RESEARCH/INVESTIGACIÓN

REPRODUCTION AND PATHOGENICITY OF ENDEMIC POPULATIONS OF *ROTYLENCHULUS RENIFORMIS* ON COTTON

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ABSTRACT

Khanal, C., E. C. McGawley, C. Overstreet, S. R. Stetina, G. O. Myers, M. T. Kularathna, B. McInnes, and F. M. C. Godoy. 2018. Reproduction and pathogenicity of endemic populations of *Rotylenchulus reniformis* on cotton. Nematropica 48:68-81.

The reniform nematode (*Rotylenchulus reniformis*) is the predominant parasitic nematode of upland cotton (*Gossypium hirsutum*) in the southern United States. Little is known about variability in geographic isolates of reniform nematode. In order to evaluate the comparative reproduction and pathogenicity of reniform nematode populations endemic in Louisiana, a series of microplot and greenhouse experiments were conducted. Reniform nematode populations derived from single-egg masses collected from West Carroll (WC), Rapides (RAP), Morehouse (MOR) , and Tensas (TEN) parishes were used in full-season (150 days) microplot, and 60-day greenhouse experiments, each repeated once. Data from two microplot trials, averaged over 2 yr, showed significant differences among isolates of reniform nematode in both reproduction and pathogenicity on upland cotton cultivars Phytogen 499 WRF, Deltapine 1133 B2RF, and Phytogen 333 WRF. Across all cotton cultivars, the MOR and RAP isolates had the greatest and the least reproduction value of 331.8 and 230.2, respectively. Reduction in plant dry weight, number of bolls, seed cotton weight, and lint weight was the greatest and the least for MOR and RAP isolates, respectively. MOR and RAP isolates lowered plant dry weights of cotton by 55%, and 9%, respectively. Reproduction and pathogenicity of the WC and TEN isolates were intermediate. Data from greenhouse trials showed results similar to that of microplot trials. In greenhouse experiments, reproduction of MOR and RAP isolates across all cotton genotypes was the greatest (reproductive value of 10.7) and the least (reproductive value of 7.9), respectively. Although reproductions of reniform nematode were lower in the germplasm lines than the cultivars, the germplasm lines sustained greater plant weight loss. The variability in reproduction and pathogenicity among endemic populations of reniform nematode in both the microplot and greenhouse experiments adds further support to the hypothesis that virulence phenotypes of *R. reniformis* exist.

Key words: pathogenicity, reniform nematode, reproduction, *Rotylenchulus reniformis*, upland cotton, virulence phenotypes

RESUMEN

Khanal, C., E. C. McGawley, C. Overstreet, S. R. Stetina, G. O. Myers, M. T. Kularathna, B. McInnes, y F. M. C. Godoy. 2018. Reproducción y patogenicidad de poblaciones endémicas de *Rotylenchulus reniformis* en algodón. Nematropica 48:68-81.

El nematodo reniforme (*Rotylenchulus reniformis*) es el nematodo parásito predominante del algodón americano (*Gossypium hirsutum*) en el sur de los Estados Unidos. Poco se sabe sobre la variabilidad en aislamientos geográficos de nematodos reniformes. Con el fin de evaluar la reproducción comparativa y la patogenicidad de las poblaciones de nematodos reniformes endémicos en Louisiana, se realizaron una serie de experimentos de microplot y de invernadero. Las poblaciones de nematodos reniformes derivadas de masas de un solo huevo recolectadas de las parroquias West Carroll (WC), Rapides (RAP), Morehouse (MOR) y Tensas (TEN) se utilizaron en microploturas de temporada completa (150 días) e invernadero de 60 días experimentos, cada uno repetido una vez Los datos de dos ensayos de microplotones, promediados

durante 2 años, mostraron diferencias significativas entre los aislados de nematodos reniformes tanto en la reproducción como en la patogenicidad en los cultivares de algodón americano (upland) Phytogen 499 WRF, Deltapine 1133 B2RF y Phytogen 333 WRF. En todos los cultivares de algodón, los aislamientos MOR y RAP tuvieron el mayor y el menor valor de reproducción de 331.8 y 230.2, respectivamente. La reducción en el peso seco de la planta, el número de cápsulas, el peso de la semilla de algodón y el peso de la fibra fue el mayor y el menor para los aislamientos MOR y RAP, respectivamente. Los aislamientos MOR y RAP disminuyeron el peso seco de la planta del algodón en un 55% y un 9%, respectivamente. La reproducción y la patogenicidad de los aislamientos WC y TEN fueron intermedios. Los datos de los ensayos de invernadero mostraron resultados similares a los de los ensayos de microplotones. En experimentos de invernadero, la reproducción de MOR y RAP aislados en todos los genotipos de algodón fue la mayor (valor reproductivo de 10.7) y la menor (valor reproductivo de 7.9), respectivamente. Aunque las reproducciones de nematodos reniformes fueron menores en las líneas de germoplasma que en los cultivares, las líneas de germoplasma sufrieron una mayor pérdida de peso de la planta. La variabilidad en la reproducción y la patogenicidad entre las poblaciones endémicas de nematodos reniformes tanto en el microplot como en los experimentos de invernadero, agrega un mayor respaldo a la hipótesis de que existen fenotipos de virulencia de *R. reniformis*.

Palabras claves: algodón americano (upland), fenotipos de virulencia, nematodo reniforme, patogenicidad, reproducción, *Rotylenchulus reniformis*

INTRODUCTION

The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) was first observed in Hawaii in 1940 attacking cowpea, pineapple, and several weeds (Linford and Oliveira, 1940). Subsequently, this nematode was reported from cotton and cowpea roots in Baton Rouge, LA, in 1941 (Smith and Taylor, 1941). Currently, reniform nematode is the predominant parasitic nematode of upland cotton in the mid-south area of the United States (Stetina and Young, 2006; Robinson, 2007; Starr *et al*., 2011). The female nematode infects cotton roots producing approximately 60 eggs per egg mass in as few as 25 days (Linford and Oliveira, 1940). Upon infection, reniform nematode adversely affects plant growth, delays flowering and fruiting times, reduces number and size of the bolls, and decreases lint quality (Robinson, 2007). Because of crop damage caused by reniform nematode, cotton production in the United States has been greatly compromised (Dighe *et al*., 2009). Each year approximately 205 thousand bales of United States upland cotton are lost to reniform nematode (Lawrence *et al*., 2017).

