IMPACT OF COTTON GENETICS AND NEMATICIDES ON RENIFORM NEMATODE POPULATIONS AND YIELD

A Thesis

by

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ABSTRACT

Rotylenchulus reniformis, the reniform nematode, is an increasingly important crop pest in *Gossypium hirsutum*, cotton. Cotton yields have suffered due to the increasing prevalence of this plant-parasitic nematode throughout the United States Cotton Belt. Two field trials were conducted at Damon, College Station, Wall, and Lubbock, Texas to evaluate the efficacy of genetic resistance and nematicides against reniform nematodes in cotton. Cotton cultivars in the genetic resistance trial included *Meloidogyne incognita* (southern root-knot nematode) and reniform nematode resistant genes and a nematodesusceptible check, each with and without an application of fluopyram (199 g ha⁻¹) and prothioconazole (199 g ha⁻¹). The nematicide trial tested three different pesticide products. A granular in-furrow application of aldicarb 15G, a liquid in-furrow combination of fluopyram and prothioconazole, foliar-applied oxamyl and all applicable combinations of these treatments were applied to two cotton cultivars with differing genetic resistance.

In 2019, the reniform nematode resistant (REN) cultivar (PHY 443 W3FE) had 26% greater lint yields than all other cultivars across the Wall and College Station sites. At Damon in 2019, PHY 443 W3FE was among the highest yielding cultivars, but was similar to some of the root-knot nematode resistant (RKN) cultivars. REN cultivars PHY 443 W3FE and PHY 332 W3FE had the highest yields among all locations in 2020.

Cultivar only impacted post-harvest nematode populations in 2020. PHY 332 W3FE and DP 18R628NR B3XF (RKN) reduced reniform nematode populations by 45% compared to DP 1747NR B2XF (RKN) (P = 0.001). In the nematicide study, yield, nematode populations, and economic return, were not impacted by the nematicide treatments or cultivar at Damon in 2019 (P > 0.05). In all other site-years combined, the combination of aldicarb and oxamyl increased yield by 14% compared to the untreated check (P = 0.0048). Aldicarb application increased partial net return \$245.37 ha⁻¹ (P = 0.02) over the combination of [fluopyram and prothioconazole] and oxamyl (which was both expensive and ineffective). However, none of the nematicide treatments resulted in a net economic gain or loss compared to the untreated check. The findings of this study indicate genetic resistance is a more effective tool than nematicide applications to mitigate negative impacts of reniform nematodes in cotton.

DEDICATION

To my grandfather, Kenneth J. Koester.

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NOMENCLATURE

DAP	Days after planting
RKN	Root-knot nematode resistant
REN	Reniform nematode resistant

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CHAPTER I

INTRODUCTION

Phytoparasitic nematodes are responsible for greater cotton yield loss than any other cotton disease. The combination of nematodes and disease contributed to a 6.5% yield reduction across the Cotton Belt in 2019 with 4.28% of that loss attributed to plant parasitic nematodes. Reniform nematodes have become one of the most detrimental pests to cotton production, specifically accounting for an estimated yield loss of 42,864 Mg (0.94% of total cotton production) across the United States Cotton Belt (Lawrence et al., 2020; United States Department of Agriculture National Statistics Service, 2020) with a projected cost to United States cotton growers of over \$62 million in 2019.

Effective tools are crucial to mitigate the damage caused by nematodes. This research focuses on two management options to reduce yield loss from reniform nematode infestations: nematicides and genetic resistance. Cotton cultivars with resistance specific to reniform nematode (commercially unavailable prior to 2021) are being developed and released by multiple commercial seed companies. Nematicides have also been used widely to suppress nematode populations but restrictions have been placed on certain products and few new products have been introduced to the market. Understanding the impact of new genetic resistance and available nematicide options is critical to optimize cotton production in the presence of reniform nematodes.

CHAPTER II

LITERATURE REVIEW

Reniform Nematodes

Rotylenchulus reniformis, reniform nematodes were first described in Hawaii, described by Linford and Oliveira (1940), and are considered a subtropical species. The females' bodies swell into a kidney shape during the reproductive stage, hence the name 'reniform'. The dispersion of the reniform nematode from 2000 to 2005 is described in depth by Robinson (2008). The infestations were reported most severe in Mississippi and Louisiana spreading outward to increase yield loss in Alabama, Arkansas, and Texas (Robinson, 2008).

The ability of reniform nematodes to reproduce has been directly linked to soil texture and potential for infestation increases in finer-textured soils relative to other plant parasitic nematodes. Koenning et al. (1996) specify the Portsmouth series (a poorly-drained sandy loam) as one soil type well-suited for reniform nematode. It can be inferred that reniform nematodes thrive in finer-textured soils due to lack of competition from other species better adapted to courser-textured soils (Koenning et al., 1996). Although soil texture plays an important role in the ability of the reniform nematode to move and infect roots, they are also resilient to a wide range of soil temperatures. This contributes to the nematodes successful capacity to overwinter in southern U.S. soils in temperatures as low as 10°C (Ayala and Ramirez, 1964; Robinson and Heald, 1989).

Lifecycle

The lifecycle of this phytoparasitic nematode starts when it molts from the first juvenile stage (J1) to the second juvenile stage (J2) in the egg. Shedding of the original cuticle is facilitated by body contractions. During this time the stylet, along with other structures, begin to appear. The nematode uses its stylet to puncture through the egg wall at a rate of around 40 times per minute, then emerges its head followed by the posterior end of the body (Nakasono, 1973). During the J2 stage, the nematode remains nonparasitic (Robinson et al., 1997).

Next, the vermiform nematode goes through three molting stages, each one represents a new juvenile classification. During the first molt outside of the egg, the nematode progresses from J2 to J3, it becomes less active and the stylet and esophageal structures lose prominence. After completing this molting process, a nematode with lesser length and body width arises. The cuticle remains visible, encapsulating the nematode, giving it the appearance of a sheath (Nakasono, 1973).

The second molt after it leaves the egg mass, reveals a newly formed J4 nematode. This juvenile has a thinner, more translucent, cuticle than the J3. The stylet has not reappeared but the oral structures progressively become more distinguishable. Like the molting before, the body length and width get smaller and the cuticle remains as another layer surrounding the nematodes body (Nakasono, 1973).

During the final molt before the nematode progresses to an adult, the cuticle is sloughed off, and the stylet and esophageal structures become easily distinguishable (Nakasono, 1973). After the completion of the fourth molt (J4 to adult), the nematodes

are equally distributed by sex. The females are plant parasitic and the males do not feed (Ganji et al., 2013).

Unlike some other genera, reniform nematodes are amphimictic and the males are needed for reproduction. The females reproduce fairly quickly, around 6-14 days after mating. After copulation, the females become sedentary semi-endoparasites that release between 60 to 200 eggs into a self-produced gelatinous matrix. The entire lifecycle can be completed fairly quickly, approximately three weeks, consequently permitting a surge in populations throughout the cotton growing season (Robinson, 2008; Koenning et al., 2004). Most plant parasitic nematodes prefer moist soils but reniform nematodes are highly adapted to dry soils and can undergo anhydrobiosis (Womersley and Ching, 1988). Due to their ability to go dormant in drought conditions, this makes them extremely capable of overwintering as well as surviving in unfavorable conditions.

Host Range

The host range of reniform nematodes includes both monocots and dicots in 77 plant families with several tropical species, and including fruits such as pineapples, apples, papaya, strawberries, and bananas (Ayala and Ramirez, 1964; Robinson et al., 1997; Wubben et al., 2010). An expansive host list compiled by Robinson and colleagues (1997) includes plants grown around the United States; *Abelmoschus esculentus* (okra), *Cicer arietinum* (chickpea), *Nicotiana tobacum* (tobacco), *Sesamum indicum* (sesame), and *Solanum tuberosum* (potato). Ornamentals such as *Bengonia semperflorens* (wax begonia) and *Euphorbia pulcherrima* (poinsettia) and various common weeds found in the southern United States, including: *Abutilon theophrasti* (velvetleaf), *Amaranthus spinosus* (spiny amaranth), *Sida spinosa* (prickly sida), *Sorhum halepense* (johnsongrass), *Xanthium* sp. (cocklebur) and *Portulaca oleracea* (common purslane).

Symptoms

Female reniform nematodes, infect cotton once growth reaches the immature fifth stage (Robinson, 2007). Symptoms from these infections include stunting due to decreased growth when the plant reaches three or four nodes. The leaves of the plant also become chlorotic or light green in color and wilted, and flowering is delayed. Although the best way to determine the species of nematodes present in the soil is to submit a soil sample to a diagnostic lab, a reniform nematode infestation may be determined by digging up suspected plants and observing the roots. The egg masses are gelatinous and soil will stick to them even after being shaken or washed. Severity of these symptoms is dependent on prior stresses on the cotton, for example lack of water.

