RESPONSE OF FIVE RESISTANT COTTON GENOTYPES TO ISOLATES OF *ROTYLENCHULUS RENIFORMIS* COLLECTED FROM RENIFORM NEMATODE INFESTED FIELDS OF LOUISIANA

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ABSTRACT

Bhandari, B., G. O. Myers, M. O. Indest, and C. Overstreet. 2015. Response of five resistant cotton genotypes to isolates of *Rotylenchulus reniformis* collected from reniform nematode infested fields of Louisiana. Nematropica 45:252-262.

Reniform nematode (*Rotylenchulus reniformis*) is a significant cotton (*Gossypium* spp.) parasite in the southern United States, causing an estimated 4% yield loss. Variation in reproduction and pathogenicity across reniform nematode isolates collected from Louisiana on susceptible cotton has been reported. This greenhouse study was conducted to determine the response of five cotton genotypes that varied in resistance to the reniform nematode to five isolates of *R. reniformis* collected from Louisiana cotton fields. Across cotton genotypes, the Evan and Avoyelles reniform nematode isolates had higher reproduction (33,793 and 27,800 juveniles/250 g of soil, respectively) than the LA, Old Crop Rotation, and Oak Tree Cut isolates. Across reniform nematode isolates, the mean number of juveniles on *G. arboreum* (A_2-190) and LONREN-2 (5,573 and 6,013 juveniles, respectively) was significantly lower than on Delta Pearl, TX-110, BARBREN-713 and LONREN-1 genotypes. There was a significant interaction between the cotton genotypes and reniform nematode isolates. However, all isolates exhibited the highest reproduction on the susceptible cultivar Delta Pearl, and less reproduction on all genotypes that had previously shown attributes of reniform nematode resistance. The LONREN-1, LONREN-2, and A_2-190 genotypes displayed a hypersensitive reaction, characterized by reduced plant height, against the Evan and Avoyelles isolates that was greater than with the other isolates. This study demonstrates that there is variability in reproduction and pathogenicity among reniform nematode isolates in Louisiana. Based upon nematode reproduction, *G. arboreum* (A_2-190) and LONREN-2 were the most resistant across all nematode isolates. With respect to pathogenicity, TX-110 and BARBREN-713 were the most tolerant to reniform nematodes and could be useful in developing cultivars that are both resistant and tolerant to the nematode.

Key words: genotypes, *Gossypium arboreum*, *G. hirsutum*, nematode resistance, *Rotylenchulus reniformis*.

RESUMEN

Bhandari, B., G. O. Myers, M. O. Indest, y C. Overstreet. 2015. Respuesta de cinco genotipos de algodón resistentes a aislados de *Rotylenchulus reniformis* recolectados en campos de Louisiana infestados por nematodos reniformes. Nematropica 45:252-262.

El nematodo reniforme (*Rotylenchulus reniformis*) es un importante parásito del algodón (*Gossypium* spp.) en el sur de los Estados Unidos de América, causando pérdidas estimadas en torno al 4% de la producción. Se han citado variaciones en la reproducción y patogenicidad en algodón susceptible de diversos aislados del nematodo reniforme recolectados en Louisiana. Este estudio en invernadero se llevó a cabo para determinar la respuesta de cinco genotipos de algodón, que variaban en la resistencia al nematodo reniforme, a cinco aislados de *R. reniformis* recolectados en campos de algodón de Louisiana. Para los genotipos de algodón en conjunto, los aislados de nematodos reniformes Evan y Avoyelles tuvieron mayor reproducción (33,793 y 27,800 juveniles/250 g de suelo, respectivamente) que los aislados LA, Old Crop Rotation, y Oak Tree Cut. Para los aislados de nematodos reniformes en conjunto, el número medio de juveniles en G. arboreum (A_2-190) y LONREN-2 (5,573 y 6,013 juveniles, respectivamente) fueron significativamente menores que en los genotipos Delta Pearl, TX-110, BARBREN-713 y LONREN-1. Hubo una interacción significativa entre los genotipos de algodón y los asilados de nematodos reniformes. No obstante, todos los aislados mostraron la mayor reproducción en el cultivar

susceptible Delta Pearl, y menor reproducción en todos los genotipos que habían mostrado previamente atributos de resistencia al nematodo reniforme. Los genotipos LONREN-1, LONREN-2, y A_2-190 mostraron una reacción de hipersensibilidad, caracterizada por una altura de la planta reducida, frente a los aislados Evan y Avoyelles, en los que la reacción fue mayor que frente a los otros aislados. Este estudio demuestra que hay variabilidad en la reproducción y patogenicidad entre aislados del nematodo reniforme en Louisiana. Basándose en la reproducción del nematodo, *G. arboreum* (A_2-190) y LONREN-2 fueron los más resistentes frente al conjunto de aislados del nematodo. En relación a la patogenicidad, TX-110 y BARBREN-713 fueron los más tolerantes a los nematodos reniformes y podrían resultar útiles en el desarrollo de cultivares que sean a la vez resistentes y tolerantes al nematodo.