With an aim of durable management of reniform nematode in upland cotton production, studies on identification of resistant cultivars and germplasm lines have been conducted since the early 1960s. Birchfield and Brister (1963) evaluated 24 upland cotton cultivars in the greenhouse and found that none were resistant to reniform nematode. Yik and Birchfield (1984) reported that *Gossypium longicalyx* was a non-host while *G. stocksii*, *G. somalense*, and *G. barbadense* 'Texas 110' showed high levels of resistance. Robinson *et al*. (1999, 2007) emphasized the absence of a source of resistance to the reniform nematode as a major constraint of upland cotton production in the United States. In 2007, two cotton germplasm lines, LONREN-1 and LONREN-2, which have a resistance gene introgressed from *G. longicalyx* Hutch. & Lee, were released by the USDA ARS, Texas Agricultural Experiment Station and Cotton Incorporated (Bell *et al*., 2014a). Reniform nematode resistance genes Ren_1 (previously Ren^{lon}), Ren_2 (previously Ren^{ari}), and Ren_2^{GB713} have been identified from *G. longicalyx*, *G. aridum* (Rose & Standl.) Skov, and *G. barbadense* L. GB713 (PI 608139), respectively (Dighe *et al*., 2009; Romano *et al.,* 2009; Fang and Stetina, 2011; Bell *et al.*, 2014b). Subsequently, reniform nematode resistant germplasm lines TAM RKRNR-9, TAM RKRNR-12, and BARBREN-713 were released (Starr *et al*., 2011; Bell *et al*., 2014b). All of these germplasm lines suppressed reniform nematode reproduction by 40-90% (Starr *et al*., 2011; Bell *et al*., 2014a; 2014b). Similarly, USDA ARS at Stoneville, MS, Mississippi State University, and College Station, TX, released several sources of reniform nematode-resistant germplasm lines that include TX 110, M713 Ren1, M713 Ren2, M713 Ren5, MT2468 Ren1, MT2468 Ren2, and MT2468 Ren3 (Wallace *et al*., 2009; McCarty *et al*., 2012; 2013).

Because no commercial cotton cultivars that are resistant to reniform nematode are available, the best management options currently available are the use of tolerant cultivars, crop rotation, and possibly nematicides. A field study conducted by Blessitt *et al*. (2012) to evaluate cotton cultivars in Mississippi identified six cultivars viz. Cropland Genetics 3520 B2RF, DynaGrow 2520 B2RF,

Stoneville 5242 BR, Stoneville 5599 BR, Deltapine 488 BG/RR, and Fibermax 960 B2R as tolerant to reniform nematodes. Tolerant cultivars are those that have the capacity to support reproduction while sustaining satisfactory yields (Schafer, 1971; Blessitt *et al*., 2012). Crop rotation with non-host crops such as corn and resistant soybean reduces the nematode populations greatly; however, the populations usually resurge quickly in a subsequent single year of cotton production (Robinson *et al*., 2007). Use of chemicals can possibly lead to better management; however, the use of nematicides is a less desirable management option for economic and environmental reasons (Khanal *et al*., 2017).

The success of management of a disease is based on understanding of variability in a pathogen (Werlemark *et al*., 2006; Silva *et al*., 2008). A few studies have reported the existence of variability in geographic populations of reniform nematode. A report from the 1960s indicated that Louisiana populations of *R. reniformis* were physiologically different from other reniform nematode populations suggesting the existence of races (Birchfield, 1962). Subsequent reports demonstrated physiological variation in reproduction and pathogenicity of geographic populations of reniform nematode (McGawley and Overstreet, 1995; McGawley *et al*., 2010; 2011). Studies have also been conducted to determine the amount of genetic variability in reniform nematode populations. While Agudelo *et al*. (2005) did not find any obvious genetic variability in reniform nematode populations collected from 10 states in the United States, other genetic studies reported variability in geographic populations of reniform nematode (Tilahun *et al*., 2008; Arias *et al*., 2009; Leach *et al*., 2012). A better understanding of variability in populations of reniform nematode can help scientists develop a better management strategy. The main objective of this research was to determine whether or not there was reproductive and pathological variation in populations of *R. reniformis* endemic in Louisiana.

MATERIALS AND METHODS

General Procedure

Isolates of reniform nematode were collected from West Carroll (WC), Rapides (RAP), Morehouse (MOR), and Tensas (TEN) parishes in Louisiana and used to establish single-egg mass cultures. The reniform nematode species was confirmed morphometrically as *R. reniformis* as described by Linford and Oliveira in 1940. Axenic cultures were maintained under greenhouse conditions on tomato (*Solanum lycopersicum* L. cultivar Rutgers PS, Seedway; Hall, NY). Reniform nematode isolates from four parishes were employed in greenhouse and microplot studies with the most widely planted upland cotton cultivars Phytogen 499 WRF, Deltapine 1133 B2RF and Phytogen 333 WRF (Anonymous, 2015). The cultivars Phytogen 499 WRF, Deltapine 1133 B2RF, and Phytogen 333 WRF hereafter will be abbreviated as PHY499, DP1133, and PHY333, respectively. Exact details of greenhouse and microplot studies are presented below under the appropriate subheadings.

A soil mixture consisting of three parts Commerce silt loam soil (fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) and one part sand, and pots, unless stated otherwise, used in all experiments were steam sterilized for 5 hr at 135°C prior to use. In each test, two cotton seeds were planted to a depth of 2.5 cm and thinned to one per pot after germination. Soils were infested by pipetting aqueous suspensions of vermiform individuals of *R. reniformis* into three depressions arranged into a triangular pattern, 0.5-cm diam. × 5- to 7.5-cm deep, surrounding a 7-d-old seedling. The infestation level for the microplot experiments was 50,000 vermiform life stages per microplot, a level which simulates the pre-plant soil level of 4-5 per gram of soil common in cotton fields in Louisiana. Similarly, the infestation level for the greenhouse experiments was 4,000 vermiform life stages per pot, which is equivalent to the number of vermiform life stages per gram of soil employed in microplot trials. Half of the inoculum was added to soil in microplots at 10 days after planting and the remainder at 21 days. Standard fertilization, weeding and insect management practices were employed in all trials.

