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Distinguishing Isolates of Rotylenchulus reniformis Endemic in Louisiana on the Basis of Root-associated Females and Egg Masses

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DISTINGUISHING ISOLATES OF *ROTYLENCHULUS RENIFORMIS* ENDEMIC IN LOUISIANA ON THE BASIS OF ROOT-ASSOCIATED FEMALES AND EGG MASSES

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ABSTRACT

The reniform nematode *Rotylenchulus reniformis* is a major pathogen of soybean and cotton in Louisiana. Previous studies have shown that populations of reniform nematode throughout the southern United States vary in reproduction and pathogenicity. Limited studies have been conducted to evaluate the reproduction and pathogenicity of populations of *R. reniformis* endemic in Louisiana. Studies with isolates of the nematode from eight cotton-producing parishes focused solely on reproduction of the root-associated infective and swollen female life stages with and without attached egg masses on the cotton genotypes MT2468 Ren3, M713 Ren5, and Stoneville 4946GLB2 and the soybean genotypes PI 548316, PI 90763, and Progeny 4930LL. Data from greenhouse-based, 30-d-duration tests showed significant differences in life stage totals per root system among the eight isolates. Data from subsequent greenhouse studies with isolates of the nematode from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes showed that on cotton there were significantly greater numbers of females with egg masses and total life stages on roots for the WC isolate than for the other 3 isolates.

Subsequent laboratory tests with durations of 14-21 days employed isolates of the nematode from WC, MOR, RAP and EC parishes. Soybean and cotton plants were grown either in steam-sterilized soil or in soilless germination pouches. Overall, genotypes of cotton were better able to distinguish populations of the nematode on roots than were the genotypes of soybean. After 14 days for both cotton and soybean, the greatest numbers of infective and swollen females and root-associated totals were observed with the WC isolate of the nematode. After 21 days, numbers of swollen females with egg masses and root-associated life stage totals

for cotton were significantly greater for the WC isolate than for the other isolates. Germination pouches showed that, on tomato, the WC and RAP isolates had greater numbers of swollen females and total root-associated stages than the other two isolates. Total egg mass contents, the sum of the numbers of eggs and hatched juveniles, were greatest for the WC isolate of the nematode and averaged 30 per egg mass.

INTRODUCTION

In the U.S., soybean and cotton are major economic crops with acreages in 2014 equaling 33.6 million hectares for cotton (Anonymous, 2015) and 4 million hectares for soybean (Anonymous, 2014). Losses in these two major crops from reniform nematode, *Rotylenchulus reniformis,* were estimated to be respectively 0.13 (Illinois Extension & Outreach, 2014) and 2.1 percent (Cotton Disease Committee, 2015).

Reniform nematode is a major pest throughout the southern U.S.A. Initially, it was identified in Hawaii in 1940 (Linford and Oliviera, 1940), but soon after was observed in the continental U.S. in Georgia on cotton (Smith, 1940). Shortly thereafter, in 1941, Smith and Taylor found *R. reniformis* associated with cotton growing near Baton Rouge, Louisiana (Smith and Taylor, 1941). By 1995 it was determined that the nematode had spread throughout the state, infesting roughly 206,000 hectares of cotton (Overstreet and McGawley, 1996). Over the past two decades *R. reniformis* has become a prominent pest in the cotton-producing areas of the southern U.S. (Robinson, 2007; Overstreet and McGawley, 1999).

A major factor contributing to the increasing economic importance of reniform nematode is a lack of commercially available resistance. In cotton there are no commercially available cultivars with resistance and in soybean there is only a limited supply. In contrast to this is the wide range of resistant cultivars of cotton and soybean that are available to combat infestations of the Southern root-knot nematode (RKN), (*Meloidogyne incognita*) and soybean cyst nematode (SCN), (*Heterodera glycines*). Plant breeders have produced cultivars of cotton and soybean with resistance to these two nematodes because nematologists have devised assays to differentiate among virulence phenotypes, traditionally called “races” or “biotypes.” These

assays are referred to as “host differential tests” for RKN and “race differential tests” for SCN. The evolution of these assays is described in the next few paragraphs.

Root-knot nematode: In 1954, Sasser developed the first differential assay for identification of species of RKN. This procedure allowed for identification of the four most common species of *Meloidogyne: M. incognita, M. hapla, M. javanica,* and *M. arenaria*. Host differentials consisting of cotton, tobacco, pepper, watermelon, peanut, and tomato were assigned a rating based on the ability of the nematode to enter the root and develop past the first parasitic juvenile stage. After 45 to 60 days, inoculated plants were scored as being positive or negative based on the number of juveniles that entered and matured on the root system. The rating scale is as follows: no galls or egg mass = 0; 1-2 galls or egg masses = 1; 3-10 = 2; 11-30 = 3; 31-100 = 4; more than 100 = 5. Hosts with a rating of 1 or 2 were designated negative and ones with a rating of 4 or 5 were designated positive.

A modified and improved assay was proposed by Taylor and Sasser in 1978. Employing cotton, tobacco, watermelon, pepper, peanut and tomato as differential hosts allowed identification of the four major species of *Meloidogyne,* four races of *M. incognita,* and two races of *M. arenaria.* This protocol also established a standardized set of guidelines that included inoculum preparation, soil characteristics, experimental duration, temperature requirements and a refined system for rating the degree of galling and egg mass production. In 1985, Hartman and Sasser proposed the host differential assay that is used currently. The main features of this assay were the use of Deltapine 61 rather than Deltapine 16 cotton and a slight modification of the gall index rating and staining of roots with Phloxine B (Hartman, 1982).

Soybean cyst nematode: In 1970, Golden et al. proposed a host differential assay for identifying virulence phenotypes, then called “races”, of *H. glycines*. This assay, unlike that for

RKN that utilizes a range of plant types, employs a range of soybean genotypes. The assay was initially ambiguous with only a few standardized guidelines. A susceptible control such as Lee soybean had to be used, inoculum concentrations had to be "high" and applied equally, and a confirmation test had to be performed by another lab using the same protocol. The genotypes Pickett, Peking, PI 88788, PI 90763, and Lee were used as the differentials. After 30 days, if reproduction on a cultivar was less than 10% of that on Lee soybean the result was considered to be negative. If reproduction was greater than 10% of that on a Lee soybean the result was considered positive.