Palabras clave: genotipos, *Gossypium arboreum*, *G. hirsutum*, resistencia a nematodos, *Rotylenchulus reniformis*.

INTRODUCTION

Cotton (primarily *Gossypium hirsutum* L. and to a lesser extent *G. barbadense*, *G. arboreum*, and *G. herbaceum*) is the leading textile fiber as well as one of the most important oilseed crops in the world. In terms of total area harvested, cotton ranks fourth after corn, soybean, and wheat in the United States. Globally, United States cotton production is ranked third after China and India. In the United States, it is estimated that 16.08 million bales were produced in 2014/2015, which is 25% higher than in 2013/2014 (USDA, 2014). The production increase in 2014/15 is largely a result in an increase in production area from 3.05 to 3.93 million hectares (USDA, 2014). As an oilseed, cotton is ranked third worldwide in terms of volume behind soybean and corn (National Cottonseed Products Association, 2014). The oil produced from cotton is largely used for human consumption. The cake left after oil extraction is a high protein animal feed principally used in the beef and dairy industries. Collectively, these uses contribute to cotton's prominence as one of the important agricultural row crops in the United States.

Cotton is vulnerable to several insect pests and diseases that decrease production. Out of a 12% loss in cotton production caused by various insects and diseases, the loss caused by reniform nematodes (*Rotylenchulus reniformis* Linford & Oliveira) is estimated to be 1.48% in the U.S. (Lawrence *et al*., 2014). The most severe yield losses (> 4%) to reniform nematode are observed in Louisiana, Arkansas, Georgia, Mississippi, Texas, and Tennessee (Lawrence *et al*., 2014). Depending upon the level of infestation, cultivars grown, and environmental conditions, yield losses caused by reniform nematode have been estimated to be as high as 40% (Farias *et al*., 2002).

The reniform nematode was first reported as a cotton parasite in Louisiana in 1941 (Smith and Taylor, 1941). Since the initial report of its occurrence in Louisiana, the reniform nematode has spread, increasing in incidence from 3 to 24 parishes during the period of 1961 to 2010 (McGawley *et al*., 2010). Compared to the root-knot nematode, *Meloidogyne incognita* Kofoid & White (Chitwood), the area infested by the reniform nematode has increased rapidly over the years because of its short life cycle (16-22 d), its ability to establish feeding sites along primary, secondary, and tertiary roots, as well as its ability to survive under desiccated soil conditions (Rebois, 1973; Gaur and Perry, 1991). Due to its aggressive nature, the reniform nematode is more competitive than root-knot nematode in cotton fields and has rapidly become the major nematode pathogen affecting cotton production in the southern United States (Robinson, 2007).

The reniform nematode is a sedentary, amphimictic, and semi-endoparasitic nematode that feeds on more than 350 plant species across 77 families in warm temperate, sub-tropical, and tropical regions of world (Gaur and Perry, 1991). The mature female is easily identified by her kidney shape, while the male is vermiform in shape and shorter than females. The life cycle of the reniform nematode is comprised of the egg and four vermiform juvenile stages (J1, J2, J3, J4) and adults. A mature female can lay from 60 to 200 eggs in a gelatinous matrix exuded on the surface of plant roots (Dasgupta and Seshadri, 1971). It takes 7 to 10 d for egg hatching before entering the different vermiform stages, which are demarcated by molting. Upon infection, the anterior portion of the female is embedded in the root, whereas the posterior portion remains outside the root surface. Infection is accompanied by the formation of a feeding site called a syncytium, which is a multinucleate cell in the host that arises from the dissolution of adjacent cell walls (Cohn, 1973; Heald, 1975). After establishing a feeding site in

the root cortex, females develop further and form the typical kidney shape (Gaur and Perry, 1991). The life cycle of the reniform nematode normally takes about 16 to 22 d, but is dependent upon the host species, temperature, and soil conditions (Bird, 1984; Gaur and Perry, 1991; Leach *et al.*, 2009). Host plant symptoms include stunting, yellowing of lower leaves, necrosis of the lower leaf margins and tips of leaves, a delay in maturity, and yield reduction (Jones *et al*., 1959; Birchfield and Jones, 1961).