Chemical Management

Nematicides are commonly used to reduce the negative impact of nematodes on crops. Although there are numerous choices on the market, the efficacy of these products can be highly dependent on environmental factors such as soil moisture and soil temperature, making it difficult for growers to get consistent results year after year (Bromilow et al., 1980; Haydock et al., 2012; Wheeler et al., 2013; Faske and Brown, 2019).

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Aldicarb is a carbamate nematistat that disrupts the acetylcholinesterase enzyme inhibiting chemoreception that can be absorbed through the cuticle of the nematode. This causes the nematode to become disoriented at low concentrations and has the ability to hamper egg hatching and induce immotility at high concentrations (Haydock et al., 2006; Ebone et al., 2019). The chemical also rapidly oxidizes and is broken down by the addition of water (Bromilow et al., 1980). Persistence and movement down the soil profile due to rainfall or irrigation differs depending on soil type (Coppedge et al., 1977). Although aldicarb has been shown to translocate into the leaves of plants for certain insect pest control, nematode control is a result of both contact and root absorption taking place in the soil (Steele and Hodges, 1975).

Like aldicarb, oxamyl is also a carbamate with a similar mode of action (Haydock et al., 2006). It is also water soluble allowing it to leach easily. It takes one to two weeks for the initial rate of oxamyl to degrade by half, and degradation rate is inversely related to the amount of organic matter in the soil (Bromilow et al., 1980). Oxamyl is a foliar spray that is translocated from the leaf tissue to the root system of the plant. Multiple spray applications made at five day intervals reduced the number of nematodes found on root samples (Rich and Bird, 1973).

Fluopyram is a fungicide with nematistatic activity and has been shown to reduce movement in reniform nematodes by 52% (Faske and Hurd, 2015). It has low water solubility and movement is dependent on both soil type and water. Finer texture soils do not allow as much movement through the soil profile, which can result in reduced efficacy of the product (Faske and Brown, 2019). Prothioconazole belongs to Group 3 fungicides (demethylation inhibitors) and has not been studied for its activity on nematodes.

Genetic Resistance

Screening for reniform nematode resistance in *Gossypium hirsutum* (Upland cotton) began nearly 60 years ago. Screenings of accessions with possible resistance to reniform nematodes were conducted by multiple scientists. Results revealed inconsistencies in host status between studies, but when results were constant, the accessions were considered resistant to moderately resistant compared to a control (Yik and Birchfield, 1984; Robinson and Percival, 1997; Weaver et al., 2007). Host status can be classified using a scale Yik and Birchfield (1984) developed based on a percentage of egg production per gram of root. The assigned scale of resistance to moderate resistance is defined as supporting 11 to 40% of egg production compared to a susceptible cultivar (Yik and Birchfield, 1984). The combination of both variable results and the level of egg production encouraged scientists to look outside of the upland cotton germplasm for sources of resistance to introgress.

Another species, *G. longicalyx*, was classified as immune to reniform nematodes based on egg production (Yik and Birchfield, 1984). However, differences in ploidy level posed a major challenge when introgressing the resistance traits from *G. longicalyx* (diploid) into upland cultivars (allotetraploid). To use *G. longicalyx* as a source of resistance, triple species hybrids were developed in a multi-step process. First, a cross was made between a tetraploid and a diploid plant which resulted in an infertile triploid.

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Next, the axillary buds were treated with colchicine to produce a fertile hexaploid (Bell and Robinson, 2004).

The HLA triple species, developed by Bell and Robinson (2004) was used in the progression of two progenies, LONREN-1 and LONREN-2. Initial results indicated reniform nematode suppression as high as 95% in the growth chamber and a range of 50 to < 80% depending on field location. Although the lines were successful at lowering reniform populations, stunting and yield loss of between 30 to 40% were recorded when plants were grown in high nematode densities (Bell et al., 2014). The negative impact on growth and development can be attributed to over-expression of cell necrosis as a response to syncytia development in the root by the female reniform nematode (Sikkens et al., 2011). Concluding, LONREN breeding lines showed promise when grown in fields with low nematode populations but suffered when introduced to high populations (Bell and Robinson, 2004).

The ease of introgression between G. *barbadense* (Sea Island Cotton) and G. *hirsutum* based on ploidy level raised interest in using it as a source of resistance. Accessions within the G. *barbadense* species, including "Texas 110", have been confirmed as highly resistant (Yik and Birchfield, 1984). Texas 110 was used as a source of resistance to release two germplasm lines TAM RKRNR-9 and TAM RKRNR-12 (Starr et al., 2011). Both germplasm lines were described as resembling G. *hirsutum* although height and maturity length were higher than commercial upland cotton. Nematode reproduction on the germplasm lines were described as "intermediate" compared to the susceptible check in the greenhouse, however when moved to the field, there were no differences in mid-season nematode populations among breeding lines (Starr et al., 2011).

G. *barbadense* GB-713 was also identified as a source of resistance to reniform nematodes (Robinson et al., 2004). In this specific study, GB-713 and Texas 110 suppressed nematode populations to 31% of the susceptible check (Deltapine 16). Numerous germplasm lines were developed using GB-713 as the source of resistance including BARBREN-713, M713 REN1, M713 REN2, and M713 REN5 (McCarty et al., 2013; Bell et al., 2015) . BARBREN-713 was evaluated across the cotton belt and resulted in inconsistent reniform nematode suppression, between 75 – 55%, and yield depending on location (Bell et al., 2015). Two of the cotton cultivars used in this study, PHY332 W3FE and PHY443 W3FE released by Corteva were bred using GB-713 as the source of resistance (McPherson, M, Personal Communication, 2021).

Identification and integration of genetic resistance has been a valuable management option for other nematode species and in other crops. A study conducted in Iowa in 2013 showed soybean yields in *Heterodera glycines*, soybean cyst nematode, infested fields resulted in 5 to 50 percent higher yields, depending on location, in cultivars with soybean cyst nematode resistance genes compared to susceptible cultivars (Tylka et al., 2013).

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Research Objectives

- I. Assess the impact of various nematode resistant cotton cultivars on cotton development and yield in fields with known reniform nematode populations.
- II. Determine the effectiveness and the economic feasibility of chemical control on cotton development and yield in reniform nematode infested fields.
- III. Evaluate influence of resistant cotton cultivars and chemicals on reniform nematode populations.

CHAPTER III

NEMATODE RESISTANCE IN COTTON

Genetic resistance is a potential tool for managing reniform nematodes in cotton. Birchfield and Brister (1963) began work in the 1960's to identify reniform nematode resistance in cotton. They screened 24 cotton cultivars to determine host status to reniform nematodes. Plant roots were examined to determine reproduction and infection rates. From there, the classification of several more cultivars was conducted by Yik and Birchfield (1984) including G. barbadense 'Texas 110' described as highly resistant. Cultivars and accessions were developed by introducing the resistant genes from G. barbadense into upland cotton (Starr et al., 2011). The mechanism of resistance in cotton with traits from G. barbadense was discovered by comparing cultivar TX 110 and accession GB 713 to a susceptible upland cultivar (DP16). The resistance genes were determined to aid mainly in early stages of cotton growth by slowing the development of nematodes from vermiform (newly attached) to swelling (body starting to enlarge). The detection of nematodes on the roots of the resistant cultivars was delayed by 1 day (9 DAP) compared to the susceptible cultivar (8 DAP) leading to fewer nematodes associated with the resistant cultivars. By approximately 11 DAP, almost all of the vermiform nematodes on all plants were starting to enlarge. By 31 DAP, all three cultivars, supported females with egg masses, but the progression of development was slowed by the resistance genes (Stetina, 2015). This reniform nematode resistance in

cotton is at the onset of commercial availability and warrants thorough assessment and comparison to chemical nematode control options.

Prior to the recent introduction of reniform nematode resistance in modern cotton cultivars, one or two cultivars with root-knot nematode resistance (PHY 417WRF and PHY 427WRF) were thought to have some resistance against reniform nematodes compared to nematode-susceptible cultivars (Woodward and Wheeler, unpublished). Studies have been conducted on other crops regarding cross resistance between nematode species such as *Heterodera glycines*, soybean cyst nematode resistance in *Glycine max*, soybeans (Lee et al., 2015). Out of the 120 accessions with proven soybean cyst nematode resistance, 64 of them reduced the severity of galling from root-knot nematodes to their standards of "resistant". Further, 24 out of the 64 accessions with resistance to root-knot nematodes also showed a reduced reproductive index of reniform nematodes compared to the susceptible cultivars.

Cotton cultivars with reniform nematode resistance have recently become available for public testing and were included in this study. The objectives of this study were to: 1) compare growth and yield of cotton cultivars with reniform nematode resistance, root-knot nematode resistance, and no nematode resistance under reniform nematode pressure, 2) assess the influence of an in-furrow nematicide on cotton growth and yield relative to varying genetic resistance, and 3) quantify the effect of genetic resistance on reniform nematode populations.

