a hard and fast rule. In addition to above-ground symptoms that can observationally appear as nutrient deficiencies, nematode damage can stunt or reduce the root system, which slows root development, and limits water and nutrient uptake. Reniform nematode infestations have also been reported to increase the susceptibility of cotton to additional seedling disease-causing organisms [10]. However, the greatest impact of the reniform nematode in cotton production systems is reduced yield [11,12].

Soil fumigants or aldicarb (AgLogic 15G or AgLogic 15GG, AgLogic Chemical LLC, Chapel Hill, NC, USA), an in-furrow insecticide with nematicidal properties, are common chemical control options for nematode management [8,13]. The use of both aldicarb and 1,3-dichloropropene (Telone II, Dow AgroSciences, Indianapolis, IN, USA) can reduce nematode populations and preserve cotton yield potential [14–16]. The main goal of using nematicides for nematode management is protection of seedling plants and promotion of a rapidly growing and healthy root system especially immediately following planting for increased tolerance to additional environmental stresses [8]. Additional alternatives for plant parasitic nematode management include crop rotation, cover crops, soil amendments, as well as seed-applied nematicides that have been more widely used in cotton production systems, but are generally reported to not be nearly as effective as soil fumigants [5,7,17,18]. While crop rotation is a beneficial strategy for nematode management, the reniform nematode has the ability to enter into an anhydrobiotic state for up to two years allowing prolonged survival in soils left fallow or planted to a non-host crop [7]. In addition, depending on the host planted, crop rotation to some hosts may result in increased reniform nematode populations (e.g., soybean (*Glycine max* (L.) Merr.)) [5]. Host plant resistance genes to the reniform nematode have been identified in cotton, but incorporation into commercial cultivars has been mostly unsuccessful. As a result, at the time of the publication of this article, and for the most part there have been no commercially available cotton cultivars with resistance to the reniform nematode [5,11,19,20]. With a general lack of commercially available resistance to the reniform nematode, studies were conducted to evaluate the response of two commonly used cotton cultivars grown in soils infested with the reniform nematode using aldicarb or 1,3-dichloropropene. A similar study was conducted by Wilson et al. (2020) focusing on the profitability of nematicides to determine the best management practices in Mississippi cotton production systems [16]. While these studies are similar in nature, the objective of this current study was to determine the value of aldicarb compared to 1,3-dichloropropene since it is commonly used for both thrips control and nematode management in many areas of Mississippi.

2. Materials and Methods

Field experiments were conducted in Hamilton, MS, and two locations in Tchula (Oswego and Shuttleworth), MS, but separated by 11.26 km, during 2017 to evaluate reniform nematode control using aldicarb and 1,3-dichloropropene on two commonly planted cotton cultivars. The field studies were implemented as a randomized complete block design with a factorial arrangement of treatments and four replications. All three fields have a history of cotton production and a non-irrigated cotton production system.

Factor A consisted of three levels of nematicide: aldicarb (as 340.5 g a.i. ha⁻¹ AgLogic 15G, AgLogic, LLC, Chapel Hill, NC, USA) [21]; 1,3-dichloropropene (as 28 L ha⁻¹ Telone II, Dow AgroSciences, Indianapolis, IN, USA) [21] injected into the soil pre-plant; and a non-treated control. Factor B consisted of two levels of cotton cultivar: Stoneville 4949 and Stoneville 4946 (Bayer CropScience, Research Triangle Park, NC, USA). Previous anecdotal observations by some researchers have suggested that Stoneville 4946 contains some inherent tolerance to the reniform nematode, while Stoneville 4949 appears to be susceptible. All seed 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) and imidacloprid (as 0.375 mg ai seed⁻¹ of Gaucho 600, Bayer Crop-Science) applied at a rate to minimize the effects of seedling diseases and thrips. Individual plots consisted of four 3.6-m rows measuring 12.2-m in length of cotton. Plots were separated by a 3 m fallow alley. Nematicide applications using 1,3-dichloropropene were applied using a four-row applicator (Mirusso Enterprises Inc., Delray Beach, FL, USA) with deep-placement coulters (Yetter Manufacturing, Colchester, IL, USA) placing the liquid at a depth of 31 cm on 13 April 2017 (Hamilton) and 17 April 2017 (Oswego and Shuttleworth). Aldicarb applications were applied in-furrow by a tractor-mounted granular insecticide box at planting. Cotton was planted at a depth of approximately 2 cm at a population of 135,850 seed ha⁻¹ on 4 May 2017, 11 May 2017, and 19 May 2017, at Hamilton, Oswego, and Shuttleworth, respectively. Standard cotton production practices were followed according to Mississippi State University Extension Service suggestions.