Cotton growers have various management options available to reduce yield loss due to reniform nematode infestation. These include crop rotation, the use of nematicides, or the planting of resistant or tolerant cultivars (Davis *et al.*, 2003; Robinson, 2007; Starr *et al*., 2007; Burris *et al.*, 2010). Crop rotation with non-host crops, such as peanut, corn, resistant soybean cultivars, or sorghum is effective in reducing the reniform nematode population (Gazaway *et al*., 2000; Davis *et al*., 2003; Koenning *et al*., 2004). Nematicides are a reliable option for growers because they can be applied at the time of planting and effectively reduce initial nematode population densities (Lawrence *et al*., 1990; Lawrence and McLean, 2000; Rich and Kinloch, 2000; Wolcott *et al*., 2005). However, there are environmental concerns associated with nematicide use and they can be expensive. Host plant resistance is an effective, and typically profitable, management option to control nematode infestations in cotton fields. Although there are currently no reniform nematode resistant cotton cultivars available commercially, to date, several cotton germplasm lines that show moderate to high levels of resistance or tolerance to the reniform nematode have been released (Yik and Birchfield, 1984; Robinson and Percival, 1997; Robinson *et al*., 2004; McCarty *et al*., 2012; McCarty *et al*., 2013; Bell *et al*., 2014a, b).

Due to increasing incidence of reniform nematodes in cotton fields, researchers undertook the screening of wild and cultivated species of cotton genotypes to identify a source of resistance. Yik and Birchfield (1984) evaluated four different species of *Gossypium* and found that the germplasm line TX-110 was highly resistant to reniform nematodes. Robinson *et al*. (2004) screened 1,866 primitive accessions of *G. hirsutum* and 907 of *G. barbendese* against reniform nematodes. They reported that a majority of the *G. hirsutum* accessions were moderately to highly susceptible to reniform nematode, while six primitive accessions of *G. barbendese* were resistant to the nematode. Out of these six accessions, GB-713 was highly resistant to reniform nematodes and has been widely used to develop reniform nematode resistant breeding germplasm (Bell *et al*., 2014b). Bell *et al*. (2014a) developed two highly resistant lines; LONREN-1 and LONREN-2 by introgression of a source of reniform nematode resistance from *G. longicalyx* into upland cotton. Stewart and Robbins (1995) evaluated Asiatic cotton germplasm and found that *G. arboreum* (A_2-190) was highly resistant to reniform nematodes. Although moderate levels of reniform nematode resistance were observed in wild species of *G. aridum* and *G. herbaceum*, they are not extensively used for breeding because of genetic incompatibility and linkage drag.

Research on the reniform nematode generally has been conducted by using a single isolate collected from a specific geographical region of the United States, typically from a local infested field. However, variation, both morphological and genetic, as well as in reproduction and pathogenicity of the isolates, has been observed (Dasgupta and Seshadri, 1971; Agudelo *et al*., 2005; Tilahun *et al.*, 2008; Arias *et al*., 2009; McGawley *et al.*, 2010). Dasgupta and Seshadri (1971) designated two races of reniform nematode, race A and race B, based on host assay and the rate of reproduction on castor, cowpea and cotton in India. Out of ten isolates, nine isolates of similar morphology reproduced on all three hosts, while one isolate reproduced only on cowpea. In Japan, Nakasono (2004) classified the reniform nematode into three categories: small, medium, and large based on body size and three different biological types, male-numerous, male-rare, and male-absent. Rao and Ganguly (1998) reported a variation in body length and width, stylet length, distance from head to vulva, and position of the dorsal esophageal gland orifice among reniform nematode populations from different geographic regions in India. Agudelo *et al.* (2005) observed variation in nematode morphology and reproduction among isolates collected from different geographical regions. They reported that a reniform nematode population collected from Hawaii has a larger body than other isolates, while a population collected from Limestone, Alabama, had a small body size. Morphological variations including size and length of stylet, position of esophagus gland orifice, and esophagus length were also observed among reniform nematode populations. A population collected from Limestone, Alabama, had a higher rate of reproduction on the hosts than isolates collected from Huxford, Alabama, Louisiana, and Hawaii.