In 1988, Riggs and Schmitt successfully characterized populations of SCN into the 16 possible virulence phenotypes that can be determined using the soybean genotypes Pickett, Peking, PI 88788, PI 90763, and Lee. In 2002, Niblack et al. developed a new characterization scheme for *H. glycines* known as the "HG Type" test. The soybean cultivars PI548402 (Pickett), PI 88788, PI 90763, PI437654, PI209332, PI89772, PI548316 (Cloud), and Lee 74 (control) were used. In this assay, females that develop are extracted from the roots and enumerated at 30 days. The HG Type test employs the 10 percent rule. That is, when the female index (FI) value, mean number of females on a test soybean line/ mean number of females on the standard susceptible, is $\geq$ 10 the host is considered susceptible while a FI < 10 represents a resistant host.

The objectives of this research were 1) to determine whether or not a greenhouse assay can distinguish endemic populations of *Rotylenchulus reniformis* and 2) to determine if root-associated life stages and a laboratory environment can also distinguish among endemic populations of *Rotylenchulus reniformis.*

MATERIALS AND METHODS

General Procedures

Isolates of reniform nematode were collected from West Carroll (WC), Rapides (RAP), Morehouse (MOR), Tensas (TEN), East Carroll (EC), Catahoula (CAT), Franklin (FRA), and Ouachita (OUA) parishes, confirmed morphometrically as *R. reniformis*, and used to establish single-egg mass cultures. Single egg masses were dissected from tomato (*Solanum lycopersicum* L. cultivar Rutgers PS, Seedway; Hall, New York 14463) roots and transferred to individual 50-ml capacity plastic centrifuge tubes containing 50 g of sterile soil and a single established Rutgers tomato seedling. Single egg mass cultures in tubes were maintained under fluorescent Gro-lux (OSRAM Sylvania Inc; Wilmington, Massachusetts 01887) bulbs in the laboratory for 4-5 weeks. Thereafter, axenic cultures were relocated to a greenhouse environment and propagated further on tomato in clay pots. These axenic cultures were the sources of nematodes for all subsequent research. Axenic cultures were maintained under greenhouse condition on tomato. Reniform nematode isolates from the eight parishes were employed in greenhouse and laboratory studies with the cotton genotypes M713 Ren5 (M7), MT2468 Ren3 (MT2), and Stoneville 4946GLB2 (STN) that represented a poor host, a intermediate host, and a good host, respectively, and the soybean genotypes PI 90763, PI 548316, and Progeny 4930LL (PROG) that represented a poor host, a intermediate host, and a good host, respectively. Exact details of greenhouse and laboratory 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 was steam sterilized for 5 hours at 135°C prior to use. The soil mixture had a pH of 6.9-7.2 and an organic matter content of 1.0-1.4 percent. In each

test, three cotton or three soybean 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.

Inoculum for all tests contained a mixture of 3,000 juveniles, pre-adult females, and males. Plants were inoculated at seven days after seed emergence or seedling establishment in germination pouches (Cyg germination pouch, Mega International; Newport, MN 55055). In all cases, nematodes were extracted from all soil using the wet-sieving (nested 425 and 38-µm-pore sieves) centrifugal/sugar flotation technique (Jenkins, 1964). Root-associated vermiform females, swollen females and females with egg masses, stained using the red-food coloring technique (Thies et al., 2002) were enumerated at x40 using a dissecting microscope.

In greenhouse experiments, 10-cm-diam. clay pots containing 500 g of steam-sterilized soil mixture were used. Laboratory-based studies employed 28 X 114 mm, 50-ml capacity, polystyrene centrifuge tubes (Sarstedt, Inc; Newton, NC 28658) containing 45 g of steam-sterilized soil mixture or soilless germination pouches that were 18 X 16.5 cm. Individual growth pouches consisted of germination paper enclosed in a transparent plastic pouch with a planting trough located at the upper edge.

All tests were repeated once. Analysis of variance was conducted using test as a fixed effect and there were no significant test by treatment interactions in any of the tests described herein. Therefore, data from all like trials was combined for analysis. Data were examined by analysis of variance (ANOVA) for a factorial design using the "Fit Model" module of SAS JMP Pro, version 13.2 (SAS Institute; Cary, NC). Means were separated by Student's t-test at the 1% and 5% levels.

Greenhouse Studies

The first greenhouse test was a 31-day duration experiment initiated to determine the host status of the three cotton and three soybean genotypes to the eight endemic isolates of Rr. A total of 240 pots were established in a randomized block design representing the six genotypes, eight isolates of reniform nematode, and 5 replications. Seed were planted and seedlings inoculated as described above. After 31 days, root systems were soaked in water for 5 minutes to dislodge soil particles and the entire root system was transported to the lab and stained with red food color (Thies et al., 2002). Total numbers of root-associated life stages per root system were then determined.

The second greenhouse test employed the three cotton and three soybean genotypes and four isolates of Reniform nematode, selected from the first experiment as representing isolates with the greatest (WC) and least (EC) reproduction and two with intermediate (RAP and MOR) levels of reproduction. A total of one hundred twenty pots were established in a randomized block design representing the six genotypes, the four isolates of reniform nematode, and 5 replications. After 31 days, root systems were handled as described for the first experiment. Root associated life stage totals were counted and subsequently separated into categories representing vermiform females, swollen females, and females with egg masses.

Laboratory Studies

The objective of the laboratory portion of this research was to determine whether or not a laboratory based, short duration test, using the same plant genotypes employed in the greenhouse trials would similarly distinguish the four isolates of Reniform nematode. For laboratory test one, a total of 144 centrifuge tubes representing the six genotypes, four isolates of reniform nematode, and six replications were established as described above and terminated after 21 days. Seed were

planted and seedlings inoculated as described above. After 21 days, root systems were removed from the centrifuge tubes, weighed, stained, and nematode were counted and separated into the three nematode life stage classes described for greenhouse test two. A second laboratory test, identical to the first one, was conducted and terminated after 14 days.

A third laboratory test was conducted using the soilless germination pouches. These tests had the objective of determining whether or not a soilless system could produce results similar to those found with the centrifuge tubes but which would have the added advantage of allowing for non-destructive and repeated observation of the developing nematode life stages. Plant species used with this procedure included cotton and soybean genotypes previously described and Rutgers tomato. In the planting trough of each pouch, seed were sown and maintained under 20 watt Gro-Lux wide spectrum fluorescent blubs. After two weeks, pouches were oriented horizontally, inoculated by pipetting an aqueous solution of 3,000 vermiform life stages onto the developed root system, and covered with aluminum foil to maintain dark conditions for 72 hours. Thereafter, pouches were returned to an erect and randomized position in the germination pouch polypap stands under the fluorescent lighting. Root system of plants were observed *in-situ* daily with a bench mounted magnifying lens. After 18 days, plants were removed from the pouches, entire plants were weighed, and a final examination was conducted using an inverted microscope at a magnification at x40.