Nematode samples were collected every two weeks starting prior to the nematicide application until first cracked boll. Nematode populations were determined by collecting 10, 20-cm deep soil cores from each individual plot using a 2.5 cm diameter soil-sampling probe. Cores were combined, gently mixed, transported in a cooler, and a sub-sample of 300 cm³ was processed by the Mississippi State University Extension Plant Diagnostic Laboratory in Starkville, MS, using a semi-automatic elutriator and sucrose extraction procedure [22,23].

Plant height and total node counts were taken at first square, first bloom, and harvest on 10 randomly selected plants per plot. Plant height was determined by measuring from the ground to the apical meristem. Nodes above white flower (NAWF) were determined by counting the nodes above the uppermost first position white flower when the majority of plants were flowering [24]. Nodes above cracked boll (NACB) were determined by counting the nodes between the uppermost first position cracked boll and the uppermost first position harvestable boll prior to defoliation. Above- and below-ground biomass samples were evaluated by uprooting five random plants from the outer two rows within each plot at the four-leaf stage. Plants were uprooted using a shovel by digging 8 to 12 cm from the mainstem in a V-shaped pattern to avoid root breakage and gently dislodging soil before bagging roots. Above- and below-ground portions were separated in the field at the soil line. 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 determine dry biomass. Cotton yield was determined by harvesting the center two rows of each plot with a spindle-type cotton picker modified for small plot research.

Data for growth parameters and yield were analyzed using analysis of variance (PROC GLIMMIX, SAS 9.4; SAS Institute; Cary, NC, USA). Location and replications were considered to be random effects, while nematicide and cotton cultivar were considered to be fixed effects. Means were separated using Fisher's Protected LSD procedure ($\alpha = 0.05$). Data for nematode populations were analyzed in the R statistical programming environment (R Core Team, 2018). To account for the non-linearity in nematode numbers, linear, quadratic, and polynomial models were fit for each location/treatment scenario. Time was used as the predictor variable for all models. The three models for each scenario were compared and the best fit was selected based on Akaike Information Criterion (AIC). Inferences from the selected models were based on likelihood ratio tests comparing models with and without the specific predictor variables (Table S1).

Weather data were collected from a weather station nearest to each field location based on weather data captured by Mississippi State University. To capture weather data, values were downloaded from the weather data repository maintained by Mississippi State University (<http://deltaweather.extension.msstate.edu/stations> (accessed on 8 October 2020)). In addition, the 30-year norms (1981–2010) were downloaded from the National Oceanic and Atmospheric Administration National Centers for Environmental information website (www.ncdc.noaa.gov (accessed on 8 October 2020)) from locations that were close to the MSU weather locations to make comparisons between the environment that occurred during 2017 and the calculated 30-year norms. Averages of the minimum and maximum

temperature for each month (April to November), and rainfall totals across the same time period for each location were calculated and compared to the 30-year norms.

3. Results

3.1. Seasonal Populations of *Reniform* Nematode

There were no significant differences among cultivars for nematode densities on any sample date at any location ($F > 0.07$; $df = 1, 282$; and $p > 0.45$), so cultivars were pooled. At the Shuttleworth location, nematode populations were similar among all treatments during the first sampling. A polynomial model was a better fit for the non-treated ($p = 0.03$), while the 1,3-dichloropropene ($p < 0.0001$) and aldicarb ($p = 0.02$) treatments were best explained by quadratic models (Figure 1A). Time improved the models fit for the non-treated and aldicarb treatments, but not the 1,3-dichloropropene treatment (Figure 1A). The nematode populations for both the aldicarb and non-treated remained stable until the sixth sample date where they began to increase from approximately 1200 nematodes per 300 cm³ to 2000 (non-treated) and 2700 (aldicarb) nematodes per 300 cm³. For the 1,3-dichloropropene, nematode populations declined from the first sample date until the seventh sample where populations then began to increase. Nematode abundances did not significantly change over time for the plots receiving 1,3-dichloropropene treatments (Figure 1A).