In all cases, nematodes were extracted from a 250 cm^3 subsample of soil from each pot and processed using a semi-automatic elutriator (Byrd *et al*., 1976) and the centrifugal/sugar flotation technique (Jenkins, 1964). Vermiform life-stages were enumerated using a dissecting microscope at 40× magnification. Eggs were extracted from whole root systems in greenhouse experiments by agitating root in 0.6% NaOCl for 4 min to dislodge eggs from egg masses (Hussey and Barker, 1973) and counting at 40× magnification. All plant materials were dried at 30-35°C for 2 wk and weighed.

Microplot studies

Terra cotta pots having top diameters of 35.6 cm were used as microplots. Microplots were placed in depressions in soil so that only the rim was exposed. Each microplot was filled with 20 kg of soil mixture. The entire microplot area was bounded by aluminum Quonset hut skeletal frame that was open at both ends. The skeletal frame was

covered with one layer of 6 mm polyethylene to protect plants in microplots from excessive summer rainfall and one layer of 20% reflective foilcloth for optimal sunlight. This cover was equipped with overhead fans and an irrigation system. The entire system allowed for maintenance of near field conditions (McGawley *et al*., 2010). A total of 75 microplots was established to evaluate 3 widely planted upland cotton cultivars (PHY499, DP1133, and PHY333), 4 isolates of reniform nematode, a non-inoculated control for each cultivar and 5 replications. Establishment of plants, inoculation with nematodes, and processing of plant and nematode materials were the same as that described previously. Additional plant data collected in microplot studies included number of harvestable bolls per plant, seed cotton weight, and lint weight.

Greenhouse studies

This study involved six genotypes of cotton: three cultivars (PHY499, DP1133, and PHY333), one susceptible cultivar (Stoneville 4946 GLB2), and two germplasm lines showing moderate to high levels of resistance (MT2468 Ren3 and M713 Ren5). The cotton genotypes Stoneville 4946 GLB2, MT2468 Ren3 and M713 Ren5 hereafter will be abbreviated as ST4946, MT2468, and M713, respectively. Terra cotta pots with a top diameter of 15 cm and containing 1.6 kg of soil mixture were used. A total of 150 pots were established to evaluate the 6 genotypes, 4 isolates of the nematode, a non-inoculated control for each genotype, and 5 replications. The experiment was terminated after 60 days and nematode life stages in soil and roots were quantified as described above.

Data analysis

Each experiment employed a factorial treatment structure and was established as a randomized block design with five replications. Each experiment was repeated once. Analysis of variance was conducted using trial as a fixed effect, and there were no significant trial by treatment interactions in any of the experiments described herein. Therefore data from all like trials were combined for analysis. Data were examined by analysis of variance (ANOVA) for a factorial design using the "Fit Y by X" module of SAS JMP Pro, version 13.0 (SAS Institute; Cary, NC). Means of data were separated by Fisher's LSD at $P \leq 0.05$.

RESULTS

Microplot studies

Data from two microplot trials were combined for analysis because of the absence of year by treatment interactions. Reniform nematode isolates, across all cotton cultivars, produced significant differences in reproduction and effect on plant dry weight, seed cotton weight, and lint weight (Tables 1 and 2). Significant interactions were not observed between cultivars of cotton and isolates of reniform nematode for any of the growth parameter measured. Statistical main effects of the isolates of the nematode across the cultivars are presented in Tables 3 and 4. Population density at harvest ranged from approximately 288 to 415 thousand individuals per 500 cm^3 of soil, representing reproductive values (number of vermiform stages per microplot divided by the infestation level) of 230.2 to 331.8. Across all cotton cultivars, reproduction of the MOR isolate was significantly greater than that by the other 3 isolates. The isolate from MOR parish significantly reduced plant dry weight, number of bolls, seed cotton weight, and lint weight compared to those of the non-inoculated control and other three nematode isolates. Cotton plant dry weight of inoculated plants ranged from 138 g to 278 g compared to 305.6 g for the non-inoculated control. MOR, WC, TEN, and RAP isolates lowered plant

Table 1. Vermiform life stages of *Rotylenchulus reniformis* as influenced by main and interactive effects (*P* values) of isolate of nematode and cultivar of cotton in a microplot environment[x].

Source	DF	Vermiform life stages
Cultivar (C)[y]	2	0.8960
Isolate (I)[z]	3	<0.0001*
C × I	6	0.7127

[x]Data were combined over two full-season trials and are means of ten replications. Data were analyzed as a 3 × 4 factorial with ANOVA ($P \leq 0.05$); * indicates *P* value was significant at the 0.01 level.
[y]Cultivars were Phytogen 499 WRF, Deltapine 1133 B2RF, and Phytogen 333 WRF that were recommended for use in Louisiana in 2015.
[z]Reniform nematode isolates were each derived from a single-egg mass from roots of cotton from West Carroll, Rapides, Morehouse, and Tensas parishes in Louisiana.

Table 2. Height, numbers of bolls and seed cotton, and lint weights of cultivars of cotton as influenced by main and interactive effects (*P* values) of isolates of *Rotylenchulus reniformis* and cultivars of cotton in a microplot environment[x].

Source	DF	Plant weight	Number of bolls	Seed cotton weight	Lint weight
Cultivar (C)[y]	2	0.0025*	0.0398*	0.0005**	0.0004**
Isolate (I)[z]	4	<0.0001**	<0.0001**	<0.0001**	<0.0001*
C × I	8	0.5983	0.2375	0.7521	0.7732

[x]Data were combined over two full-season trials and are means of ten replications. Plant material was dried at 30-35°C. Data were analyzed as a 3 × 5 factorial with ANOVA ($P \leq 0.05$); * and ** indicate *P* values significant at the 0.05 and 0.01 level, respectively.
[y]Cultivars were Phytogen 499 WRF, Deltapine 1133 B2RF, and Phytogen 333 WRF that were recommended for use in Louisiana in 2015.
[z]Isolates were non-inoculated controls, and nematode populations each derived from a single-egg mass from roots of cotton from West Carroll, Rapides, Morehouse, and Tensas parishes in Louisiana.