In a fourth series of laboratory trials designed to determine whether or not differences in reproduction of the WC, RAP, MOR, and EC isolates were related to differences in numbers of eggs per egg mass, fifteen egg masses of reniform nematode per isolate were individually dissected from root material and placed into a 45-mm-diam. petri dish containing 15 ml of sterile distilled water. Subsequently for each egg mass, contents were categorized into numbers of eggs

and hatched juveniles. Eggs were further classified as containing or not containing a developed juvenile.

A fifth series of laboratory tests also focused on eggs of the WC, RAP, MOR, and EC isolates and evaluated their viability. Fresh root tissue was collected from greenhouse cultures and immediately washed and extracted using a 0.6% sodium hypochlorite solution (Byrd et al., 1972) for 10 minutes. For each isolate, the number of eggs per ml of aqueous solution were determined and a suspension necessary to contain 1,000 eggs was pipetted on a Baermann funnel containing 75 ml of sterile distilled water and a heat-sterilized supporting screen fitted with a double layer of Kimwipe (Kimberly-Clark Corportation; Roswell, GA 30076), and covered with a 90-mm-diam. petri dish cover. After 24 hours and daily for four days, 20 ml of liquid was removed from the bottom of each funnel, the number of juveniles was counted and the 75 ml volume of the funnel was reestablished.

RESULTS

Greenhouse Studies

Data for the first greenhouse test showed significant main and interactive effects, which influenced root-associated life stage totals on both soybean and cotton (Table 1). Effects of reniform nematode isolate were significant at the 1% level on both soybean and cotton. Main effects of genotype were significant at the 5% level for soybean and the 1% level for cotton. Isolate by genotype effects on life stage totals for reniform nematode were significant at the 1% level on soybean and the 5% level on cotton. Across the three soybean genotypes, root-associated life stage totals for reniform nematode ranged from 45.8-95.5 (Table 2). The lowest total was for the EC isolate but it was not statistically different from the numbers for TEN, CAT,

and WC parishes. Across the cotton genotypes, life stage totals ranged from 36-126.3. The highest life stage total for the nematode occurred with the WC isolate and it was significantly greater than that for all other isolates except those from OUA and RAP.

Table 1. Main and interaction effects (P values) of eight isolates of *Rotylenchulus reniformis* endemic in Louisiana and three soybean and three cotton genotypes on totals[w] in a greenhouse environment[x].

		Root-associated nematode life stage	
Source	DF	Soybean	Cotton
Isolate (I)[y]	2	<0.0001**	0.0002**
Genotype (G)[z]	7	0.0383*	0.0031**
I x G	14	0.0080**	0.0153*

[w] Root-associated life stages include vermiform females, swollen females, and swollen females with egg masses.
[x] Data were combined over two 30-day duration trials and are means of ten replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[y] Isolates of *R. reniformis* were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, Rapides, Catahoula, Ouachita, Tensas, and Franklin parishes.
[z] Genotypes of soybean were Progeny 4930LL, PI 90763, and PI 548316 and genotypes of cotton were MT2468 Ren3, M713 Ren5, and Stoneville 4946GLB2.

Table 2. Across three soybean or cotton genotypes[w], main effects on root-associated nematode life stage totals of eight isolates[x] of *Rotylenchulus reniformis* in a greenhouse environment[y].

	Root-associated life nematode stage totals[z]	
Isolate	Soybean	Cotton
West Carroll	66 abc	126 a
Ouachita	96 ab	102 ab
Rapides	81 ab	90 abc
Morehouse	80 ab	80 bc
Franklin	83 ab	76 bcd
East Carroll	46 c	66 bcd
Catahoula	59 bc	60 cd
Tensas	49 bc	36 d

[w] Genotypes of soybean were Progeny 4930LL, PI 90763, and PI 548316 and genotypes of cotton were MT2468 Ren3, M713 Ren5, Stoneville 4946GLB2.
[x] Isolates of *R. reniformis* were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[y] Data were combined over two 30-day duration trials and are means of ten replications. Within columns, means followed by a common letter are not significantly different based on Student's t-test.
[z] Root-associated life stages include vermiform females, swollen females, and swollen females with egg masses.

Across the eight isolates of reniform nematode, root-associated life stage totals for soybean ranged from 5.5-130 (Table 3). For PROG, PI 548316 and PI 90763 there were significant and stepwise decreases in root life stage totals averaging 130, 74, and 6, respectively. For cotton, life stage totals ranged from 55-110. Numbers for STN averaged 110 and were significantly greater than those for MT2 and M7, which averaged 73 and 55, respectively.

Table 3. Across eight isolates[x] of *Rotylenchulus reniformis*, main effects on root-associated life stage totals on three soybean or cotton genotypes in a greenhouse environment[y].

Soybean		Cotton	
Genotype	Root-associated life stage total[z]	Genotype	Root-associated life stage total
Progeny 4930LL	130 a	Stoneville 4946GLB2	110 a
PI 548316	74 b	MT2468 Ren3	73 b
PI 90763	6 c	M713 Ren5	55 b

[x] Isolates of *R. reniformis* were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, Rapides, Catahoula, Ouachita, Tensas, and Franklin parishes.
[y] Data were combined over two 30-day duration trials and are means of ten replications. Means followed by a common letter are not significantly different based on Student's t-test.
[z] Root-associated life stages include vermiform females, swollen females, and swollen females with egg masses.

Interactions of isolate of reniform nematode and genotype of soybean from are presented as Figure 1. The highest totals for root-associated life stages of Reniform nematode, which averaged 207 per root system, were observed with the isolate from OUA parish on PROG; life stage totals for isolates from WC, MOR, and FRA parishes were lower, 149, 151, and 174, respectively, but not significantly different than OUA on PROG. On PI 548316, the highest numeric total for Reniform nematode stages on roots, that averaged 117, occurred with the RAP isolate and this value was different than totals of 44, 61, and 40 per root system for isolates from WC, EC, and TEN parishes. Except for values of TEN isolate on PI 548316 and the CAT isolate

on PROG, life stage totals for PI 90763 were significantly lower than those for the other two genotypes, ranging from 2-7 per root system, and there were no significant differences in totals for each isolate on this genotype.