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Methods

Field trials were conducted at four locations across Texas: Damon, College Station, and Wall (2019 and 2020), and Lubbock (2020). The distribution of locations across the state represent an array of soil types (Table 3.1) (USDA-NRCS, 2019). Irrigation varied across years and locations. Both years at the Damon were rain fed (nonirrigated), tilled conventionally, planted on beds, and in a corn to cotton rotation. College Station was furrow irrigated, tilled conventionally, planted on beds, and was planted continuously to cotton. Wall in 2019 received deficit furrow irrigation, tilled conventionally, planted on beds, and in continuous cotton. In2020, Wall received ample sub-surface drip irrigation. The field was strip-tilled with corn residue on the surface from the previous years crop and was planted flat. At each site, a randomized complete block design with four replications was used. Plots were 4.1 meters wide, 12.2 meters long, consisting of four rows on 102-cm spacing, and cotton was planted at 106,251 seeds ha⁻¹. Cotton cultivar treatments varied by year with six cultivars in 2019 and eight cultivars and breeding cultivars in 2020, comprising various levels of nematode resistance (Table 2.3). Each cultivar was planted with a split-plot application of fluopyram (199 g ha⁻¹) and prothioconazole (199 g ha⁻¹) (hereafter referred to as "nematicide treatment") applied in-furrow at 994 mL ha⁻¹. PHY 440 W3FE was planted as the susceptible check at Damon in 2019, however a more suitable susceptible cultivar (PHY 340 W3FE) was planted in College Station and Wall. All locations in 2020 were planted with the same cultivars with the susceptible check remaining PHY 340 W3FE.

In-season measurements generally included stand establishment (plants per 4 m²) (early season), plant height (80 and 100 DAP), and total nodes (end of season). Plant measurements were collected from the middle two rows, to avoid any border effect that may have occurred. Total nodes were not measured in Wall in 2020. All in-season growth measurements were not recorded at Lubbock.

At all locations and site years, except Damon 2019, the middle two rows were harvested. Seed cotton weights were measured per harvest area and subsamples from each plot were collected to gin. Seed cotton samples from Damon and College Station (approximately 120 g) were weighed and ginned, to determine lint percentage. The harvesters used at Wall and Lubbock did not have a bur extractor, so samples from these sites were processed using the same methods, except approximately 160 g of bur cotton was weighed, de-burred by hand, weighed again, and then ginned. All samples were processed using the same twenty saw tabletop laboratory-scale gin.

Soil samples were collected twice during the growing season, pre-plant and postharvest, to determine nematode populations. The pre-plant collection was done within a week of planting. The timing of the final sampling varied by site and field conditions (Table 3.1). A composite of four samples was taken from each replication pre-plant and from each plot post-harvest. A shovel was angled towards the root zone to collect the soil sample from the area between 15-30 cm depth approximately.

Extractions were conducted using a combination of the methods described by USDA-ARS (Handoo and Ellington, 2017) and Whitehead and Hemming (1965). This consisted of a PVC sieve layered over a plastic pan with one single-ply tissue placed into the PVC sieve. Then 200 mL of soil was poured inside the PVC sieve followed by 250 mL of water poured over the soil sample. The entire arrangement was placed inside a plastic bag and placed inside a cabinet at approximately 21°C for 48 hours. The nematodes moved from the soil, through the tissue and plastic sieve, into the pan. The water from the plastic pan was strained using a set of three metal sieves, with pore sizes of 250-µm, 45-µm, and 38-µm. The final sieve in the protocol from USDA was 45-µm. The finer sieve used in this study may have resulted in reduced nematode counts.

First, the PVC sieve and pie-pan was rinsed with water ten times into the sieves. Then the sieves with pore sizes of 230-µm and 43.2-µm were also rinsed ten times and set aside. Finally, the nematodes and soil particles that collected on the sieve with a pore size of 37-µm were washed to one side, then 15mL of water was used to rinse the slurry into a serrated petri dish for counting. A stereo microscope was used to identify and count only the reniform nematodes.

Data were analyzed with SAS 9.4 using a mixed model analysis. Response variables from some locations were analyzed separately due to inconsistencies in cultivar or stand establishment. Response variables at Damon 2019 were analyzed separately because of differences in treatment. Yield at Wall 2020 was analyzed separately due to stand establishment. All other variables from College Station and Wall in 2019, and Damon, College Station, and Lubbock in 2020 were combined for analysis. Cultivars DP 18R628 B3XF and DG 3651NR B2XF were excluded from the yield analysis at Wall in 2020 due to insufficient stand establishment, so this site was analyzed separately.

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Fixed effects included location, cultivar, nematicide, and all possible interactions. Block nested within location was treated as random. Power transformations were applied to post-harvest nematode populations and height at 80 and 100 DAP for the combined locations in 2020 to normalize data using the Box-Cox method (Box and Cox, 1964).

The Damon location in 2019 was analyzed separately as treatments were different from the other sites. Fixed effects included cultivar, nematicide, and the corresponding interaction, with block treated as random. Differences were identified at α = 0.05 and means were separated using Tukey's HSD post-hoc means separation test. Post-harvest nematode populations were transformed using the Box-Cox method and all means were back-transformed for presentation.

Location	Soil series		20	19		2020				
		Pre-plant nematode sampling	Plant	Harvest	Late- season nematode sampling	Pre-plant nematode sampling	Plant	Harvest	Late- season nematode sampling	
Damon	Lake Charles Clay	22-Apr	22-Apr	12-Sept	28-Sept	15-Apr	15-Apr	1-Sept	5-Sept	
College Station	Belk Clay	10-May	14- May	24-Sept	25-Sept	16-Apr	21-Apr	18-Sept	22-Sept	
Wall	Angelo Clay Loam	22-May	27- June	25-Nov	26-Nov	26-May	27-May	11-Nov	12-Nov	
Lubbock	Acuff Loam and Olton Clay Loam	_	-	-	-	-	20-May	2-Nov	17-Aug	

Table 3.1. Soil series, nematode sampling, planting, and harvest dates of all reniform nematode locations in 2019 and 2020

	Dam	Damon		College Station		all	Lubbock		
Month	2019	2020	2019	2020	2019	2020	2019	2020	
					mm				
January	94 (7)	135 (48)	122 (37)	63 (-22)	8 (-15)	28 (5)	0 (-17)	9 (-7)	
February	55 (-4)	13 (-47)	53. (-14)	54 (-14)	7 (-25)	44 (12)	1 (-18)	13 (-6)	
March	15 (-64)	19 (-60)	32 (-58)	63 (-26)	17 (-19)	79 (43)	29 (3)	60 (34)	
April	47 (-49)	110 (13)	141 (74)	98 (31)	76 (39)	43 (6)	44 (11)	0.5 (-33)	
May	141 (28)	146 (34)	201 (91)	104 (-6)	136 (61)	56 (-19)	101 (36)	52 (-12)	
June	250 (131)	121 (2)	126 (30)	62 (-34)	81 (23)	22 (-36)	52 (-13)	47 (-18)	
July	43 (-51)	107 (12)	5 (-51)	70 (14)	34 (5)	25 (-5)	115 (66)	47 (-1)	
August	68 (-28)	39 (-57)	53 (-26)	15 (-64)	8 (-53)	47 (-15)	54 (9)	14 (-32)	
September	277 (175)	178 (77)	65 (-24)	97 (8)	7 (-61)	124 (56)	152 (89)	26 (-37)	
October	138 (34)	20 (-84)	78 (-44)	11 (-111)	13 (-47)	13 (-48)	28 (-9)	20 (-18)	
November	58 (-40)	100 (1)	32 (-51)	24 (-59)	29 (-0.4)	1 (-29)	27 (7)	2 (-18)	
December	25 (-61)	180 (94)	14 (-78)	117 (25)	33 (12)	27 (6)	17 (-2)	2 (-17)	

Table 3.2. Monthly total precipitation in 2019 and 2020 and deviation from 30-year averages in Damon, College Station, Wall, and Lubbock, TX.

* Values in parenthesis show departure from 30-year average

20)19	2020			
Damon	College Station & Wall	All Locations	Nematode resistance		
-	PHY 340 W3FE	PHY 340 W3FE	Susceptible		
PHY 440 W3FE	PHY 440 W3FE	-	Root-knot		
PHY 480 W3FE	PHY 480 W3FE	PHY 480 W3FE	Root-knot		
PHY 443 W3FE	PHY 443 W3FE	PHY 443 W3FE	Reniform & Root-knot		
-	-	PHY 332 W3FE	Reniform & Root-knot		
DG 3651NR B2XF	DG 3651NR B2XF	DG 3651NR B2XF	Root-knot		
DP 1747NR B2XF	DP 1747NR B2XF	DP 1747NR B2XF	Root-knot		
DP 18R628 B3XF	DP 18R628 B3XF	DP 18R628 B3XF	Root-knot		
-	-	DP 2143NR B3XF	Reniform & Root-knot		

Table 3.3. Cotton cultivars tested and corresponding resistance traits included in reniform evaluation trials at the Damon and College Station in 2019 and all locations (Damon, College Station, Wall, and Lubbock) in 2020.