Table 3. Vermiform life stages as influenced by main effect of isolates of *Rotylenchulus reniformis* across cultivars of cotton in a full season microplot environment[w].

Isolate[x]	Vermiform life stages (1000s) per 500 cm^3 of soil[y]	Reproduction value[z]
WC	337 b	269.8
RAP	288 b	230.2
MOR	415 a	331.8
TEN	333 b	266.6

[w]Data were combined over two full season trials and are means of ten replications. Cultivars of cotton were Phytogen 499 WRF, Deltapine 1133 B2RF, and Phytogen 333 WRF.
[x]Reniform nematode isolates were each derived from single-egg masses isolated from roots of cotton from West Carroll (WC), Rapides (RAP), Morehouse (MOR), and Tensas (TEN) parishes in Louisiana.
[y]Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Within columns, means followed by a common letter are not significantly different.
[z]Reproduction values were calculated by dividing the estimated number of vermiform stages per microplot (20 kg of soil) by the infestation level of 50,000 vermiform life stages.

dry weight of cotton by 55%, 25%, 21%, and 9%, respectively. The RAP isolate produced numerically the least reduction in plant dry weight, number of bolls and lint weight. Only seed cotton weight was significantly reduced as compared to the control.

Statistical main effects of the 3 varieties of cotton across the 4 isolates of the nematode and non-inoculated controls are presented in Table 5. Across all 4 isolates of the nematode and non-inoculated controls, plant, seed cotton, and lint weights for DP1133 were reduced significantly more than were those for the 2 Phytogen cultivars. Results for numbers of bolls were similar, except that PHY499 did not differ significantly from DP1133.

Greenhouse studies

Because there were no significant trial by treatment interactions, data from the two greenhouse trials were also combined. For almost all parameters, there were highly significant genotype and isolate main effects as well as genotype by isolate interactions (Tables 6 and 7). Genotype main effects on nematode vermiform stages and eggs as well as shoot and plant dry weights were significant at the 0.01 level, and root dry weight was significant at the 0.05 level. Isolate main effects were significant at the 0.01 level across both nematode and plant parameters. Genotype by isolate interactions were significant at the 0.01 levels for both nematode and plant values

Table 4. Plant weight, number of bolls, seed cotton and lint weights as influenced by main effect of isolates of *Rotylenchulus reniformis* across cultivars of cotton in a full season microplot environment[w].

Isolate[x]	Plant weight (g)[y]	Numbers of bolls	Seed cotton weight (g)	Lint weight (g)
WC	228.4 b	16.5 b	82.6 b	35.6 b
RAP	278.0 ab	23.9 a	99.7 b	45.2 ab
MOR	138.0 c	9.5 c	48.5 c	19.7 c
TEN	241.9 b	17.3 b	89.5 b	39.6 ab
Control	305.6 a	27.7 a	117.4 a	55.5 a

[w]Data were combined over two full-season trials and are means of ten replications. Plant material was dried at 30-35 °C. Cultivars of cotton were Phytogen 499 WRF, Deltapine 1133 B2RF, and Phytogen 333 WRF.
[x]Reniform nematode populations were each derived from single-egg masses isolated from roots of cotton from West Carroll (WC), Rapides (RAP), Morehouse (MOR), and Tensas (TEN) parishes in Louisiana; control was not inoculated with nematodes.
[y]Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Within columns, means followed by a common letter are not significantly different.

Table 5. Plant weight, number of bolls, seed cotton and lint weights of cultivars of cotton as influenced by main effect of cultivars of cotton across isolates of *Rotylenchulus reniformis* and non-inoculated controls in a full season microplot environment[x].

Cultivar[y]	Plant weight (g)[z]	Numbers of bolls	Seed cotton weight (g)	Lint weight (g)
Phytogen 499 WRF	268.7 a	20.2 ab	100.9 a	45.3 a
Deltapine 1133 B2RF	196.7 b	15.7 b	64.4 b	28.2 b
Phytogen 333 WRF	251.4 a	21.1 a	98.2 a	44.3 a

[x]Data were combined over two full season trials and are means of ten replications. Plant material was dried at 30-35°C. Reniform nematode isolates were each derived from single-egg masses isolated from roots of cotton from West Carroll (WC), Rapides (RAP), Morehouse (MOR), and Tensas (TEN) parishes in Louisiana; control was not inoculated with nematodes.
[y]Cultivars were recommended for use in Louisiana in 2015.
[z]Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Within columns, means followed by a common letter are not significantly different.

Table 6. Vermiform life stages and eggs per root system as influenced by main and interactive effects (*P* values) of isolates of *Rotylenchulus reniformis* and genotypes of cotton in a greenhouse environment[x].

Source	DF	Vermiform life stages	Eggs/root system
Genotype (G)[y]	5	<0.0001**	<0.0001**
Isolate (I)[z]	3	0.0002**	0.1363
G × I	15	<0.001**	0.7279

[x]Data were combined over two 60-day trials and are means of ten replications. Data were analyzed as a 6 × 4 factorial with ANOVA ($P \leq 0.05$); ** indicate *P* values were significant at the 0.01 level.
[y]Genotypes were the cultivars Phytogen 499 WRF, Deltapine 1133 B2RF, Phytogen 333 WRF, and Stoneville 4946 GLB2, and the germplasm lines MT2468 Ren3 and M713 Ren5.
[z]Reniform nematode isolates were each derived from a single-egg masses from roots of cotton from West Carroll, Rapides, Morehouse and Tensas parishes in Louisiana.

Table 7. Root, shoot, and plant dry weights of genotypes of cotton as influenced by main and interactive effects (*P* values) of isolates of *Rotylenchulus reniformis* and genotypes of cotton in a greenhouse environment[x].