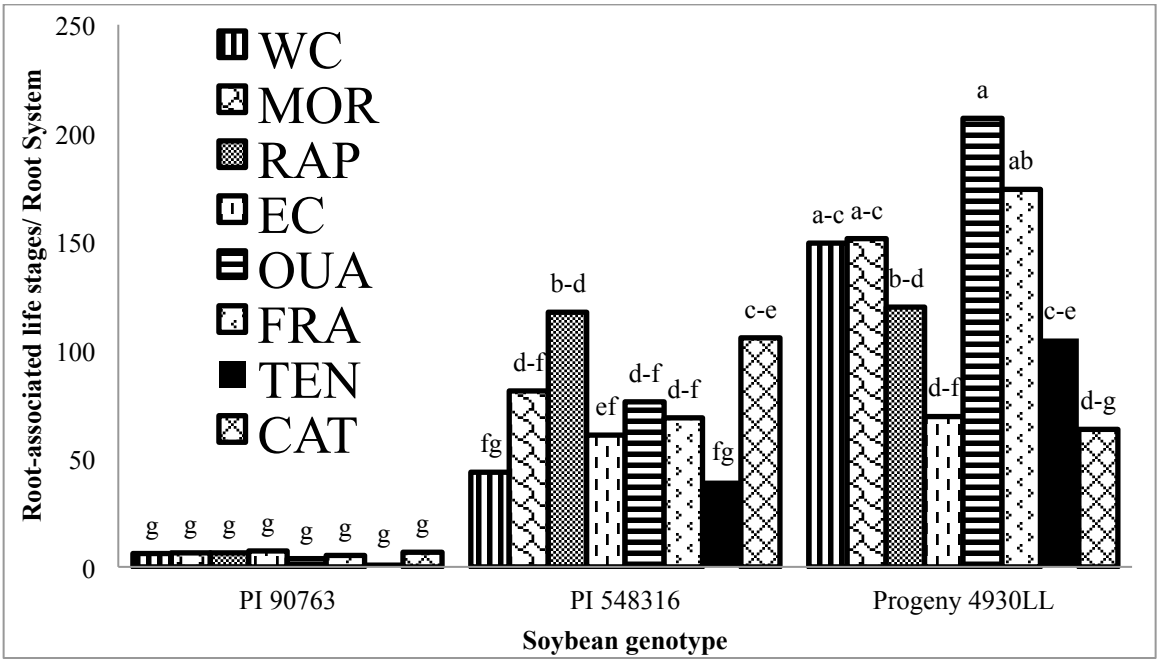

Figure 1. Total numbers of root-associated life stages of isolates of *Rotylenchulus reniformis* from West Carroll (WC), Morehouse (MOR), Rapides (RAP), East Carroll (EC), Ouachita (OUA), Franklin (FRA), Tensas (TEN), and Catahoula (CAT) parishes recovered from root systems of the soybean genotypes PI 90763, PI 548316, and Progeny 4930LL. Data are means of ten replications combined over two 30-day duration trials conducted in a greenhouse environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

Interactions of isolate of reniform nematode and genotype of cotton from Table 1 are presented as Figure 2. On STN, the isolate from OUA parish had life stage totals that averaged 201 per root system and were significantly greater than totals for the other seven isolates of the nematode, which range from a low of 45 for CAT to a high of 122 for RAP. On MT2 life stage totals ranged from a high of 138 per root system for WC to a low of 13 per root system for TEN. Values of 83 for MOR, 89 for RAP, 75 for FRA, and 100 for CAT were not significantly different than the total for WC. The cotton genotype M7 supported a high value of 145 reniform

nematode life stages for the WC isolate and a low of 12 life stages per root system for the TEN isolate. The 145 life stages per root system for WC on M7 were significantly greater than those for the other seven isolates of the nematode.

On the basis of this first set of greenhouse tests, four isolates of reniform nematode were selected for subsequent research. The isolates selected were from WC, RAP, MOR, and EC parishes. Across both crops, the greatest reproduction was by the isolate from WC and the least was by the isolate from EC. Reproduction by the isolates from RAP and MOR was intermediate to these levels.