Figure 1. Reniform nematode (*Rotylenchulus reniformis*) abundances over time from planting (0) to harvest (12) for Shuttleworth, Tchula, MS, (A), Hamilton, MS, (B), and Oswego, Tchula, MS, and (C) containing in-furrow nematicide applications conducted during 2017.

At the first sampling, all treatments at the Hamilton location had similar populations (Figure 1B). A polynomial model was a better fit for the non-treated ($p < 0.001$) and aldicarb treatments ($p = 0.01$) (Figure 1B). Time improved the fit of the models for the non-treated and aldicarb treatments, but not the 1,3-dichloropropene treatment ($p = 0.46$) (Figure 1B). The non-treated and aldicarb followed similar trends throughout the season. Nematode abundances did not significantly change over time for 1,3-dichloropropene treatments and the resulting reniform nematode populations decreased over the course of the season (Figure 1B). Populations decreased from approximately 4400 nematodes per 300 cm³ at the first sample to 1900 at harvest.

Nematode populations at the Oswego location were similar among all treatments during the first sampling (Figure 1C). A polynomial model was a better fit for the non-treated ($p = 0.005$) and 1,3-dichloropropene treatments ($p = 0.01$), while aldicarb ($p = 0.008$) was best

explained by a quadratic model (Figure 1C). Time improved the models fit for the non-treated and 1,3-dichloropropene treatments (Figure 1C). The aldicarb and non-treated had similar nematode populations across all sample dates. Both the aldicarb and non-treated began to decrease slightly until the sixth sample date where populations then began to increase. 1,3-dichloropropene followed a similar trend except the decrease in population was much greater up until the sixth sample where it then began to increase more dramatically than the aldicarb and non-treated. These results show that in Oswego, reniform nematode abundances were minimal early in the season, but reached maximum concentrations at later time points for the non-treated and aldicarb treatments. Nematode abundances did not significantly change over time as a result of aldicarb treatment (Figure 1C).

3.2. Cultivar and In-Furrow Nematicide Response on Agronomic Plant Characteristics

No significant interactions between cultivar and nematicide were observed for plant height or number of nodes at first square, first bloom, or harvest, nor were there any interactions for NAWF, NACB, above-ground biomass, or below-ground biomass (Table 1). At first square, Stoneville 4946 was 6.5% taller than 4949 ($p = 0.0411$) and 6.2% greater number of nodes than Stoneville 4949 ($p = 0.017$; Table 1). At first bloom, there were no significant differences between cultivars for plant height ($p = 0.2711$) or NAWF ($p = 0.2320$; Table 1). Also, there were no significant differences in plant height ($p = 0.8$) or NACB between cultivars at maturity ($p = 0.622$; Table 1). 1,3-dichloropropene resulted in a 17.4% and 16.1% increase in plant height at first square ($p = 0.0001$) and a 14.7% and 13.5% increase at first bloom, compared to the non-treated and aldicarb-treated cotton, respectively ($p = 0.0001$; Table 1). Also, 1,3-dichloropropene resulted in 7.3% more main stem nodes at first square compared to non-treated and aldicarb-treated cotton ($p = 0.016$). Significant differences were not observed for NACB or plant height at harvest (Table 1).

Table 1. Impacts of cotton cultivar and in-furrow nematicide treatment on plant growth at first square, first bloom, and harvest in Tchula (Shuttleworth and Oswego), MS, and Hamilton, MS, during 2017.