Source	DF	Root weight	Shoot weight	Plant weight
Genotype (G)[y]	5	0.0027*	<0.0001**	<0.0001**
Isolate (I)[z]	4	<.0001**	<0.0001**	<0.0001**
G × I	20	0.5167	0.0001**	0.0002**

[x]Data were combined over two 60-day trials and are means of ten replications. Plant material was dried at 30-35°C. Data were analyzed as a 6 × 5 factorial with ANOVA ($P \leq 0.05$); * and ** indicate *P* values were significant at the 0.05 and 0.01 level, respectively.
[y]Genotypes were the cultivars Phytogen 499 WRF, Deltapine 1133 B2RF, Phytogen 333 WRF, and Stoneville 4946 GLB2, and the germplasm lines MT2468 Ren3 and M713 Ren5.
[z]Isolates were non-inoculated controls, and nematode populations each derived from a single-egg mass from roots of cotton from West Carroll, Rapides, Morehouse and Tensas parishes in Louisiana.

except root dry weight where they were not significant.

Statistical main effects of the four isolates of the nematode across the six genotypes of cotton in the greenhouse environment are presented in Table 8. Reniform nematode population density in soil ranged from approximately 10 thousand to 13 thousand individuals per 500 cm^3 of soil with corresponding reproductive values of 7.9 to 10.7. The numbers of eggs per root system was similar among the isolates and averaged 4 to 5 thousand per root system (data not shown).

Plant growth parameters of cotton as influenced by main effect of isolates of nematode and non-inoculated controls across genotypes of cotton in a greenhouse environment are presented in Table 9. Across all genotypes, all isolates of the nematode caused significant reductions in root weight compared with controls, but there were no differences among the isolates. Results for weights of shoots was similar except that the MOR isolate caused greater reductions than the other 3 isolates. The isolate of *R. reniformis* from MOR parish also caused a reduction in final plant weight, which was greater than that caused by the other 3 isolates, 8.6 g compared with 9.5 g for the WC isolate, 10.3 g for the RAP isolate and 9.9 g for the TEN isolate.

The two Phytogen cultivars, along with DP1133 and ST4946, supported the highest, but not significantly different, numbers of nematodes that ranged from 9,688 to 13,022 individuals per 500 cm^3 of soil (Table 10). Significantly fewer numbers, 7,315, were recovered for MT2468 Ren3. Vermiform stages per 500 cm^3 of soil averaged 4,573 for M713 Ren5 and were significantly less than the averages across the 4 isolates for all other genotypes.

Egg production by the nematode, across isolates, was similar and not significantly different for PHY499, DP1133, PHY333, and ST4946 (Table 10). Respectively, eggs per root system averaged 4,667, 4,534, 4,637, and 5,181. Significantly fewer eggs, 1,360 and 642, were collected from roots of MT2468 Ren3, and M713 Ren5.

There was significant main effect of genotypes of cotton on root, shoot and plant weights across four isolates of *Rotylenchulus reniformis* and non-inoculated controls in a greenhouse environment (Table 11). Root dry weights of the germplasm lines were either lower or equal to that of the cultivars, however, not always significantly different. The cultivar DP1133 had significantly greater plant dry weight than any genotypes. Plant dry weights of the other three cultivars were not significantly different among each other. The germplasm lines had significantly lower plant dry weights compared to that of the cultivars. The pattern of shoot weights followed that of plant dry weights.

There were highly significant genotype by isolate interactions which influenced soil stages of the nematode (Table 6). Individual treatment means illustrating soil population levels of the nematode across the 6 genotype x 4 isolate combinations are presented in Fig. 1. Nematode numbers associated with PHY499 ranged from 12,795 for the TEN isolate to 15,520 per 500 cm^3 of soil for the MOR isolate with intermediate values of 13,232 for WC and 14,454 for RAP and no significant differences in numbers among the 4 isolates. An average soil population level of 15,592 individuals per 500 cm^3 of soil was estimated for the WC isolate on DP1133, and this was significantly greater than the 8,907 for the RAP isolate but not the 11,829 and 12,112 per 500 cm^3 with the MOR and TEN isolates. With the genotype PHY333, nematode populations in soil

Table 8. Vermiform life stages as influenced by main effect of isolates of *Rotylenchulus reniformis* across genotypes of cotton in a greenhouse environment[w].

Isolate[x]	Vermiform life stages (1000s) per 500 cm^3 of soil[y]	Reproduction value[z]
WC	11 ab	9.1
RAP	10 b	7.9
MOR	13 a	10.7
TEN	10 b	8.8

[w]Data were combined over two 60-day trials and are means of ten replications. Genotypes were the cultivars Phytogen 499 WRF, Deltapine 1133 B2RF, Phytogen 333 WRF, and Stoneville 4946 GLB2, and the germplasm lines MT2468 Ren3 and M713 Ren5.
[x]Reniform nematode isolates were each derived from single egg masses isolated from roots of soybean from West Carroll (WC), Rapides (RAP), Morehouse (MOR), and Tensas (TEN) parishes in Louisiana.
[y]Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Within columns, means followed by a common letter are not significantly different.
[z]Reproduction values were calculated by dividing the estimated number of vermiform stages per pot (1.6 kg of soil) by the infestation level of 4,000 vermiform life stages.

Table 9. Root, shoot, and plant dry weights of genotypes of cotton as influenced by main effect of isolates of *Rotylenchulus reniformis* and non-inoculated controls across genotypes of cotton in a greenhouse environment[w].

Isolate[x]	Root weight (g)	Shoot weight (g)	Plant weight (g)
WC	2.0 b	7.5 b	9.5 c
RAP	2.2 b	8.2 b	10.3 b
MOR	1.9 b	6.6 c	8.6 d
TEN	2.1 b	7.8 b	9.9 bc
Control	2.7 a	9.1 a	11.7 a

[w]Data were combined over two 60-day trials and are means of ten replications. Plant material was dried at 30-35°C. Genotypes were the cultivars Phytogen 499 WRF, Deltapine 1133 B2RF, Phytogen 333 WRF and Stoneville 4946 GLB2, and the germplasm lines MT2468 Ren3 and M713 Ren5.
[x]Reniform nematode isolates were each derived from single egg masses isolated from roots of soybean from West Carroll (WC), Rapides (RAP), Morehouse (MOR) and Tensas (TEN) parishes in Louisiana; control was not inoculated with nematodes.
[y]Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Within columns, means followed by a common letter are not significantly different.