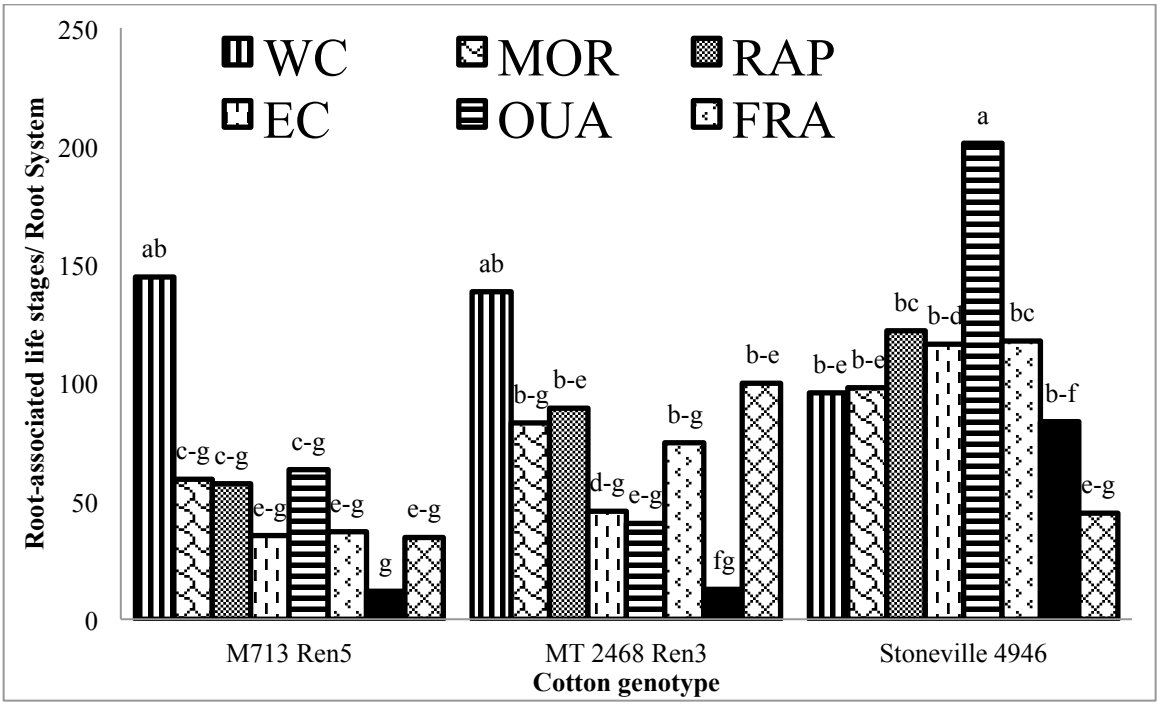

Figure 2. Total numbers of root-associated life stages of isolates of *Rotylenchulus reniformis* from West Carroll (WC), Morehouse (MOR), Rapides (RAP), East Carroll (EC), Ouachita (OUA), Franklin (FRA), Tensas (TEN), and Catahoula (CAT) parishes recovered from root systems of the cotton genotypes M713 Ren5, MT 2468 Ren3, and Stoneville 4946GLB2. Data are means of ten replications combined over two 30-day duration trials conducted in a greenhouse environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

In the second greenhouse test, there were no significant main effects of isolate but there were significant main effects of genotype that influenced numbers of swollen females, females with egg masses, and total numbers of root-associated stages of the nematode (Table 4). Main effects of soybean genotype on swollen females, females with egg masses, and root-associated stage totals were significant at the 1% level. Isolate by genotype interactions were significant at the 5% level and influenced females with egg masses and totals for root-associated life stages.

Table 4. Main and interaction effects (P values) of four isolates of *Rotylenchulus reniformis* endemic in Louisiana and three soybean genotypes on fresh root weight and root-associated nematode life stages in a greenhouse environment[x].

Source	DF	Fresh root weight	Root-associated nematode life stages			
			Vermiform females	Swollen females	Females with egg masses	Total
Isolate (I)[y]	3	0.95	0.80	0.95	0.17	0.28
Genotype (G)[z]	2	0.94	0.15	<0.0001**	<0.0001**	<0.0001**
I x G	6	0.93	0.98	0.07	0.019*	0.030*

[x] Data were combined over two 30-day duration trials and are means of ten replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[z] Genotypes were Progeny 4930LL, PI 90763, and PI 548316.

With PI 548316, swollen females, females with egg masses, and totals for their sum averaged 33, 133, and 174, respectively (Table 5). These values were not significantly different respectively from the 28, 116, and 153 counted for PROG. Significantly lower numbers, 6, 9, and 28 were recovered for swollen females, females with egg masses, and total numbers per root system, respectively for PI 90763.

The interactions of isolate of Reniform nematode and soybean genotype in the second greenhouse test and their influence on females with egg masses are presented as Figure 3. There was no consistent pattern in the number of females with egg masses across the four isolates of reniform nematode on the three soybean genotypes. With PI 548316 numbers of females with

egg masses were higher for WC, 176 per root system, than for RAP and EC, which averaged 107 and 75, respectively, but numbers were not significantly different, averaging 172, than those for MOR. With PROG the greatest numbers of females with egg masses, 167 per root system, were observed for the RAP isolate which was significantly greater than MOR and EC isolates, averaging 91 and 97 females with egg masses, respectively. With PI 90763, very few females with egg masses were observed for any of the four isolates of reniform nematode. Numbers per root system averaged 8, 12, 7, and 9 for WC, MOR, RAP, and EC and were not significantly different.

Table 5. Across four isolates[y] of *Rotylenchulus reniformis* and three soybean genotypes, main effects on root-associated nematode life stages in a greenhouse environment[z].

Genotype	Root-associated nematode life stages			
	Vermiform females	Swollen females	Females with egg masses	Total
PI 548316	8 a	33 a	133 a	174 a
Progeny 4930LL	9 a	28 a	116 a	153 a
PI 90763	13 a	6 b	9 b	28 b

[y] Isolates were each derived from a single egg mass and were collected from cotton fields from West Carroll, East Carroll, Morehouse, and Rapides parishes.
[z] Data were combined over two 30-day duration trials and are means of ten replications. Means followed by a common letter are not significantly different based on Student's t-test.

Unlike results with soybean in the second greenhouse test, there were significant main effects of both isolate and genotype on cotton (Table 6). Isolate main effects significant at the 1% level impacted swollen females, females with egg masses, as well as total root-associated life stages. Genotype main effects significant at the 1% level influenced fresh root weight and vermiform females. The significant influence of genotype on females with egg masses and root-associated totals was significant at the 5% level. Isolate by genotype interactions influenced only vermiform females and at the 5% level of significance.