*Damon, College Station, Wall, and Lubbock

Results

Site Conditions

Average monthly temperatures were similar (within 1°C) to the 30-yr averages at each site. All average monthly precipitation totals compared to 30-yr averages are shown in Table 3.2. In 2019, the Damon site received 110% greater than normal precipitation in June, and May precipitation at College Station was 83% above normal. College Station, Wall, and Lubbock generally received below-normal precipitation throughout the 2020 growing season. A notably heavy rainfall event occurred at Wall in 2020 one day after planting resulted in reduced stand establishment for all cultivars, but especially DP 18R628 B3XF and DG 3651NR B2XF. Pre-season reniform nematode assays indicated 41, .25, 26 nematodes (200 mL soil⁻¹) at Damon, College Station, and Wall, respectively in 2019, and 34, 43, and 56 nematodes (200 mL soil⁻¹) at Damon, College Station, and Wall, respectively in 2020.

Damon 2019

Yield was affected by cotton cultivar at Damon in 2019 (P = 0.001). PHY 443 W3FE (reniform resistant), PHY 480 W3FE, DP 18R628 B3XF, and PHY 440 W3FE were all among the highest yielding (Figure 3.1). DG 3651NR B2FX and DP 1747NR B2XF (mean = 905 kg ha⁻¹) yielded 533 kg ha⁻¹ less than PHY 443 W3FE (mean = 1340 kg ha⁻¹). The addition of fluopyram and prothioconazole and the interaction between the chemical application and cultivar did not impact yield (P > 0.05).

Nematode populations were not affected by cultivar, the application of fluopyram and prothioconazole, nor the combination (P > 0.05). Cotton cultivar did impact stand establishment (plants per 4 m²), height at 80 DAP, and total nodes (Tables 3.4). Plant height at 100 DAP were different between both cultivar and the chemical application, where the application of fluopyram and prothioconazole increased plant height by 4.2 cm. Total nodes were also different for the cultivar (P = 0.02), where DP 18R628 B3XF had two more nodes than PHY 480 W3FE, PHY 440 W3FE, and PHY 443 W3FE (Table 3.5). The application of nematicide did not impact total nodes (P > 0.05)

College Station and Wall Combined 2019

Cotton yield was affected by location (P = 0.0001), cultivar (P = 0.0001), and the interaction between cultivar and location (P = 0.0008), across Wall and College Station in 2019. Yields were nearly 10 times higher in College Station (mean = 1,749 kg ha⁻¹) than Wall (mean = 180 kg ha⁻¹). PHY 443 W3FE was the highest yielding cultivar (1,102 kg ha⁻¹), producing 288 kg ha⁻¹ more cotton, than PHY 340 W3FE (mean = 814 kg ha⁻¹) (Figure 3.1). The application of nematicide did not impact yield (P > 0.05).

Nematode populations were not affected by cultivar or the application of chemicals (P > 0.05). Stand establishment was impacted by location, cultivar, and the interaction between location and cultivar (Table 3.4). Plant establishment averaged 32 and 19 plants per 4 m² for College Station and Wall, respectively. The PHY 443 W3FE resulted in more plants per 4 m² than, PHY 340 W3FE, DP 1747NR B2XF, DP 18R628 B3XF, and DG 3651NR B2XF (Table 3.5). Total nodes in most root-knot nematode

resistant (RKN) cultivars produced more nodes than PHY 340 W3FE and an interaction between location and cultivar was observed (Table 3.5). At both plant height timings, cultivar and location were influential where PHY 443 W3FE grew taller than PHY 340 W3FE (Table 3.5).

All Locations Combined 2020

Yield was affected by cultivar for Damon, College Station, and Lubbock combined. PHY 443 W3FE and PHY 332 W3FE were the highest yielding (mean = 1,209 kg ha⁻¹), however PHY 332 W3FE yielded similarly to DP 2143NR B3XF (mean = $1,102 \text{ kg ha}^{-1}$). The susceptible check yielded similarly to PHY 480 W3FE, DP 1747NR B2XF, DP 18R628 B3XFB3XF (mean = 830 kg ha⁻¹). DG 3651NR B2XF $(\text{mean} = 574 \text{ kg ha}^{-1})$ yielded the least in 2020 (Figure 3.2). The application of nematicide decreased yields by 5% compared to no treatment (P = 0.04). Location also affected cotton yield (P = 0.005) where College Station and Damon (mean = 993 kg ha⁻ ¹) yielded higher than Lubbock (mean = 773 kg ha^{-1}). Then interaction between location and cultivar also impacted yield (P = 0.001). In Damon, PHY 443 W3FE, PHY 332 W3FE, DP 2143NR B3XF, PHY 480 W3FE, and PHY 340 W3FE yielded higher than DG 3651NR B2XF (Table 3.6). In College Station, PHY 332 W3FE and DP 2143NR B3XF yielded higher than PHY 480 W3FE and all cultivars yielded higher than DG 3651NR B2XF. In Lubbock, PHY 443 W3FE and PHY 332 W3FE yielded the highest, followed by DP 2143NR B3XF.

Cultivar impacted yield (P = 0.001) in Wall where PHY 332 W3FE and PHY 443 W3FE yielded the greatest (mean = 3,246 kg ha⁻¹) while PHY 443 W3FE yielded similarly as DP 2143NR B3XF (mean = 2,943 kg ha⁻¹). DP 2143NR B3XF yields were similar to PHY 480 W3FE, PHY 340 W3FE, and DP 1747NR B2XF (mean = 2,321 kg ha⁻¹) (Figure 3.2). The addition of nematicide did not impact yield (P > 0.05) at Wall in 2020.

Reniform nematode populations were impacted by cultivar (P = 0.005), location (P > 0.001), and the interaction between location and cultivar (P > 0.001). PHY 443 W3FE (mean = 170 nematodes 200 mL⁻¹ soil) suppressed nematode populations compared to PHY 480 W3FE and DP 1747NR B2XF (mean = 355 nematodes 200 mL⁻¹ soil) by approximately 52%. The application of fluopyram and prothioconazole did not impact nematode populations (P > 0.05) in 2020.

Multiple in season growth measurements were impacted by the main effects as well as their interactions. Plant stands were impacted by location, cultivar, and the interaction between location and cultivar (Table 3.4). PHY 332 W3FE had greater emergence than DP 2143NR B3XF, DP 1747NR B3XF and DP 18R628 B3XF and DG 3651NR B2XF (Table 3.5). Location, cultivar, the interaction between location and cultivar, and the interaction between location and nematicide all impacted height at 80 DAP (Table 3.4). Plant heights fluctuated among cultivars, with PHY 443 W3FE and PHY 332 W3FE growing the tallest (Table 3.5). Plant height at 100 DAP was impacted by location, cultivar, and the interaction between location and cultivar (Table 3.4). PHY while PHY 480 W3FE and PHY 340 W3FE were the shortest (Table 3.5). Total nodes were impacted by location, cultivar, and the interaction between location and cultivar (Table 3.4). DP 18R628 B3XF, DG 3651NR B2XF, DP 2143 NR B3XF grew more nodes than PHY 340 W3FE and PHY 332 W3FE (Table 3.5).

Source of	Dependent variable												
variation		Damo	on 2019			Combined 2019 ⁺ All locations combined							
	P◆	H 1	H 2	Т	Р	H 1	H 2	Т	Р	H 1	H 2	Т	
L^{\dagger}	-	-	-	-	0.0001	<.0001	<.0001	0.06	<.0001	0.001	0.01	0.02	
Ν	0.48	0.11	0.02	0.44	0.31	0.93	0.57	0.94	<.0001	<.0001	<.0001	0.26	
L x N	-	-	-	-	0.31	0.92	0.53	0.63	<.0001	<.0001	<.0001	0.88	
		<.000	<.0001	0.02	<.0001	<.0001	<.0001	<.0001	0.91	0.32	0.61	<.000	
С	0.001	1										1	
					0.01	0.09	0.06	0.001	0.76	0.02	0.15	<.000	
L x C	-	-	-	-								1	
C x N	0.98	0.51	0.319	0.86	0.36	0.93	0.79	0.12	0.29	1	0.59	0.49	
L x C x N	-	-	-	-	0.7	0.92	0.69	0.83	0.99	0.75	0.82	0.91	

Table 3.4. Significance of fixed effects for stand establishment (plants per 4 m^2), height 80 days after planting (DAP), height 100 DAP, and total nodes at Damon, College Station, Wall, and Lubbock, TX in 2019 and 2020.