Table 10. Main effect of genotypes of cotton on vermiform life stages and eggs per root system across four isolates of *Rotylenchulus reniformis* in a greenhouse environment[x].

Genotype[y]	Vermiform life stages[z]	Eggs/root system
Phytogen 499 WRF	11,200 a	4,667 a
Deltapine 1133 B2RF	9,688 ab	4,534 a
Phytogen 333 WRF	10,174 a	4,637 a
Stoneville 4946 GLB2	13,022 a	5,181 a
MT2468 Ren3	7,315 b	1,360 b
M713 Ren5	4,573 c	642 b

[x]Data were combined over two full-season trials and are means of ten replications. Plant material was dried at 30-35 °C.
[y]Genotypes were the cultivars Phytogen 499 WRF, Deltapine 1133 B2RF, Phytogen 333 WRF, and Stoneville 4946 GLB2, and the germplasm lines MT2468 Ren3, and M713 Ren5.
[z]Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Within columns, means followed by a common letter are not significantly different.

averaged 13,728, 10,520, 14,768, and 11,856 nematodes per 500 cm^3 for the WC, RAP, MOR, and TEN isolates, respectively. Among these four isolates, population levels did not differ significantly from one another for WC, MOR and TEN; however, MOR and RAP were different. For ST4946 there were significantly greater numbers of the MOR and TEN isolates (20,856 and 17,728, respectively) in soil than for the other 2 isolates with averages of 8,672 for WC and 12,056 for RAP. There were no significant differences in population levels of the nematode in soil for any of the isolates with the genotype MT2468. Numbers per 500 cm^3 of soil ranged from 6,776 for the TEN isolate to 10,192 for the MOR isolate. Overall, the lowest populations of the nematode in soil were found with the genotype M713. Nematode numbers for the RAP and TEN isolates were similar and not significantly different averaging 3,488 and 4,992, respectively. Greater population levels, 7,016 for the WC isolate and 7,369 for MOR, were associated with M713.

There were also highly significant genotype by isolate interactions indicated in Table 7 that influenced the cotton shoot and plant dry weights. Inspection of individual treatment means for both plant parameters reveal a similar pattern and, therefore, only those for plant weight are presented as Fig. 2. Overall, the figure illustrates clearly that the isolate of the nematode from MOR parish was the most pathogenic and it was significantly more so on the germplasm lines MT2468 and M713. Relative to the non-inoculated control at 60 days after inoculation, weights of PHY499 plants were reduced significantly by the reniform nematode isolates from WC, MOR, and TEN parishes but not by the one from RAP. Control plant weight averaged 11.9 g, those for WC, MOR and TEN were 10.1, 8.8, and 9.6 g, respectively and that for RAP was 10.9 g. Three of the 4 isolates, WC, RAP and TEN, did not cause significant reductions in plant weight for DP1133 when compared with the average for the control. Respectively, these plant dry weights averaged 11.2, 12.2, 11.6 and 12.6 g. The WC and TEN isolates caused significant damage to PHY333 and the RAP and MOR isolates did not. The mean plant weight for non-inoculated PHY333 was 11.7 g. Weights for PHY333 inoculated with isolates from WC and TEN were 9.2 and 9.9 g, respectively and those for RAP and MOR were 10.4 and 10.9 g. With ST4946, the isolates from RAP and MOR reduced weights of plants significantly below the 11.6 g value of the non-inoculated control. Isolates from WC and TEN did not reduce weights of ST4946 significantly. Weights were 8.6 g for RAP, 9.2 g for MOR, and 10.3 g for WC and TEN. For both MT2468 and M713, three of the four isolates of the nematode reduced weights of plants significantly relative to the control. Plant weights for non-inoculated MT2468 and M713 each averaged 10.8 g. For MT2468, significant reductions in plant weight were caused by WC, MOR, and TEN isolates with mean plant weights of 7.7, 5.8, and 8.0 g, respectively. Plant weight for the RAP isolate was 10.2 g and not significantly different from the control. For M713 the isolates which caused significant plant damage were from WC, RAP, and MOR parishes. Respectively, plant weights were 8.2, 9.6, and 6.3 g, and the isolate, which did not elicit significant plant damage, was from TEN parish. The final plant weight averaged 10.0 g.

DISCUSSION

Nematologists have documented the existence of reproductive, pathogenic, and/or genetic variability in a range of plant-parasitic nematodes including burrowing nematode (Ducharme and Birchfield, 1956; Huettel and Yaegashi, 1988),

Table 11. Main effect of genotypes of cotton on root, shoot and plant weights across four isolates of *Rotylenchulus reniformis* and non-inoculated controls in a greenhouse environment[x].

Genotype[y]	Root weight (g)[z]	Shoot weight (g)	Plant weight (g)
Phytogen 499 WRF	2.2 abc	8.0 b	10.3 b
Deltapine 1133 B2RF	2.4 a	9.2 a	11.6 a
Phytogen 333 WRF	2.3 ab	8.2 b	10.4 b
Stoneville 4946 GLB2	2.1 bc	7.9 b	9.9 b
MT2468 Ren3	2.0 c	6.6 c	8.6 c
M713 Ren5	2.1 bc	7.1 c	9.1 c

[x]Data were combined over two full season trials and are means of ten replications. Plant material was dried at 30-35 °C.

[y]Genotypes were the cultivars Phytogen 499 WRF, Deltapine 1133 B2RF and Phytogen 333 WRF, and Stoneville 4946 GLB2, and the germplasm lines MT2468 Ren3, and M713 Ren5.