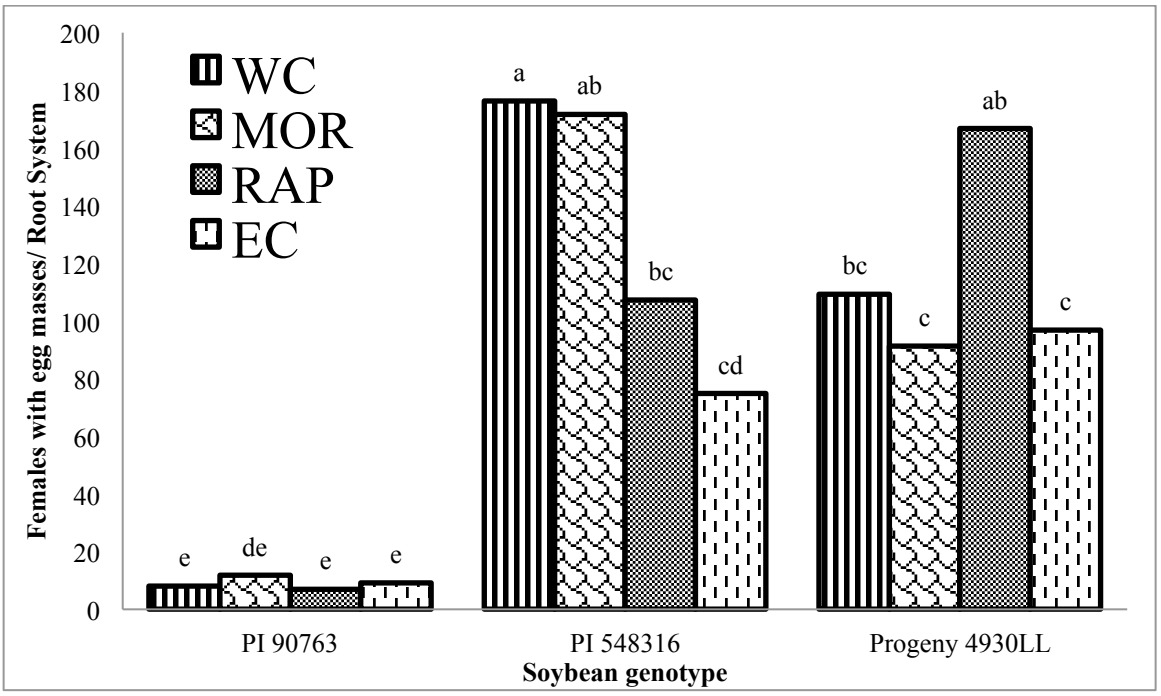

Figure 3. Numbers of females of *Rotylenchulus reniformis* with egg masses from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from root systems of the soybean genotypes PI 90763, PI 548316, and Progeny 4930LL. Data are means of ten replications combined over two 30-day duration trials conducted in a greenhouse environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

Across the three cotton genotypes, the greatest numbers of swollen females, 16 per root system, were found with the isolate from WC parish and a significantly lower number, 9 per root system, were recovered for the EC isolate (Table 7). Numbers of swollen females for RAP and MOR parishes averaged 13 and 10 per root system and were not significantly different from one another. Numbers of females with egg masses averaged 117 per root system for the WC isolate. Significantly lower numbers, 77, 54, and 50, were recovered from the RAP, MOR, and EC isolates, respectively. For root totals also, the WC isolate produced the highest numbers which averaged 136 per root system and significantly lower totals were found for RAP, MOR, and EC isolates, which averaged 92, 66, and 62, respectively.

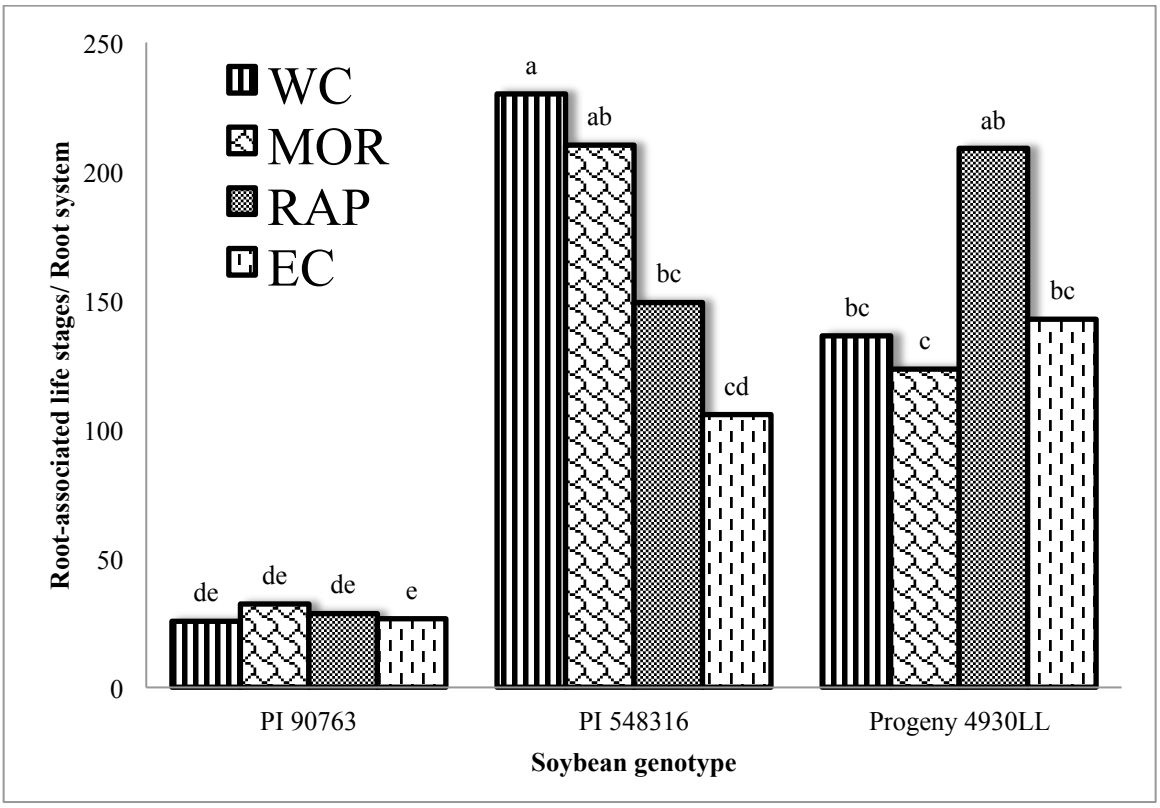

Figure 4. Total numbers of root-associated life stages of isolates of *Rotylenchulus reniformis* from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from root systems of the soybean genotypes PI 90763, PI 548316, and Progeny 4930LL. Data are means of ten replications combined over two 30-day duration trials conducted in a greenhouse environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

Table 6. Main and interaction effects (P values) of four isolates of *Rotylenchulus reniformis* endemic in Louisiana and three cotton genotypes on fresh root weight and root-associated nematode life stages in a greenhouse environment[x].

Source	DF	Fresh root weight	Root-associated nematode life stages			
			Vermiform females	Swollen females	Females with egg masses	Total
Isolate (I)[y]	3	0.24	0.18	0.0072**	<0.0001**	<0.0001**
Genotype (G)[z]	2	0.0003**	0.0032**	0.33	0.024*	0.021*
I x G	6	0.082	0.037*	0.21	0.23	0.17

[x] Data were combined over two 30-day duration trials and are means of ten replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[z] Genotypes were Stoneville 4946GLB2, MT 2468 Ren3, and M713 Ren5.

Fresh root weights for MT2, M7, and STN averaged only 1.0, 1.2, and 1.5g 30-d after planting (Table 8). The weight for STN was significantly greater than that for the other two genotypes. Numbers of vermiform females recovered from MT2 and M7, 4 and 3 respectively, were significantly greater than the 1 per root system found with STN. Across the four isolates of Reniform nematode the greatest number of females with egg masses occurred with MT2 and averaged 91 per root system. Numbers of females for M7 averaged 57 while those for STN averaged 76 per root system. Numbers from M7 and STN were not significantly different. Root totals for MT2, which averaged 108, were not different from the numbers 89 per root system from STN but were significantly greater than the numbers recovered from M7, which averaged 70.