+ College Station and Wall

*Damon, College Station, and Wall 2020

† L, location; N, nematicide; C, cultivar

♦P, plants per 4 m²; H1, height 80 DAP (cm); H2, height 100 DAP (cm); T, total nodes

Cultivar		Damo	n 2019		(Combine	d 2019	+	All le	ocations c	combined	2020*
	P◆	H 1	H 2	Т	Р	H 1	H 2	Т	Р	H 1	H 2	Т
PHY 443 W3FE	53 a	99 a	101 a	19 b	34 a	85 a	89 a	19 с-е	34 ab	90 e	90 d	19 d-e
PHY 332 W3FE	-	-	-	-	-	-	-	-	39 a	81 de	80 bc	18 e
DP 2143NR B3XF	-	-	-	-	-	-	-	-	32 b	80 cd	87 cd	20 bc
PHY 480 W3FE	47 a-c	73 c	80 c	19 b	28 a-c	72 bc	72 b	19 b-d	33 ab	66 a	69 a	19 cd
PHY 440 W3FE	51 ab	75 c	81 bc	19 b	33 ab	64 c	70 b	19 de	-	-	-	-
PHY 340 W3FE	-	-	-	-	26 bc	71 bc	73 b	18 e	34 ab	68 ab	72 a	18 de
DP 1747NR B2XF	39 c	88 b	95 a	19 ab	22 cd	79 ab	88 a	20 a-c	30 bc	70 ab	79 b	21 ab
DP 18R628 B3XF	40 bc	88 b	94 a	20 a	21 cd	77 ab	82 a	20 ab	24 cd	75 b-d	82 b-d	22 a
DG 3651NR B2XF	38 c	83 bc	92 ab	19 ab	15 d	75 b	83 a	21 a	23 d	73 а-с	83 b-d	21 ab

Table 3.5. Cotton cultivar effects on stand establishment (plants per 4 m²), height 80 days after planting (DAP), height 100

+ College Station and Wall

*Damon, College Station and Wall 2020

◆ P, plants per 4 m²; H1, height 80 DAP (cm); H2, height 100 DAP (cm); T, total nodes

DAP, and total nodes at Damon, College Station, Wall, and Lubbock, TX in 2019 and 2020.

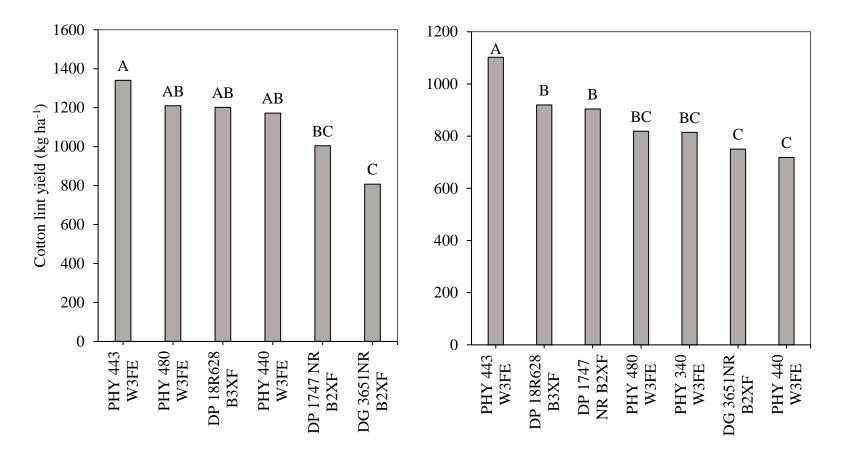


Figure 3.1. Left panel: cotton yield (kg ha⁻¹) at Damon in 2019, right panel: cotton yield (kg ha⁻¹) from College Station and Wall combined 2019.

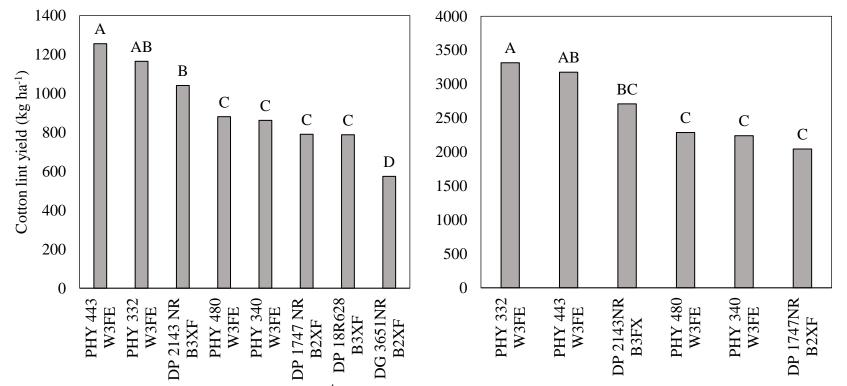


Figure 3.2. Left panel: cotton yield (kg ha⁻¹) from Damon, College Station and Lubbock 2020 combined, right panel: cotton yield (kg ha⁻¹) from Wall 2020.

Cultivar	Damon	College Station	Lubbock	
		kg ha ⁻¹		
PHY 443 W3FE	1302 a	1154 abc	1310 a	
PHY 332 W3FE	1164 ab	1153 ab	1177 a	
DP 2143NR B3XF	1060 ab	1198 a	865 b	
PHY 480 W3FE	1061 ab	856 c	724 bc	
PHY 340 W3FE	1000 ab	939 abc	647 bcd	
DP 1747NR B2XF	896 bc	916 bc	558 cd	
DP 18R628 B3XF	969 bc	955 abc	440 d	
DG 3651NR B2XF	701 c	557 d	465 cd	

Table 3.6. Cotton yield (kg ha⁻¹) from Damon, College Station, and Lubbock in 2020

Table 3.7. Pre-plant average nematode populations with standard deviation and cultivar impact on post-harvest nematode populations at Damon, College Station, Wall, and Lubbock, TX in 2019 and 2020.

Cultivar	Damon	2019	Combine	ed 2019 ⁺	All locations c	All locations combined 2020*	
	A^*	В	А	В	Α	В	
			200	0 mL soil ⁻¹			
Average	41 (27)		13 (17)		45 (25)		
PHY 443 W3FE		69 a		42 a		215 а-с	
PHY 332 W3FE		-		-		170 c	
DP 2143NR B3XF		-		-		203 а-с	
PHY 480 W3FE		83 a		43 a		329 ab	
PHY 440 W3FE		93 a		72 a		-	
PHY 340 W3FE		-		63 a		216 а-с	
DP 1747NR B2XF		76 a		58 a		380 a	
DP 18R628 B3XF		72 a		58 a		175 bc	
DG 3651NR B2XF		76 a		55 a		291 а-с	

+ College Station and Wall

◆Damon, College Station, Wall, and [Lubbock (post-harvest)]

*A, pre-plant ; B, post-harvest

Values in parenthesis show departure from average

Discussion

Cultivar had the greatest impact on yield at all locations over both years. At the Damon location in 2019, the reniform nematode resistant (REN) cultivar (PHY 443 W3FE) did not yield higher than most of the RKN cultivars. This lack of yield difference relative to the resistance traits warrants consideration of confounding abiotic factors, nematode pressure, or potential for cross resistance. Excessive rainfall at this site-year may have influenced plant growth and/or nematode activity in a way that diminished the ultimate effect of genetic resistance. Certain soybean accessions with soybean cyst nematode resistance have shown resistance to root-knot and reniform nematodes (Lee et al., 2015). However, in all other locations and years, the REN cultivars yielded among the top with the exception of Wall in 2020 where DP 2143NR B3XF yielded similarly to all of the RKN cultivars and the susceptible check. In a similar study conducted in the Tennessee valley PHY 332 W3FE increased yield by 1,895 kg ha⁻¹ compared to PHY 340 W3FE (Lawrence, 2020). Although the findings of this research show less drastic differences, they support the findings of similar work across the cotton belt.

Common effects were also observed among yield and plant height. The cultivars with REN and RKN resistance were consistently among the tallest measured. As stunting is a common symptom of reniform nematode parasitism, we can draw linkage between nematode resistance suppressing infection rates during the growing season, taller cotton plants, and higher yields.

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The negative effect nematicide had on yields in the combined sites in 2020 is not readily explained by the in-season growth measurements taken because they were not impacted by nematicide applications. Phytotoxicity due to fluopyram as a seed treatment has been documented in soybeans causing symptoms such as necrosis of plant tissue, although impacts on yield were not observed (Spinks et al., 2001). Other phytotoxicity symptoms include poor emergence, seedling death, stunting and poor plant development (Moorman, 2011). Although plant stand measurements would have detected poor germination and seedling death, measuring plant height earlier in the season may have detected possible early-season stunting. Root measurements were also not taken in this study. If root growth was inhibited by the application of fluopyram and prothioconazole compared to the untreated treatments, yields may have been affected.

Although the resistance trait did not consistently affect post-harvest nematode populations, the observed reduction in nematodes with PHY 332 W3FE agrees with findings reported by Lawrence, K (2020) linking nematode population suppression with resistant genetics by 83% compared to the susceptible check. Eggs (g root⁻¹) were reported in her research, which differs from the vermiform numbers reported in this study (Lawrence, 2020). This difference in measurement could account for the lack of nematode reduction measured in this study. The nematode populations reported in this study are also lower than other reports of vermiform reniform nematode populations which could be due to sample timing where nematode populations are highest during the growing season and start to decline after harvest. However, it is difficult to sample properly if soil moisture is inadequate. Also, dry conditions can result in more or the

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population tied up in the egg stage, which was not extracted in these studies.