[z]Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Within columns, means followed by a common letter are not significantly different.

stem and bulb nematode (Seinhorst, 1957), soybean cyst nematode (Riggs *et al*., 1981; Niblack *et al*., 2002), and root-knot nematode (Barker *et al*., 1985; Noe, 1992; Van der Beek *et al*., 1999; Khanal *et al*., 2016). The existence of morphological, physiological, and/or genetic variability among geographic isolates of *R. reniformis* have been proposed by some studies as well. Dasgupta and Seshadri (1971) designated two races (Race A, Race B) of *R. reniformis* based on their differential reproduction in castor, cotton, and cowpea. A study published in 1983 in Japanese and translated in English by Nakasono (2004) designated three distinct biological types (male-numerous, male-rare, and male-absent) of *R. reniformis* that originated from Japan and the United States. McGawley and Overstreet (1995) studied 17 populations of reniform nematode collected from Louisiana, Arkansas, Hawaii, Mississippi, and Texas in greenhouse and laboratory tests and found variation among populations with respect to reproduction on and/or damage to cotton and soybean. A study conducted by Agudelo *et al*. (2005) on selected cotton and soybean cultivars involving 13 amphimictic populations of reniform nematode collected from major cotton growing areas in the United States showed that considerable variation in reproduction and morphology exists within and among the geographic populations. Agudelo *et al*. (2005) further evaluated the ribosomal internal transcribed spacer region-1 (ITS1) of populations of reniform nematodes from the United States as well as from Brazil, Colombia, Honduras, and Japan. They found that all the populations, except one from Japan, did not differ genetically for the studied nuclear region. The population from Japan, which was parthenogenetic, showed a considerable amount of nucleotide variation (41/348 bp), suggesting that a difference in genotypic make up can introduce considerable variation into a population. They suggested further that other molecular markers such as amplified fragment length polymorphism and microsatellites would be useful in assessing variation in nematode populations. In contrast to the results from Agudelo *et al*. (2005), Tilahun *et al*. (2008) found significant amount of variation in ITS1 and 18S ribosomal DNA of seven reniform nematode populations in Alabama. Such contrasting results have created confusion about the suitability of ribosomal DNA for assessment of genetic variability in this nematode.

As the genetic variability studies specifically focused on ITS and 18s rDNA has been elusive, Arias *et al*. (2009) readily distinguished reniform nematode populations from Texas, Louisiana, Mississippi, and Georgia using microsatellite markers. Furthermore, a greenhouse experiment by Arias *et al*. (2009) supported the notion of variability in geographic isolates. Studies of the variability of geographic isolates described heretofore were short-duration greenhouse or lab studies that did not always employ populations derived from single-egg mass cultures, and the experiment may or may not have been repeated. In contrast to the methodology employed in previous research, McGawley *et al*. (2010; 2011) conducted microplot tests involving cotton and soybean to assess reproduction and pathogenicity of reniform nematode populations derived from single-egg mass cultures collected from Alabama, Arkansas, Hawaii, Louisiana, Mississippi, and Texas. They collected data from two full-season microplot trials and found significant differences among isolates of reniform nematode in both reproduction and pathogenicity. Microplot trials conducted by McGawley *et al*. (2010; 2011) reported that reniform nematode inoculated cotton and soybean cultivars sustained 38.6% and 27.9%, respectively, plant dry weigh reduction compared to those of the non-inoculated controls. Further research, including several isolates of reniform nematode endemic in Louisiana, is necessary to support that reniform nematode is more damaging to cotton than soybean. Greenhouse study conducted by Bhandari *et al*. (2015) reported significant variability in reproduction and pathogenicity of Louisiana populations, although not derived from single egg mass cultures, of reniform nematode on susceptible cotton genotypes and resistant germplasm lines.

Research detailed in this report provides an indication of the amount of variation in endemic populations of reniform nematode from cotton growing regions in Louisiana. Over the course of this research, two microplot and two greenhouse experiments were conducted to determine the pathogenicity and reproduction of four endemic populations of reniform nematode on cotton genotypes. Data obtained from both greenhouse and microplot experiments demonstrated that the MOR and RAP isolates caused the greatest and the least damage, respectively. Furthermore, least reproduction and lower pathogenicity of reniform nematode isolates, especially MOR and RAP, was evident on the germplasm lines rather than on commercial cultivars.

This research is the first report that employs a series of microplot and greenhouse experiments to demonstrate reproductive and pathogenic variation on cotton among populations of *R. reniformis* endemic to Louisiana. Evidence strongly suggesting the existence of virulence phenotypes in endemic populations makes it essential for