Treatment	1st Square [†]		1st Bloom [†]			Harvest [†]	
	Height (cm) (±SE)	# Nodes (±SE)	Height (cm) (±SE)	# Nodes (±SE)	# NAWF (±SE) [‡]	Height (cm) (±SE)	# NACB (±SE) ^{††}
Stoneville 4946	27.5 a (0.63)	8.1 a (0.11)	79.3 (1.06)	14.9 a (0.12)	6.9 (0.15)	106.5 (3.08)	6.5 (0.41)
Stoneville 4949	25.7 b (0.61)	7.6 b (0.12)	77.6 (1.04)	14.2 b (0.11)	7.2 (0.17)	107.6 (3.01)	6.9 (0.47)
<i>p</i> -value	0.0411	0.0170	0.2711	0.0009	0.2320	0.8000	0.6220
Non-treated	24.7 b (0.71)	7.8 ab (0.14)	73.9 b (1.21)	14.3 b (0.15)	6.8 (0.20)	102.9 (3.50)	6.6 (0.52)
1,3-dichloropropene	29.9 a (0.81)	8.2 a (0.16)	86.6 a (1.38)	15.1 a (0.16)	7.1 (0.19)	112.8 (3.98)	6.5 (0.60)
Aldicarb	25.1 b (0.75)	7.6 b (0.13)	74.9 b (1.28)	14.2 b (0.13)	7.1 (0.19)	105.5 (3.69)	6.9 (0.51)
<i>p</i> -value	0.0001	0.016	0.0001	0.0030	0.4560	0.1720	0.8880

[†] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD ($\alpha = 0.05$).

[‡] NAWF = nodes above white flower. ^{††} NACB = Nodes above cracked boll. # Number.

3.3. Cultivar and In-Furrow Nematicide Response on Plant Biomass and Yield

Stoneville 4946 produced 14.9% more above-ground biomass ($p = 0.017$) and 16.1% below-ground biomass ($p = 0.038$) when compared with Stoneville 4949 (Table 2). In terms of yield, Stoneville 4946 produced 6% more yield than Stoneville 4949 ($p = 0.031$). Treatment with 1,3-dichloropropene resulted in 33.5% and 25.8% more above-ground biomass than the non-treated and the aldicarb-treated cotton ($p = 0.002$), respectively (Table 2). The response of the below-ground biomass was similar with 1,3-dichloropropene producing 25% and 37.5% more cotton than the non-treated and aldicarb-treated cotton, respectively ($p = 0.022$). The 1,3-dichloropropene treatment resulted in 3% and 8.5% greater cotton yield compared to the non-treated and the aldicarb-treated cotton, respectively ($p = 0.05$; Table 2).

Table 2. Impact of cotton cultivar and in-furrow nematicide on above- and below-ground dry plant biomass at the fourth leaf stage and cotton yield in Tchula (Shuttleworth and Oswego), MS, and Hamilton, MS, during 2017.

Treatment	Biomass (g) ^{†,‡}		Yield (kg ha ^{−1}) ^{††}
	Above (±SE)	Below (±SE)	Lint (±SE)
Stoneville 4946	19.5 a (0.85)	3.1 a (0.14)	1356 a (31.3)
Stoneville 4949	16.6 b (0.82)	2.6 b (0.16)	1274 b (30.0)
<i>p</i> -value	0.017	0.038	0.031
Non-treated control	15.5 b (0.95)	2.4 b (0.16)	1326 ab (33.3)
1,3-dichloropropene	23.3 a (0.85)	3.2 a (0.16)	1367 a (41.3)
Aldicarb	17.3 b (1.03)	2.0 ab (0.21)	1251 b (38.3)
<i>p</i> -value	0.002	0.022	0.050

[†] Means within the column that are followed by the same letter are not different according to Fisher's Protected LSD (alpha = 0.05). [‡] Dried plant biomass at the fourth leaf stage. ^{††} Cotton yield was taken from the center two rows of each plot.