CHAPTER IV

CHEMICAL CONTROL OF RENIFORM NEMATODES

The application of nematicides is another method to manage reniform nematodes in cotton fields. A common issue with nematicides is the level of dependency these chemicals have on environmental factors, resulting in efficacy varying by year and location (Bromilow et al., 1980; Haydock et al., 2012; Wheeler et al., 2013; Faske and Brown, 2019). The combination of growing a resistant cultivar with the application of nematicides is recommended as long as it is economically feasible (Davis et al., 2009).

Field trials were conducted to compare the efficacy of three nematicides and assess potential interaction with root-knot nematode resistance in reniform nematode infested fields. The objectives of this study were to 1) evaluate the efficacy of different nematicides on reniform nematode populations and cotton growth and yield, 2) evaluate the interaction between genetic root-knot nematode resistance and nematicides relative to cotton growth, yield, and nematode populations, and 3) determine potential return on investment for growers relative to product and application cost.

Methods

The chemical trial was conducted at the same sites with the same experimental design, plot size and planting, sampling, and harvest methods as the genetic resistance trial (reference Chapter III methods). Treatments included: AGLOGIC 15G (active ingredient: aldicarb ([2-methyl-2-(methylthio) propionaldehyde 0-(methylcarbamoyl)oxime]) 15%) applied in-furrow at planting using granular insecticide boxes at 5.6 kg ha⁻¹, Propulse (AI: fluopyram 17.4% and prothioconazole 17.4%) applied in-furrow at planting at 994 mL ha⁻¹ using XR8002 EVS (Teejet, Glendale Heights, IL) nozzles perpendicular to the row, Vydate CLV (AI: oxamyl ([methyl N'N'dimethyl-N-[(methylcarbamoyl)oxy]-1-thioxamimidate]) 42%) foliar broadcasted at 1108 mL ha⁻¹ approximately 30 and 45 days after planting using nozzle type Q8003 (Teejet) with spray volume ranging 122-154 L ha⁻¹ relative to site years, a combination of AGLOGIC 15G and Vydate CLV, and a combination of Propulse and Vydate CLV (Table 4.1). Two cotton cultivars were planted, one susceptible to nematodes (PHY 340 W3FE/PHY 440 W3FE) and the other with resistance to root-knot nematodes (PHY 480 W3FE). Each cultivar received all chemical treatments to determine how each chemical performed alone and in combination with a level of genetic nematode resistance. In 2019 at Damon, PHY 440 W3FE was used as the susceptible check but the check was changed to PHY 340 W3FE at all other locations and years. In season growth factors, yield, , and nematode samples were collected in the same manner as the genetic resistance study. A partial net economic analysis was conducted using cotton lint value calculated using

Cotton Incorporated's upland loan calculator, chemical cost, and estimated cost of application (Klose, 2020).

Similar to the genetic resistance study, the data were analyzed using SAS 9.4. Fixed effects included cultivar, nematicide, and all possible interactions. Random effects were site-year and block nested within site-year. Total nodes and post-harvest nematode populations were transformed using a Box-Cox power transformation, then means were back-transformed for presentation (Box and Cox, 1964). Damon 2019 was analyzed separately. Fixed effects included cultivar, nematicide, and their interaction and block and the interaction between block and main fixed effects treated as random. Differences were identified at $\alpha = 0.05$ and means were separated using Tukey's HSD post-hoc means separation test.

Results

Precipitation and temperature data for the nematicide trial was the same as the genetic resistance trial as they were conducted at the same locations (reference Chapter III results).

Damon 2019

Neither yield nor nematode populations were impacted by nematicide treatment or cultivar (P > 0.05). The application of nematicides, cultivar, and the interaction between the two impacted plant height at 80 DAP (Tables 4.2). The application of aldicarb and oxamyl (82 cm) increased plant height by 10 cm compared to the untreated check (72 cm) (Table 4.3). Plant height at 100 DAP, plant emergence per 4 m², and total nodes were not impacted by the nematicide treatments or cultivar (Table 4.2). The use of nematicides showed no partial economic gain or loss compared to the untreated check, based on yield and loan value.

All Other Locations Combined

The application of nematicides increased yield (P = 0.005). The application of aldicarb and oxamyl increased yield by 187 kg ha⁻¹ compared to the untreated check (Figure 4.1). Cultivar and the interaction of nematicides and cultivar did not impact yield (P > 0.05). Post-harvest reniform nematode populations were not impacted by nematicide treatment or cultivar (P > 0.05).

The application of nematicides, cultivar, and the interaction between these two factors did not impact either plant height at either timing or stand establishment (Table 4.2). Total nodes per plant was impacted by cultivar but not by nematicide applications nor their interaction. PHY 480 W3FE produced more nodes (20) than PHY 340 W3FE (18.5). Post-harvest reniform nematode populations were not impacted by nematicide treatment or cultivar. Nematicide treatment affected partial net return (Table 4.2). Based on lint value and costs, the application of aldicarb (mean= \$2141.05 ha⁻¹) increased net return by \$254.37 ha⁻¹ compared to the application of fluopyram and prothioconazole and oxamyl (mean = \$1895.68 ha⁻¹) (Figure 4.2). When compared to the untreated check, none of the nematicide treatments resulted in a net gain or loss

Treatment	Rate	Cost ($\$ ha ⁻¹) (chemical + application [†])
Aldicarb 15G •	5.6 kg ha ⁻¹	83.47
Aldicarb 15G + Oxamyl		159.89
Fluopyram and Prothioconazole•	993.9 mL ha ⁻¹	105.09
Fluopyram and Prothioconazole + Oxamyl		181.50
Oxamyl*	1108 mL ha ⁻¹	76.41
Untreated Check (UTC)		0

Table 4.1. Nematicide treatments, application rates, and cost including chemical price and average application costs across cultivars.

† Application cost according to 2020 Texas Agriculture Custom Rate Chart (Klose, 2020)

♦ All aldicarb 15G and fluopyram + prothioconazole treatments were applied in-furrow at planting.

*All oxamyl treatments were foliar broadcast at approximately 30 and 45 days after planting.

Table 4.2. Significance of fixed effects for stand establishments (plants per 4 m²), height 80 days after planting (DAP), height 100 DAP, and total nodes PHY 440 W3FE/PHY 340 W3FE and PHY 480 at College Station, and Wall in 2019 and Damon, College Station, and Wall, TX 2020.

Dependent variable			Source of	of variation			
	Damon 2019			All site-years combined*			
	\mathbf{N}^{\dagger}	С	C x N	Ν	С	C x N	
			P	P > F			
Plant stand	0.891	0.850	0.599	0.897	0.602	0.385	
Height 80 DAP	0.032	0.986	0.713	0.085	0.132	0.646	
Height 100 DAP	0.090	0.782	0.076	0.919	0.070	0.624	
Total nodes	0.776	0.452	0.884	0.505	<.0001	0.441	
Partial net return	0.452	0.259	0.611	0.019	0.104	0.969	

*College Station and Wall in 2019, and College Station, Damon, and Wall in 2020.

† N, nematicide; C, cultivar.

Nematicide Treatment	Dependent variables				
	Damon, 2019	All site-years combined*			
	Height 80 DAP	Partial net return ⁺			
	cm	\$ ha ⁻¹			
Aldicarb	78.86 ab	2141.05 a			
Aldicarb + Oxamyl	81.57 a	2108.69 ab			
Fluopyram and prothioconazole	73.43 ab	1961.16 ab			
Fluopyram and prothioconazole + Oxamyl	73.54 ab	1895.68 b			
Oxamyl	71.20 ab	1995.36 ab			
UTC	72.51 b	1996.01 ab			

Table 4.3. Nematicide treatment impact on plant height (80 days after planting, DAP) and economic analysis PHY 440 W3FE/PHY 340 W3FE and PHY 480 for Damon, College Station, and Wall, TX in 2019 and 2020.

*College Station and Wall in 2019, and College Station, Damon, and Wall in 2020.

⁺Partial net return = ((lint yield (kg ha⁻¹)*1.43 kg⁻¹ +(((lint yield/turnout) – lint yield)/2000) * \$165.35 tonne⁻¹)-cost Lint value calculated using the Cotton Loan Calculator (Cotton Incorporated, 2019, 2020) Cost equals the sum of chemical cost and application cost (Klose, 2020)

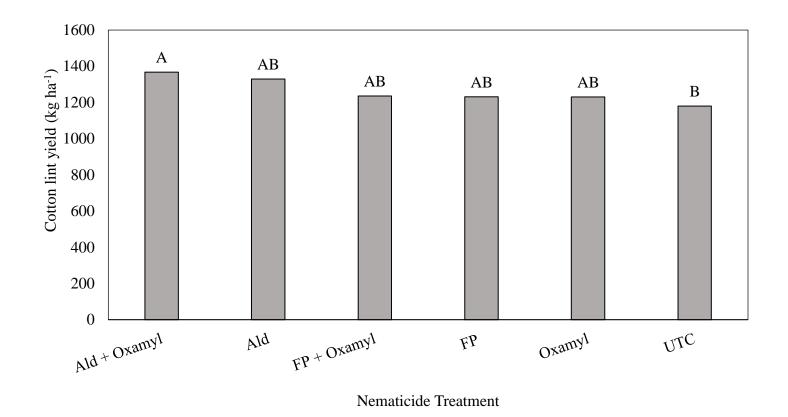


Figure 4.1. Cotton yield (kg ha⁻¹) resulting from nematicide treatments: aldicarb (Ald), oxamyl, fluopyram and prothioconazole (FP), and the untreated check (UTC), at College Station and Wall 2019 and Damon, College Station, and Wall 2020 combined.