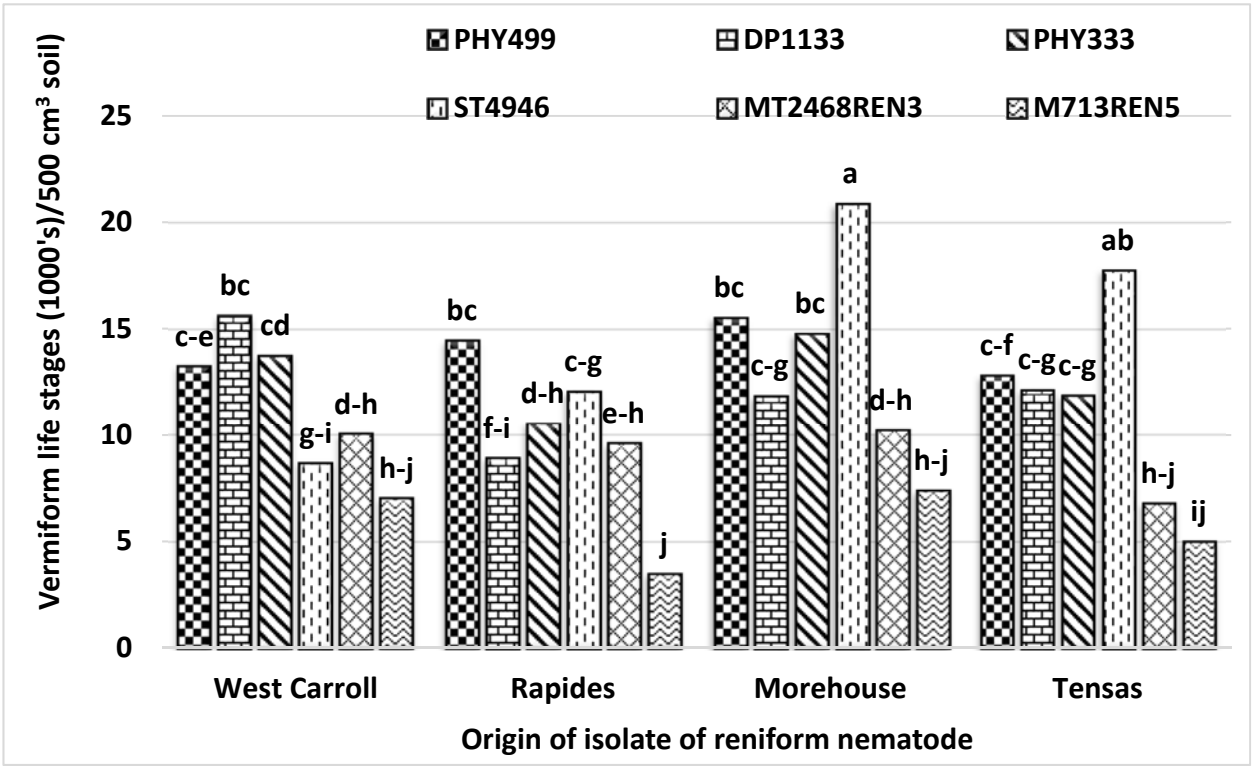

Fig. 1. Vermiform life stages of *Rotylenchulus reniformis* per 500 cm^3 of soil, after 60 days in a greenhouse environment from cotton genotypes Phytogen 499 WRF (PHY499), Deltapine 1133 B2RF (DP1133), Phytogen 333 WRF (PHY333), Stoneville GLB2 (ST4946), MT2468 Ren3, and M713 Ren5. Data are means of 10 replications averaged over two trials. Bars with common letters are not significantly different based on Fisher's LSD test ($P \leq 0.05$).

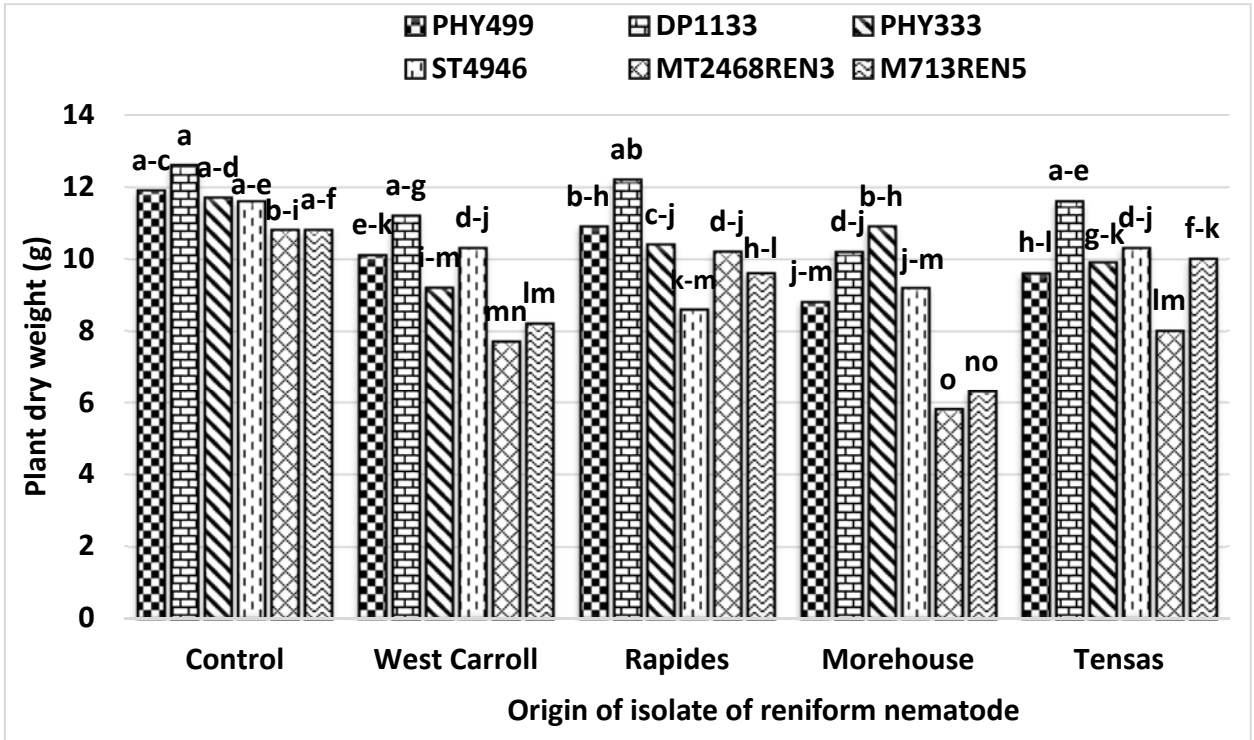

Fig. 2. Effect of four isolates of *Rotylenchulus reniformis* on plant dry weight of cotton genotypes Phytogen 499 WRF (PHY499), Deltapine 1133 B2RF (DP1133), Phytogen 333 WRF (PHY333), Stoneville 4946 GLB2 (ST4946), MT2468 Ren3, and M713 Ren5. Nematode isolates each were derived from a single-egg mass isolated from West Carroll, Rapides, Morehouse, and Tensas Parishes of Louisiana; control was not inoculated with nematodes. Plant material was dried at 30-35°C. Data were combined over two 60-day greenhouse trials and are means of ten replications. Data were analyzed with ANOVA and Fisher's LSD test ($P \leq 0.05$). Means followed by a common letter are not significantly different.

breeding programs aimed at developing reniform nematode-resistant cultivars employ as many isolates as possible in order to produce durable sources of resistance.

Parallel research conducted at Louisiana State University (Kularathna *et al*., 2017) employed the same populations of reniform nematode, but used soybean as the host plant. Data from that research also show differences in reproduction and pathology of the nematode on soybean. A major difference in results from these two parallel lines of research involve the level of reproduction of MOR isolate on two different hosts. Across soybean genotypes, the MOR isolate exhibited the lowest level of reproduction, but caused the greatest amount of damage. Conversely, with cotton, the MOR isolate exhibited the greatest level of reproduction and caused the greatest level of damage. Across all cotton and soybean genotypes, respectively, MOR isolate reduced plant dry weight by 54.8% and 29.8% relative to those of the non-inoculated controls. This difference in pathogenicity of MOR isolate on cotton and soybean is possibly a function of host.

DISCLAIMER

Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Louisiana State University (LSU) or the United States Department of Agriculture (USDA). LSU and USDA are equal opportunity providers and employers.

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