Based on the 18S ribosomal DNA and first internally transcribed space (ITS1), genetic variation was observed among the populations collected within reniform nematode infested

fields of Alabama (Tilahun *et al.*, 2008). Arias *et al.* (2009) reported that 88 microsatellite markers are polymorphic across six isolates collected from Texas, Louisiana, Mississippi, and Georgia. The reniform nematode isolate collected from Georgia had the highest reproduction and pathogenicity compared to other isolates. McGawley *et al.* (2010) showed that reniform nematode populations collected from Mississippi and Louisiana had higher reproduction than populations collected in Arkansas, Texas, Hawaii, and Alabama. Xavier *et al.* (2014) reported variation among different isolates of reniform nematode on susceptible cotton cultivars. A common feature of all of these studies, however, is that the reproduction and pathogenicity tests were conducted upon a single-host genotype, although not necessarily the same one across the studies.

There is now ample evidence that there is variability in reproduction and pathogenicity among reniform nematode isolates from different regions of the United States. This variation may have an impact on host-plant resistance development and deployment. It is unknown if there is variation among reniform nematode isolates within Louisiana or if variation is detectable by the use of different host genotypes of the same genus. It would be valuable to establish a differential response of resistant and tolerant lines of cotton to different reniform nematode isolates if such variation exists. Therefore, this study seeks to evaluate the response of resistant cotton genotypes to reniform nematode isolates collected from reniform nematode-infested fields in Louisiana and provide information useful to plant breeders for future research to develop cotton genotypes with resistance and tolerance to the reniform nematode.

MATERIALS AND METHODS

Reniform nematode isolates and cotton genotypes

Five reniform nematode isolates collected from reniform nematode-infested fields in Louisiana were used in this study (Table 1). Using a dissecting microscope, 25 egg masses were collected from each isolate and transferred to previously established tomato seedlings (*Solanum lycopericum* L. cv. 'Rutgers') planted in 20.3-cm terra cotta pots filled with steam-sterilized sandy loam soil in a greenhouse under natural light conditions. The reniform nematode isolates were carefully handled and maintained in the greenhouse in a manner to maintain nematode isolate purity by minimizing the chance of cross contamination. Inoculum was extracted on the day of inoculation by using the centrifugal sugar flotation technique (Jenkins, 1964).

General information

Seed of resistant and susceptible cotton genotypes were planted in 3.8-L plastic pots filled with steam-sterilized sandy loam soil in summer 2013 at a seeding rate of two seeds per pot. The pots were arranged in randomized complete block design (RCBD) with a factorial arrangement of treatments. Treatments consisted of five reniform nematode isolates and five cotton genotypes, and each treatment combination was replicated five times in a greenhouse. The experiment was repeated in the early fall of 2013. The cotton variety "Delta Pearl" (PVP 20000061, Delta & Pine Land, Co., Scott, MS) was used as the susceptible check. Plants without reniform nematode inoculation were used as controls. After seed germination, pots were thinned to one seedling per pot. At 7 d after germination, 10,000 vermiform nematodes from each isolate were used to inoculate appropriate pots. The inoculum was injected 2 to 5 cm deep into the soil at two spots 1 to 2 cm away from the plant stem to facilitate access to the host root system. The pots were watered via drip irrigation as required to maintain adequate soil moisture to support the plant growth. The plants were fertilized as needed for adequate growth. The pots were harvested at 9 wk

Table 1. Reniform nematode isolates and cotton genotypes used in this study.

Isolate	Parish of origin of isolates	Cotton genotypes	References
Evan	Evangeline	LONREN-1	Bell *et al.* (2014)
LA	Rapides	LONREN-2	Bell *et al.* (2014)
Avoyelles	Avoyelles	BARBREN-713	Bell *et al.* (2014)
Oak Tree cut	Tensas	TX-110	Yik and Birchfield (1984)
Old Crop rotation	Tensas	A_2-190 Delta Pearl	Stewart and Robbins (1995)

(63 d) after inoculation.

Plant height from cotyledon scar to the terminal was recorded. Harvested shoots and roots of each genotype were oven dried at 65°C for 72 hr, and the weight was recorded. Soil from individual pots was carefully transferred to a flat plastic pan and roots and root fragments removed. After thoroughly mixing the soil, 250 g of soil was taken for extraction using an elutriator (Byrd *et al*., 1976). A soil suspension was poured through the elutriator and collected on stacked sieves arranged with a sieve of 140-µm pore size over a sieve with a 37-µm pore size. The nematodes were washed from the finer mesh sieve, extracted by sucrose centrifugation, (Jenkins, 1964) and enumerated.

Data analysis

Analysis of variance (ANOVA) was conducted using SAS 9.3 (SAS Institute Inc., Cary, NC) for number of vermiform nematodes per 250 g soil, plant height, dry shoot and root weight. Prior to ANOVA, the numbers of vermiform nematodes were transformed (log_{10}) to meet an assumption of normality. To determine the difference among isolates and genotypes, T-grouping was used for mean comparisons.