Nematicide treatment	Damon	2019	Combined ⁺	
	A^*	В	Α	В
		2	200 mL soil ⁻¹	
Average	48 (27)		35 (37)	
Aldicarb		70 a	· · · ·	109 a
Aldicarb + Oxamyl		65 a		118 a
Fluopyram and prothioconazole		68 a		124 a
Fluopyram and prothioconazole + Oxamyl		67 a		113 a
Oxamyl		66 a		124 a
UTC		68 a		145 a

Table 4.4. Nematicide treatment impact on post-harvest nematode populations and average with standard deviation on preplant populations at Damon, College Station, and Wall, TX in 2019 and 2020.

+ College Station and Wall in 2019, and College Station, Damon, and Wall in 2020.

*A, pre-plant average ; B, post-harvest

Discussion

The lack of yield differences between nematicide treatments in Damon 2019 could be attributed to environmental factors. Above average rainfall in May and June accumulating 392 mm may have led to rapid degradation of the products, resulting in less efficacy against reniform nematodes (Bromilow et al., 1980; Haydock et al., 2012; Wheeler et al., 2013; Faske and Brown, 2019). The Lake Charles Clay series is moderately well drained with very slow permeability and has 45-60 % clay (National Cooperative Soil Survey, 2014). These factors coupled with above average rainfall likely resulted in the soil remaining saturated for longer periods of time.

The impact nematicide application had on height 80 DAP was between oxamyl and the untreated check, but none of the other treatments. This could be attributed to application timing vs. product degradation, more favorable environmental conditions, and that oxamyl is absorbed and translocated within the plant (in contrast to the other nematicide treatments). The last application of oxamyl was the nearest nematicide treatment timing (35 days) to the first height measurements. Oxamyl generally suppresses stunting due to reniform nematodes for 7 to 14 days following application (Bromilow et al., 1980), so height differences detected at 80 DAP followed the peak activity of the product and dissipated by 100 DAP.

A factor contributing to the lack of differences in nematode populations due to nematicide treatments could be sample timing. The efficacy of the aldicarb and oxamyl decrease as the growing season progresses (Harvey et al., 1978; Bromilow et al., 1980). Analyzing soils post-harvest may not have captured an earlier change in nematode populations (Bromilow et al., 1980). Although end of season nematode populations were not impacted by nematicide applications, other studies have confirmed nematode suppression with aldicarb by 59% compared to the untreated check at 30 DAP (Schrimsher et al., 2014). Other treatment combinations in this study such as aldicarb and oxamyl have also been shown to reduce mid and late season reniform nematode populations (Lawrence and McLean, 2000).

The yield increase with the aldicarb and oxamyl treatment compared to the untreated check was not sufficient to offset the chemical and application costs and resulted in no partial economic gain or loss. The net increase from this treatment was \$113 ha⁻¹and the treatment cost was \$159.89 ha⁻¹. The added cost of the oxamyl application did not justify the observed yield increase relative to the untreated check. The application of aldicarb alone was more profitable than the application of fluopyram + prothioconazole and oxamyl that did not benefit yield.

This work compared chemical applications with susceptible and RKN cultivars. As cultivars with specific REN resistance are released, growers have the option to combine this resistance with chemical applications. A study published in 2014 compared REN resistant to susceptible genotypes with and without the addition of nematicide treatments including in-furrow aldicarb (Schrimsher et al., 2014). They reported significant yield increases with nematicide applications across both REN and susceptible genotypes. Therefore, combining nematicides with genetic resistance warrants consideration and further investigation.

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CHAPTER V

CONCLUSION

Reniform nematode resistant cotton cultivars have shown potential to preserve yield compared to cultivars with RKN resistance and those without nematode resistance in reniform nematode infested fields. In both years and all locations, the reniform nematode resistant cotton yielded among the top. Specifically, PHY 443 W3FE and PHY 332 W3FE yielded higher than all other cultivars except at Damon in 2019. DP 2143NR B3XF also resulted in a 16% yield increase compared to the susceptible cultivar, although it was not as consistently among the highest yielding. This study did not result in drastic differences in nematode population suppression, although one cultivar with reniform resistance did reduce reniform nematode populations more effectively than cultivars with only RKN resistance. Among nematicide treatments, the application of aldicarb and oxamyl resulted in higher yields. However, observed yield increases were not great enough to benefit the producer economically. The findings of both studies combined show reniform nematode resistance in cotton is likely a more effective tool in nematode management than application of the nematicides tested.

REFERENCES

- Ayala, A., and C.T. Ramirez. 1964. Host-range, distribution, and bibliography of the reniform nematode, *Rotylenchulus reniformis*, with special reference to Puerto Rico. J. Agric. Univ. Puerto Rico 48(2): 140–161.
- Bell, A.A., A. Forest Robinson, J. Quintana, N.D. Dighe, M.A. Menz, et al. 2014. Registration of LONREN-1 and LONREN-2 Germplasm Lines of Upland Cotton Resistant to Reniform Nematode. J. Plant Regist. 8(2): 187–190. doi: 10.3198/jpr2013.11.0069crg.
- Bell, A., and A.F. Robinson. 2004. Development and characteristics of triple species hybrids used to transfer reniform nematode resistance from *Gossypium longicalyx* to *Gossypium hirsutum*. Proceedings of the Beltwide Cotton Conferences. p. 422– 426
- Bell, A.A., A.F. Robinson, J. Quintana, S.E. Duke, J.L. Starr, et al. 2015. Registration of BARBREN-713 Germplasm Line of Upland Cotton Resistant to Reniform and Root-Knot Nematodes. J. Plant Regist. 9(1): 89–93. doi: 10.3198/jpr2014.04.0021crg.
- Birchfield, W., and L.R. Brister. 1963. Susceptibility of Cotton and Relatives to Reniform Nematode in Louisiana. Plant Dis. Report. 47(11): 990–992.
- Box, G.E., and D.R. Cox. 1964. An analysis of transformations. J. Am. Stat. Assoc. 26(2): 211–252. doi: 10.1080/01621459.1982.10477788.
- Bromilow, R.H., R.J. Baker, M.A.H. Freeman, and K. Görög. 1980. The degradation of aldicarb and oxamyl in soil. Pestic. Sci. 11(4): 371–378. doi: 10.1002/ps.2780110402.
- Coppedge, J.R., D.L. Bull, and R.L. Ridgway. 1977. Movement and persistence of aldicarb in certain soils. Arch. Environ. Contam. Toxicol. 5(1): 129–141. doi: 10.1007/BF02220896.
- Cotton Incorporated. 2019. 2019/2020 Upland Loan Calculator.
- Cotton Incorporated. 2020. 2020/21 Upland Loan Calculator.
- Davis, R.F., G.B. Jagdale, P. Bertrand, J.D. Gay, R.E. Baird, et al. 2009. Guide for Interpreting Nematode Assay Results.

Ebone, L.A., M. Kovaleski, and C. Cardoso Deuner. 2019. Nematicides: History, mode,

and mechanism action. Plant Sci. Today 6(2): 91–97. doi: 10.14719/pst.2019.6.2.468.

- Faske, T.R., and K. Brown. 2019. Movement of seed- And soil-applied fluopyram in soil columns. J. Nematol. 51(1): 1–8. doi: 10.21307/jofnem-2019-045.
- Faske, T.R., and K. Hurd. 2015. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to fluopyram. J. Nematol. 47(4): 316–321.
- Ganji, S., M.J. Wubben, and J.N. Jenkins. 2013. Two simple methods for the collection of individual life stages of reniform nematode, *Rotylenchulus reniformis*. J. Nematol. 45(2): 87–91. https://www-ncbi-nlm-nih-gov.srvproxy2.library.tamu.edu/pmc/articles/PMC3700741/pdf/87.pdf (accessed 3 September 2019).
- Handoo, Z.A., and D. Ellington. 2017. Some procedures for collecting and preparing nematodes for study. https://www.ars.usda.gov/northeast-area/beltsville-md-barc/beltsville-agricultural-research-center/mycology-and-nematology-genetic-diversity-and-biology-laboratory/people/zafar-handoo/nematode-extraction-procedures/.
- Harvey, J., J.C.Y. Han, and R.W. Reiser. 1978. Metabolism of Oxamyl in Plants. J. Agric. Food Chem. 26(3): 529–536. doi: 10.1021/jf60217a007.
- Haydock, P.P.J., E.L. Ambrose, A. Wilcox, and T. Deliopoulos. 2012. Degradation of the nematicide oxamyl under field and laboratory conditions. Nematology 14(3): 339–352. doi: 10.1163/156854111X597485.
- Haydock, P.P.J., S.R. Woods, I.G. Grove, and M.C. Hare. 2006. Chemical control of nematodes. In: Perry, R.N. and Moens, M., editors, Plant Nematology. p. 392–410
- Klose, S. 2020. 2020 Texas Agricultural Custom Rates.
- Koenning, S.R., S.A. Walters, and K.R. Barker. 1996. Impact of soil texture on the reproductive and damage potentials of *Rotylenchulus reniformis* and *Meloidogyne incognita* on cotton. J. Nematol. 28(4): 527–536.
- Koenning, S.R., J.A. Wrather, T.L. Kirkpatrick, N.R. Walker, J.L. Starr, et al. 2004. Plant-parasitic nematodes attacking cotton in the United States old and emerging production challenges. https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.2004.88.2.100 (accessed 14 August 2019).