Interaction of isolate of reniform nematode and genotype of cotton are presented as Figure 5. Of the three categories of root-associated females the isolate by genotype interaction influenced only the vermiform females and their numbers per root system were very low. The greatest number of vermiform females, 8 per root system, represented the EC isolate on MT2 genotype. On this genotype significantly lower numbers were found and they averaged 3 for WC and MOR and 2 for RAP. With the cotton genotype M7 the greatest number of vermiform females, 6 per root system, were observed for the WC isolate and significantly fewer numbers, 2 for MOR and RAP and 1 for the EC isolate, were detected. Numbers of vermiform females averaged less than 1 for the four isolates on STN and did not differ significantly from each other.

In summary of the greenhouse portion of this research, the two runs of the first greenhouse test employed eight endemic isolates of the nematode and three genotypes each for soybean and cotton as well as a census which indicated a total for the number of root-associated life stages. In both runs of the second greenhouse experiment the number of isolates of reniform

nematode was reduced from eight to the four on the basis of reproduction levels across both crops. The WC isolate that had the greatest amount of reproduction, the EC isolate had the least reproduction, and the RAP and MOR isolates had intermediate levels of reproduction. Additionally a more complete census of the root-associated nematode life stages was obtained by further categorizing them into vermiform females, swollen females, swollen females with egg masses as well as a total for the root-associated stages.

Table 7. Across three cotton genotypes[x] and four isolates[y] of *Rotylenchulus reniformis*, main effects on root-associated nematode life stages in a greenhouse environment[z].

Isolate	Root-associated nematode life stages			
	Vermiform females	Swollen females	Females with egg masses	Total
West Carroll	3 a	16 a	117 a	136 a
Rapides	2 a	13 ab	77 b	92 b
Morehouse	2 a	10 b	54 b	66 b
East Carroll	3 a	9 b	50 b	62 b

[x] Genotypes were MT 2468 Ren3, M713 Ren5, and Stoneville 4946GLB2.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[z] Data were combined over two 30-day duration trials and is means of ten replications. Means followed by a common letter are not significantly different based on Student's t-test.

Table 8. Across four isolates[y] of *Rotylenchulus reniformis* and three cotton genotypes, main effects on fresh root weight and root-associated nematode life stages in a greenhouse environment[z].

Genotype	Fresh root weight (g)	Root-associated nematode life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
MT2468 Ren3	1.0 b	4 a	13 a	91 a	108 a
M713 Ren5	1.2 b	3 a	10 a	57 b	70 b
Stoneville 4946GLB2	1.5 a	1 b	12 a	76 ab	89 ab

[y] Isolates were each derived from a single egg mass and were collected from cotton fields from West Carroll, East Carroll, Morehouse, and Rapides parishes.
[z] Data were combined over two 30-day duration trials and is means of ten replications. Means followed by a common letter are not significantly different based on Student's t-test.

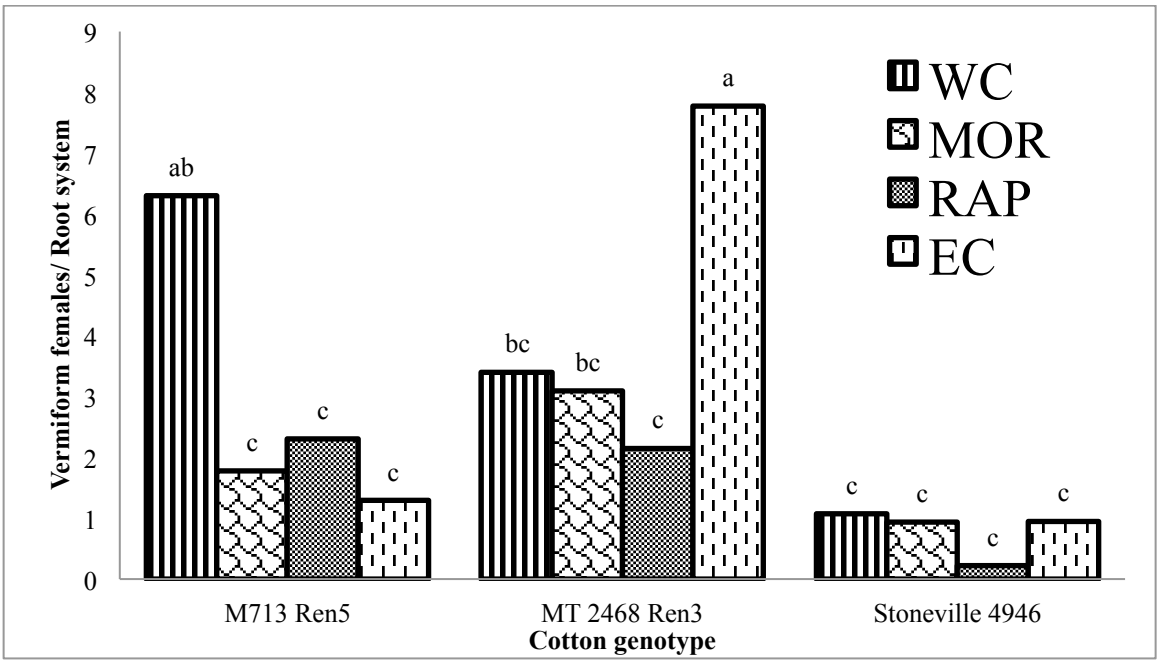

Figure 5. Numbers of vermiform females of *Rotylenchulus reniformis* from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from root systems of the cotton genotypes M713 Ren5, MT 2468 Ren3, and Stoneville 4946GLB2. Data are means of ten replications combined over two 30-day duration trials conducted in a greenhouse environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$)

Reproduction of the four isolates used in the second greenhouse test was consistent with that shown in the first greenhouse test. Data from both runs of the second greenhouse test indicated that of the three root-associated stages the females with egg masses were the best method to distinguish the isolates since the statistical analysis most closely reflected that for the total root-associated nematode life stages.