RESULTS

Reproduction of reniform nematode isolates on cotton genotypes

There were differences ($P < 0.01$) among reniform nematode isolates and cotton genotypes. There was a significant interaction ($P < 0.01$) between cotton genotype and reniform nematode isolate with respect to reniform nematode reproduction (Table 2). Reproduction over all the cotton genotypes was higher for the Evan and Avoyelles isolates than for the other isolates, and reproduction was lowest for the Oak Tree Cut isolate (Fig. 1). There was higher reproduction by all reniform nematode isolates on the susceptible cultivar Delta Pearl than on all other cotton genotypes (Fig. 2). Reproduction of all isolates on LONREN-2 and *G. arboreum* (A_2-190) was significantly lower than on LONREN-1, TX-110, and BARBREN-713 genotypes (Fig. 2).

On Delta Pearl, the Evan isolate had the highest reproduction (107,600 vermiform nematodes/250

Table 2. Number of vermiform nematodes as affected by reniform nematode isolate and cotton genotype.[x]

Source	DF	Mean square	F value	Pr > F
Isolates[y]	4	1.52	36.54	<0.01
Genotypes[z]	5	8.41	201.89	<0.01
Isolates x Genotypes	20	0.11	2.73	<0.01

[x]Data combined over two 9-wk duration experiments with a total of ten replications. ANOVA and T-grouping were used to analyze the data ($P \leq 0.05$).

[y]Isolates were collected from Evangeline, Rapides, Avoyelles, and Tensas Parishes.

[z]Genotypes are *Gossypium arboreum* (A_2-190), LONREN-1, LONREN-2, BARBREN-713, TX-110, and Delta Pearl.

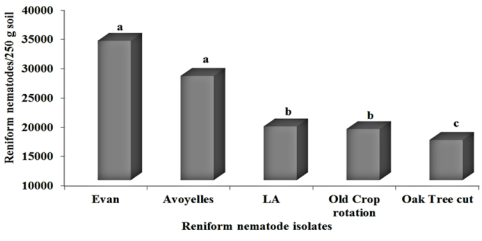

Fig. 1. Reproduction of the five reniform nematode isolates across the six cotton genotypes. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

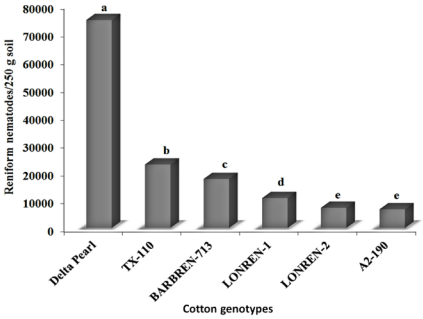

Fig. 2. Reproduction on six cotton genotypes across five reniform nematode isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping)

Table 3. Number of vermiform nematodes as affected by reniform nematode isolates and cotton genotypes.[w]

	Reniform nematode isolates[y]				
Genotypes[x]	Evan	Avoyelles	LA	Oak Tree cut	Old Crop rotation
Delta Pearl	107,600 aw[z]	86,080 abw	66,800 bcw	61,760 bcw	51,040 cw
TX-110	29,520 abx	32,000 ax	14,960 cx	17,000 cx	20,280 bcx
BARBREN-713	29,320 ax	21,520 ay	10,560 by	12,720 bx	13,760 by
LONREN-1	17,720 ay	10,640 bz	10,400 byz	3120 cy	11,760 abxy
LONREN-2	9360 az	7400 az	7520 az	3360 by	8440 ay
A_2-190	9240 az	9160 az	4760 bcz	3200 cy	7080 abz

[w]Data combined over two 9-wk duration experiments with a total of ten replications.
[x]Genotypes are *Gossypium arboreum* (A_2-190), LONREN-1, LONREN-2, BARBREN-713, TX-110, and Delta Pearl.
[y]Isolates were collected from Evangeline, Rapides, Avoyelles, and Tensas Parishes.
[z]Means followed by same letter in each row (a-c) and each column (w-z) do not differ significantly according to T-grouping, $P \leq 0.05$. T-grouping was used to analyze the data ($P \leq 0.05$).