- Lawrence, K. 2020. 2020 Alabama Row Crops Short Course. https://cses.auburn.edu/2020-alabama-row-crops-short-course/ (accessed 2 May 2021).
- Lawrence, K., A. Hagan, R. Norton, J. Hu, T. Faske, et al. 2020. Cotton Disease Loss Estimate Committee Report, 2019. Proc. Beltwide Cott. Conf.: 117–119.
- Lawrence, G.W., and K.S. McLean. 2000. Effect of foliar applications of oxamyl with aldicarb for the management of Rotylenchulus reniformis on cotton. J. Nematol. 32: 542–549.
- Lee, J.-D., H.-J. Kim, R.T. Robbins, J.A. Wrather, J. Bond, et al. 2015. Reaction of Soybean Cyst Nematode Resistant Plant Introductions to Root-Knot and Reniform Nematodes. Plant Breed. Biotechnol. 3(4): 346–354. doi: 10.9787/pbb.2015.3.4.346.
- Linford, M.B., and J.M. Oliveira. 1940. *Rotylenchulus reniformis*, nov. gen., n. sp., a nematode parasite of roots.'. Proceeding Helminthol. Soc. Washingt. 7: 35–42.
- McCarty, J.C., J.N. Jenkins, M.J. Wubben, O.A. Gutierrez, R.W. Hayes, et al. 2013. Registration of Three Germplasm Lines of Cotton Derived from *Gossypium barbadense L*. Accession GB713 with Resistance to the Reniform Nematode. J. Plant Regist. 7(2): 220–223. doi: 10.3198/jpr2012.08.0024crg.
- Moorman, G.W. 2011. Phytotoxicity. https://extension.psu.edu/phytotoxicity (accessed 2 May 2021).
- Nakasono, K. 1973. Studies on post-embryonic development of the reniform nematode, *rotylenchulus reniformis* linford and oliveira (nematoda : Rotylenchulidae) : I.
 Morphological changes of the molting larva of a parthenogenetic population. Appl. Entomol. Zool. 8(2): 83–96. doi: 10.1303/aez.8.83.
- National Cooperative Soil Survey. 2014. Lake Charles Series. https://soilseries.sc.egov.usda.gov/OSD_Docs/L/LAKE_CHARLES.html (accessed 4 February 2021).
- Rich, J.R., and G.W. Bird. 1973. Inhibition of *Rotylenchulus reniformis* Penetration of Tomato and Cotton Roots with Foliar Applications of Oxamyl effective in controlling *Meloidogyne incognita*. J. Nematol. 5(3): 221–224.
- Robinson, A.F. 2007. Nematode Management in Cotton. In: Ciancio, A. and Mukerji, K.G., editors, Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Springer. p. 149–182

- Robinson, A.F. 2008. Reniform in U.S. cotton: When, where, why, and some remedies. Annu. Rev. Phytopathol. 45(1): 263–288. doi: 10.1146/annurev.phyto.45.011107.143949.
- Robinson, A.F., A.C. Bridges, and A.E. Percival. 2004. New sources of resistance to the reniform (*Rotylenchulus reniformis* Linford and Oliveira) and root-knot (*Meloidogyne incognita* (Kofoid & White) Chitwood) nematode in upland (*Gossypium hirsutum L.*) and Sea Island (*G. barbadense L.*) cotton. J. Cotton Sci. 8(3): 191–197.
- Robinson, A.F., and C.M. Heald. 1989. Accelerated movement of nematodes from soil in baermann funnels with temperature gradients. J. Nematol. 21(3): 370–378.
- Robinson, A.F., R.N. Inserra, E.P. Caswell-Chen, N. Vovlas, and A. Troccoli. 1997. *Rotylenchulus* species: Identification, distribution, host ranges, and crop plant resistance. Nematropica 27(2): 127–180.
- Robinson, A.F., and A.E. Percival. 1997. Resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in wild accessions of *Gossypium hirsutum* and *G. barbadense* from Mexico. J. Nematol. 29(4 SUPPL): 746–755.
- Schrimsher, D.W., K.S. Lawrence, R.B. Sikkens, and D.B. Weaver. 2014. Nematicides enhance growth and yield of *rotylenchulus reniformis* resistant cotton genotypes. J. Nematol. 46(4): 365–375.
- Sikkens, R.B., D.B. Weaver, K.S. Lawrence, S.R. Moore, and E. Van Santen. 2011. Lonren upland cotton germplasm response to *rotylenchulus reniformis* inoculum level. Nematropica 41(1): 68–74.
- Spinks, C.S., M. Emerson, and T.R. Faske. 2001. Assessment of seed-applied fluopyram for management of *meloidogyne incognita* in soybean. Nematropica 50(1): 118– 126.
- Starr, J.L., C.W. Smith, K. Ripple, E. Zhou, R.L. Nichols, et al. 2011. Registration of TAM RKRNR-9 and TAM RKRNR-12 Germplasm Lines of Upland Cotton Resistant to Reniform and Root-Knot Nematodes. J. Plant Regist. 5(3): 393–396. doi: 10.3198/jpr2011.01.0048crg.
- Steele, A.E., and L.R. Hodges. 1975. In-Vitro and In-Vivo Effects of Aldicarb on Survival and Development of *Heterodera schachtii* of potato roots by *Heterodera rostochiensis* effects of aqueous solutions of aldicarb on hatching and emergence of larvae from cysts : Effects o f aldicarb and its. J. Nematol. 7(3): 305–312.
- Stetina, S.R. 2015. Postinfection development of *rotylenchulus reniformis* on resistant *gossypium barbadense* accessions. J. Nematol. 47(4): 302–309.

- Tylka, G.L., G.D. Gebhart, C.C. Marett, and M.P. Mullaney. 2013. Evaluation of Soybean Varieties Resistant to Soybean Cyst Nematode in Iowa—2019.
- United States Department of Agriculture National Statistics Service. 2020. Crop Production 2019 Summary.
- USDA-NRCS. 2019. Websoil Survey. https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx.
- Weaver, D.B., K.S. Lawrence, and E. Van Santen. 2007. Reniform nematode resistance in upland cotton germplasm. Crop Sci. 47(1): 19–24. doi: 10.2135/cropsci2006.02.0130.
- Wheeler, T.A., K.S. Lawrence, D.O. Porter, W. Keeling, and B.G. Mullinix. 2013. The relationship between environmental variables and response of cotton to nematicides. J. Nematol. 45(1): 8–16.
- Wheeler, T.A., K.T. Siders, M.G. Anderson, S.A. Russell, J.E. Woodward, et al. 2014. Management of *meloidogyne incognita* with Chemicals and Cultivars in Cotton in a Semi-Arid Environment. J. Nematol. 46(2): 101–7. http://www.ncbi.nlm.nih.gov/pubmed/24987161%0Ahttp://www.pubmedcentral.ni h.gov/articlerender.fcgi?artid=PMC4077170.
- Whitehead, A.G., and J.R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55(1): 25–38. doi: 10.1111/j.1744-7348.1965.tb07864.x.
- Womersley, C., and C. Ching. 1988. Natural dehydration regimes as a prerequisite for the successful induction of anhydrobiosis in the nematode *rotylenchulus reniformis*. J. Exp. Biol. 143(01): 359–372.
- Wubben, M.J., S. Ganji, and F.E. Callahan. 2010. Identification and molecular characterization of a β -1,4-endoglucanase gene (Rr-eng-1) from *rotylenchulus reniformis*. J. Nematol. 42(4): 342–351.
- Yik, C.P., and W. Birchfield. 1984. Resistant germplasm in gossypium species and related plants to *rotylenchulus reniformis*. J. Nematol. 16(2): 146–53. http://www.ncbi.nlm.nih.gov/pubmed/19295892%0Ahttp://www.pubmedcentral.ni h.gov/articlerender.fcgi?artid=PMC2618364.