4. Discussion

At present, a single commercially available cotton cultivar with confirmed resistance to the reniform nematode is available, therefore a cultivar response to nematode infestation stress would be unlikely. Differences in plant performance are more likely due to cultivar responses to various biotic and abiotic factors throughout the season. The early season biomass and plant growth response to in-furrow nematicide application did not translate into increased cotton yield. Previous research evaluating cultivar performance with the presence of reniform nematode populations found differential yield responses when infestations were severe [25]. One major advantage of aldicarb, the most commonly used nematicide in the history of cotton production, is the additional control of important insect pests, including tobacco thrips, *Frankliniella fusca* (Hinds) [10,26]. Numerous studies have demonstrated effective plant parasitic nematode suppression and increased yield with 1,3-dichloropropene and aldicarb, with soil fumigants generally being more effective than other chemical nematicide options [16,27,28]. Therefore, it is important to weigh the cost of nematicide usage to cotton yield responses in Mississippi. Yield losses associated with reniform nematode in the U.S. are estimated to be <10% on average annually; however, with additional stresses, such as water stress, yield losses >50% have been observed [8]. Generally, nematicide applications are warranted when yield losses are expected to be >5%; however, it is not possible to accurately predict the environmental conditions for the remainder of the growing season or the amount of yield losses that may occur as a result [29]. To determine the potential benefit of nematicide usage in Mississippi cotton production systems, a knowledge of production history and the ability to minimize additional environmental stresses, such as water stress, would be needed. While 1,3-dichloropropene did suppress reniform nematode densities early in the season, and resulted in an early-season plant response, yield responses were not great enough to warrant the cost, generally considered to be estimated at \$130 per ha [16]. Other studies support the use of 1,3-dichloropropene for root protection, nematode suppression, and position yield responses; however, the economic analysis conducted by Wilson et al. (2020) suggested that the use of 1,3-dichloropropene did not provide an economic advantage compared to other nematicide options [16,30,31]. Late-season environmental conditions during the current study were favorable for plant compensation, and the amount of stress throughout the season was relatively low resulting in yield that was comparable (Table 3). If greater environmental stresses had been observed, yield responses great enough to warrant nematicide use may have been observed. Additional research is needed to better understand the relationship between reniform nematode infestation, environmental conditions, and possibly consider the role of soil properties to allow for development of the best management practices for reniform nematode in Mississippi cotton production systems.

Table 3. Environmental variables, minimum and maximum temperature (°C), total precipitation (mm), and the deviation from the 30-year normal (values in parentheses) for the locations where nematicide trials were conducted in Mississippi during 2017 ^{††}.

	Minimum Air Temp (°C)		Maximum Air Temp (°C)		Precipitation (mm)	
	Hamilton, MS ‡	Tchula, MS ‡	Hamilton, MS ‡	Tchula, MS ‡	Hamilton, MS ‡	Tchula, MS ‡
April	12.8 (2.7)	14.7 (−2.5)	26.3 (1.6)	14.7 (−2.5)	101.6 (−20.1)	96.3 (−36.6)
May	14.9 (−0.3)	16.6 (−1.1)	27.5 (−1.2)	16.6 (−1.1)	152.9 (46.0)	144.3 (−16.5)
June	19.8 (0.5)	21.1 (−0.6)	29.6 (−2.5)	21.1 (−0.6)	184.9 (61.5)	149.6 (55.9)
July	22.1 (0.7)	23.1 (0.1)	33.5 (−0.3)	23.1 (0.1)	11.9 (−95.8)	75.7 (−33.3)
August	21.4 (0.5)	22.5 (0.1)	31.3 (−2.4)	22.5 (0.1)	152.7 (50.5)	170.2 (80.8)
September	17.4 (0.2)	17.8 (−0.7)	29.4 (−1.1)	17.8 (−0.7)	60.7 (−32.0)	40.9 (−39.4)
October	11.7 (1.0)	12.6 (0.0)	25.2 (0.3)	12.6 (0.0)	18.0 (−83.1)	24.9 (−90.9)
November	6.2 (0.9)	8.0 (0.4)	19.6 (0.5)	8.0 (0.4)	0.3 (−124.5)	23.1 (−96.0)

[†] Tchula, MS corresponds to the two field locations, Oswego and Shuttleworth, that were conducted a short distance from Tchula.

[‡] Weather data for the Hamilton, MS, location were downloaded from the Mississippi State University weather system website (<http://deltaweather.extension.msstate.edu/stations>) (accessed on 8 October 2020)) and correspond to the Prairie, MS, location which would be approximately 21 miles from the field location in Hamilton, MS. ^{††} 30-year normalized weather data was downloaded from the National Oceanic and Atmospheric Administration National Centers for Environmental Information website (www.ncdc.noaa.gov) (accessed on 8 October 2020)).

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11112166/s1>, Table S1. Model selection and statistical inferences for each scenario. Model selections were made based on Akaike Information Criterion (AICc). Inferences from the models are based on likelihood ratio tests comparing models with and without the specific predictor variables.

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