Table 4. Impact of cotton genotypes and reniform nematode isolates on plant height, dry shoot, and root weight.[v]

		Plant height		Dry shoot weight[y]		Dry root weight[z]	
Source	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Isolates[w]	5	15.04	<0.01	7.50	<0.01	17.47	<0.01
Genotypes[x]	5	74.65	<0.01	26.25	<0.01	43.60	<0.01
Isolates[w] Genotypes	25	2.55	<0.01	1.35	0.12	1.20	0.24

[v]Data combined over two 9-wk duration experiments with a total of ten replications. ANOVA and T-grouping were used to analyze the data ($P \leq 0.05$).
[w]Isolates were collected from Evageline, Rapides, Avoyelles, and Tensas Parishes.
[x]Genotypes are *Gossypium arboreum* (A_2-190), LONREN-1, LONREN-2, BARBREN-713, TX-110, and Delta Pearl.
[y]Dry shoot weight was obtained by oven drying at 72°C for 72 hr.
[z]Dry root weight was obtained by oven drying at 72°C for 72 hr.

g soil) followed by the Avoyelles isolate (86,080 vermiform nematodes/250 g of soil), and both were significantly higher than the Old Crop Rotation isolate (51,040 vermiform nematodes/250 g soil) (Table 3). In contrast to reproduction on Delta Pearl, the Avoyelles isolate had the highest reproduction on TX-110 (32,000 vermiform nematodes/250 g soil) followed by the Evan isolate (29,520 vermiform nematodes/250 g soil) and was significantly different from the Oak Tree Cut and LA isolates. On BARBREN-713, the Evan isolate had the highest number of vermiform nematodes (29,320/250 g soil), but it was not significantly different from the Avoyelles isolate (21,520/250 g soil). The LA isolate had the lowest number of vermiform nematodes (10,560/250 g soil) and was not significantly different than the Old Crop Rotation and Oak Tree Cut isolates (Table 3).

On LONREN-1, the Evan isolate had the highest numerical number of vermiform nematodes (17,720/250 g soil), but was not significantly different from the Old Crop Rotation isolate (11,760/250 g soil). The Oak Tree Cut isolate (3,120 vermiform nematodes/250 g soil) had significantly lower reproduction than the other isolates (Table 3). The Evan isolate had the highest number of vermiform nematodes on LONREN-2 (9,360/250 g soil), but it was not significantly different compared to the Old Crop Rotation, LA, and Avoyelles isolates. Both LONREN-1 and LONREN-2 have resistance from similar sources, and they demonstrated a similar pattern of response to the different reniform nematode isolates. Compared to the other tetrapoloid cotton genotypes, both LONREN-1 and LONREN-2 suppressed reproduction the most across all isolates. Even the order of the isolates is generally preserved though LONREN-2 limited reproduction almost twice as much as LONREN-1. On the diploid cotton genotype, *G. arboreum* (A_2-190), the Evan isolate had the highest reproduction (9,240 vermiform nematodes/250 g soil) followed by the Avoyelles isolate (9,160/250 g soil), but they were not different from each other or from the Old Crop Rotation isolate (Table 3).

Effect of reniform nematode isolate on plant height

As was true for reproduction, there were significant differences ($P < 0.01$) among reniform nematode isolates and cotton genotypes for plant height (Table 4). There was also a significant interaction ($P < 0.01$) between the genotypes and isolates for plant height suggesting that there is a differential pathogenicity of reniform nematode isolates across the cotton genotypes. Across cotton genotypes, control plants that were not inoculated with reniform nematode isolates had the highest plant height (112.0 cm) and this difference was significant ($P < 0.01$). The tallest inoculated plants were in the Old Crop Rotation treatment (103.0 cm). Across cotton genotypes, the Evan isolate resulted in the shortest plant height (92.9 cm), followed by the Avoyelles isolates (94.8 cm), but they were not significantly different from each other (Fig. 3). Mirroring the reproduction numbers, the Evan and Avoyelles isolates, were the most aggressive on cotton and reduced plant height the most across the cotton genotypes (Fig. 3).

The LA isolate suppressed plant height to the greatest degree (88.1 cm) on Delta Pearl but was not significantly different from the other isolates (Fig. 4). On LONREN-1, LONREN-2, and *G. arboreum* (A_2-190), the Evan isolate reduced the plant height the most followed by the Avoyelles isolate, and both were significantly shorter in relation to control. The Avoyelles isolate had the biggest impact on BARBREN-713, but there were no significant differences among reniform nematode isolates for plant height (Fig. 4).

Effect of reniform nematode isolate on dry shoot weight

For dry shoot weight, there were significant differences among reniform nematode isolates and cotton genotypes, but the interaction between isolates and genotypes was not significant ($P = 0.12$) (Table 4). The uninoculated control had the highest dry shoot weight (18.6 g) and was significantly higher than cotton genotypes inoculated with the nematode isolates (Fig. 5). Although the cotton genotypes inoculated with the Avoyelles had the lowest mean dry shoot weight, the weights were

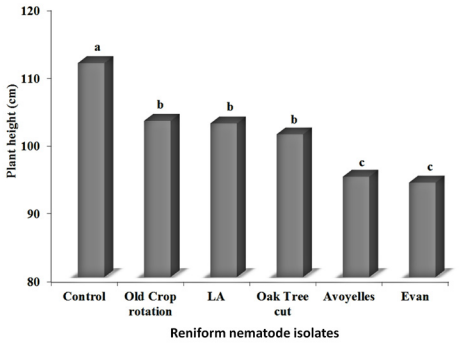

Fig. 3. Average height of six cotton genotypes across the five reniform nematode isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

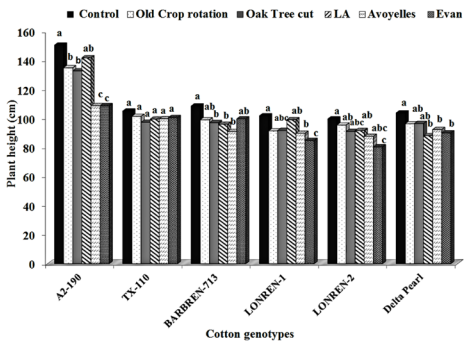

Fig. 4: Height of six cotton genotypes across five reniform nematode isolates. Within genotypes, means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

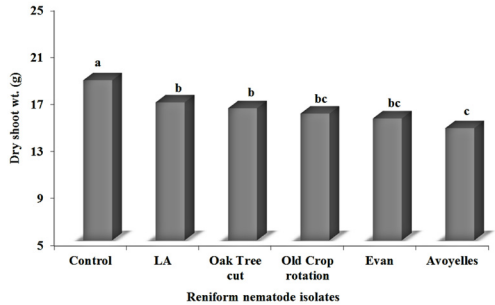

Fig. 5: Average dry shoot weight of six cotton genotypes across the five reniform nematode isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

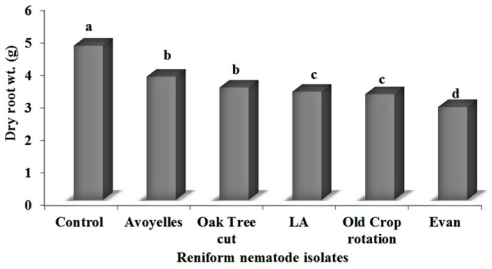

Fig. 6: Average dry root weight of six cotton genotypes across the five reniform nematode isolates. Means with same letter do not differ significantly ($P \leq 0.05$, T-grouping).

not different from those receiving the Evan and Old Crop Rotation isolates (Fig. 5).

Effect of reniform nematode isolate on dry root weight

There were significant differences among reniform nematode isolates and cotton genotypes with respect to dry root weight, but no interaction between reniform nematode isolates and cotton genotypes (P = 0.24) (Table 4). Dry root weight was higher for the uninoculated cotton genotypes (4.7 g) than for all inoculated cotton genotypes (Fig. 6). Across cotton genotypes, those inoculated with the Evan isolate had the lowest (P = 0.01) dry root weight (2.9 g) and were significantly lower than the Old Crop Rotation, LA, Oak Tree Cut, and Avoyelles isolates (Fig. 6).

DISCUSSION

This study revealed significant variation in reproduction and pathogenicity among reniform nematode isolates collected from cotton fields within Louisiana on a common cotton genotype. It was also found that the response of cotton genotypes that have been reported to be resistant were different across reniform nematode isolates. Variation in reproduction among isolates collected from different infested fields might be due to their adaptation to different soil textures (Koenning *et al*., 1996; Sturhan, 1971). Differences in reproduction and pathogenicity might also occur because of genetic variation in reniform nematode isolates (Tilahun *et al*., 2008; Arias *et al*., 2009). In addition to the polymorphism across the reniform nematode populations collected from different states in the United States, Arias *et al.* (2009) reported that 22 SSR markers showed the polymorphism among three reniform nematode populations collected from reniform nematode-infested fields in Mississippi.

Phenotyping and the identification of polymorphic molecular markers within segregating progenies are essential for successful quantitative trait loci (QTL) mapping and eventual marker-assisted selection. After identifying reniform nematode resistant germplasm, cotton breeders have been mapping populations and identifying QTL linked to reniform nematode resistance loci. Robinson *et al*. (2007) reported a single dominant gene was associated with reniform nematode resistance in *G. longicalyx*. Dighe *et al.* (2009) mapped a single dominant QTL locus, designated (*Ren*^{lon}), on chromosome 11 in *G. longicalyx*. Romano *et al*. (2009) reported that a single dominant QTL locus (*Ren*^{ari}) on chromosome 21 is responsible for reniform nematode resistance in *G. aridium*. Gutiérrez *et al.* (2011) found two major QTLs linked to reniform nematode resistance on chromosome 21 (*Ren*^{barb1}, *Ren*^{barb2}) and one minor QTL on chromosome 18 (*Ren*^{barb3}) in the *G. barbadense* L. accession GB 713. The underlying assumption in all these studies was that there is no variation among reniform nematode populations regardless of geographic origin, and (or) that the response of cotton genotypes across reniform nematode isolates is uniform. There is still a lack of information about whether these QTLs are stable across different reniform nematode isolates. In this study, the reproduction of reniform nematode isolates on LONREN-2 and *G. arboreum* (A_2-190) was significantly lower than on other cotton genotypes. LONREN-2 and *G. arboreum* (A_2-190) also had significantly different responses across the multiple reniform nematode isolates. It would be valuable to investigate if QTL map differences for reniform nematode resistance across diverse reniform nematode isolates exist.

Based on the reproduction potential, cotton fields infested with the Evan isolate may increase reniform nematode populations faster than fields infested with the Old Crop Rotation or the Oak Tree Cut isolates. It is anticipated that cotton fields infested with the Evan isolate may require a longer crop rotation with corn, sorghum, resistant soybean or peanut non-hosts than other reniform nematode isolates to suppress the population densities. Due to differential reproduction and host preferences, Kirkpatrick and Sasser (1984) recommended a specific crop rotation scheme for each race of root-knot nematode (*Meloidogyne incognita*) to suppress root-knot populations in cotton. Also, because of a differential rate of reproduction, application rates of nematicides could possibly be varied to manage specific reniform nematode isolates in a cotton fields in different regions.

With respect to development of reniform nematode resistant cotton cultivars, understanding the effectiveness of a source of resistance across nematode isolates will be vital to managing the reniform nematodes in infested fields. Based on the reproduction seen on TX-110 and BARBREN-713, improved cotton cultivars derived from these two sources are likely to increase reniform nematode populations faster than other, less susceptible cotton genotypes. Fortunately, neither TX-110 nor BARBREN-713 displayed a hypersensitive reaction to all of reniform nematode isolates studied. This was not the case for the LONREN-1, LONREN-2, and A_2-190 cotton genotypes. This study supports current utilization of reniform nematode resistance sources from TX-110 and BARBREN-713 to develop the reniform nematode resistance commercial

cotton cultivars. Although utilization of reniform nematode resistance sources; A_2-190, LONREN-2, and LONREN-1 provide better resistance than TX-110 and BARBREN-713, they also displayed a hypersensitive reaction to some extent depending upon the reniform nematodes isolate.

This study found that A_2-190, LONREN-2, and LONREN-1 cotton genotypes displayed a hypersensitive reaction to the Evan and Avoyelles isolates, the most aggressive reniform nematode isolates tested. Although they display a hypersensitive reaction, improved cultivars from these sources could be utilized to manage the LA, Oak Tree Cut, and Old Crop Rotation isolates. The reproduction of these isolates on these cotton genotypes was quite low and could sufficiently limit reproduction to levels below that which cause hypersensitivity.

Crop rotation with reniform nematode resistant/tolerant cultivars is recommended to manage reniform nematode infested cotton fields because it maintains the reniform nematode population density below an economic threshold level and could lower vulnerability of cultivars to the development of resistance-breaking reniform nematode populations in the field. The reproduction and pathogenicity of specific reniform nematode isolates as well as a degree of resistance among cotton genotypes will dictate the type of management needed for acceptable control.

This study implies a need for further investigation of the relationship between the reproduction of reniform nematode isolates and different sources of resistance in cotton. It also highlights the fact that different management strategies may need to be applied to reduce crop damage from reniform nematode isolates that are specific to some geographical regions. Both LONREN-2 and *G. arboreum* (A_2-190) exhibit a high level of resistance regardless of reniform nematode isolate geographic origin. Within a cotton breeding program, both LONREN-1 and LONREN-2 (both tetraploids) are good sources of resistance, although they both have other agronomic performance deficiencies (Bell *et al*., 2014). The diploid cotton *G. arboreum* (A_2-190) exhibited the highest level of resistance across the reniform nematode isolates, but would be more problematic to use within a breeding program. With respect to pathogenicity, TX-110 and BARBREN-713 provided a higher level of tolerance across reniform nematode isolates than other cotton genotypes and could be used to develop resistant/tolerant cultivars.

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