Laboratory Studies

In the first laboratory test there were no significant main effects of isolate but there were significant main effects of genotype which impacted fresh root weight, vermiform females, swollen females with egg masses, and total root stages (Table 9). Genotype main effects for fresh

root weight, females with egg masses, and total root-associated nematode stages were significant at the 1% level except those for vermiform females, which were significant at the 5% level. There were no isolate by genotype interactions.

Table 9. Main and interaction effects (P values) of four isolates of *Rotylenchulus reniformis* endemic in Louisiana and three soybean genotypes grown in polystyrene centrifuge tubes[w] on fresh root weight and root-associated nematode life stages in a laboratory environment[x].

Source	DF	Fresh root weight	Root-associated nematode life stages			
			Vermiform females	Swollen females	Females with egg masses	Total
Isolate (I)[y]	3	0.27	0.098	0.26	0.50	0.30
Genotype (G)[z]	2	0.0031**	0.039*	0.063	<0.0001**	<0.0001**
I x G	6	0.98	0.44	0.89	0.82	0.90

[w] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[x] Data were combined over two 21-day duration trials and are means of twelve replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[z] Genotypes were Progeny 4930LL, PI 90763, and PI 548316.

Fresh root weight for PI 548316 was 2.8 g and was significantly greater than PROG, averaging 2.5 g, and PI 90763 that averaged 2.4 g (Table 10). Numbers of vermiform females for PI 90763 and PI 548316, 10 and 8, respectively, were not significantly different but those for PROG, 5 per root system, were significantly lower than those for PI 90763. Significantly greater values for both females with egg masses and root-associated nematode life stage totals were obtained for PI 548316 and PROG than for PI 90763. Numbers of females with egg masses ranged from 14 to 64 and root totals ranged from 52 to 114 per root system.

In the cotton portion of the first 21 day duration laboratory test there was no significant main effect of isolate on fresh root weight but there was a genotype main effect significant at the 1% level (Table 11). Also, there were no isolate by genotype interactions that influenced root weight. There were significant main effects of both isolate and genotype on root-associated life stages. The main effect of isolate did not significantly influence vermiform females, but

significantly influenced swollen females, females with egg masses, and the total for life stages. Isolate main effects on females with egg masses and root totals were significant at the 1% level and on swollen females at the 5% level. As was the case with the isolate main effect, the main effect of genotype did not significantly affect vermiform females but significantly influenced swollen females, females with egg masses, and the root total. All effects were significant at the 1% level. Isolate by genotype interactions significantly influence all root-associated life stages of reniform nematode. The effect on vermiform females was significant at the 5% level and the interaction effect on swollen females; those with egg masses, and total life stages were significant at the 1% level.

Table 10. Across four isolates[x] of *Rotylenchulus reniformis* and three soybean genotypes grown in polystyrene centrifuge tubes[y], main effects on fresh root weight and root-associated nematode life stages in a laboratory environment[z].

Genotype	Fresh root weight (g)	Root-associated nematode life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
PI 548316	2.8 a	8 ab	42 a	64 a	114 a
Progeny 4930LL	2.5 b	5 b	34 ab	73 a	112 a
PI 90763	2.4 b	10 a	28 b	14 b	52 b

[x] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[y] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[z] Data were combined over two 21-day duration trials and is means of twelve replications. Means followed by a common letter are not significantly different based on Student's t-test.

Across the cotton genotypes, numbers of swollen females were greatest, averaging 13 per root system for the WC isolate and were significantly greater than the 8 per root system that were recovered for the EC isolate but not significantly different than the numbers for the RAP and MOR isolates which averaged 13 and 11 per root system, respectively (Table 12). The trend for the main effect of the three genotypes on females with egg masses and total nematodes per root system were the same. That is the highest numbers were observed for the WC isolate which averaged 71 females with egg masses and a root-associated nematode life stage total of 85. The

lowest numbers were found with the EC isolate which averaged 36 females with egg masses and 46 total root stages. Numbers for RAP and MOR averaged 53 and 47 females with egg masses and were significantly lower than the WC isolate. A similar trend is seen for totals per root system with averages of 68 and 59 for the RAP and MOR isolates, respectively.

Table 11. Main and interaction effects (P values) of four isolates of *Rotylenchulus reniformis* endemic in Louisiana and three cotton genotypes grown in polystyrene centrifuge tubes[w] on fresh root weight and root-associated nematode life stages in a laboratory environment[x].

Source	DF	Fresh root weight	Root-associated nematode life stages			
			Vermiform females	Swollen females	Females with egg masses	Total
Isolate (I)[y]	3	0.79	0.28	0.019*	<0.0001**	<0.0001**
Genotype (G)[z]	2	<0.0001**	0.12	<0.0001**	<0.0001**	<0.0001**
I x G	6	0.14	0.023*	<0.0001**	<0.0001**	<0.0001**

[w] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[x] Data were combined over two 21-day duration trials and are means of twelve replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[z] Genotypes were Stoneville 4946GLB2, MT 2468 Ren3, and M713 Ren5.

Across the isolates of Reniform nematode, fresh root weights of MT2 and M7 cotton both averaged 1.0 g and were significantly greater than STN cotton averaging, 0.7 g (Table 13). Numbers of swollen females for MT2 and M7 averaged 15 and 17 per root system and were significantly greater than STN, which averaged 2 per root system. A similar trend was observed for both females with egg masses and total root stages with values averaging 3 to 78 females with egg masses for STN and M7, respectively, and having subsequent root totals of 5 to 97.

Four of the five parameters that were examined with the first laboratory test on cotton showed significant isolate by genotype interactions. Those for females with egg masses and root stage totals are presented as Figures 6 and 7; histograms for each parameter showed an identical pattern. Overall, Figure 6 shows that of the three cotton genotypes M7 but not MT2 or STN can distinguish among the four isolates of reniform nematode. Numbers of swollen females on MT2

did not significantly differ across the four nematode isolates with values of 62, 76, 77, and 81 for RAP, MOR, WC, and EC, respectively. Similarly, there were no statistical differences in swollen females representing each of the four isolates on STN with numbers for WC, MOR, RAP, and EC averaging 3, 1, 2, and 4 females per root system, respectively. With the M7 genotype there were significant and stepwise decreases in the numbers of swollen females with egg masses that averaged 133 for WC, 95 for RAP, 64 for MOR, and 21 for EC.

Table 12. Across three cotton genotypes[w] and four isolates[x] of *Rotylenchulus reniformis* grown in polystyrene centrifuge tubes[y], main effects on root-associated nematode life stages in a laboratory environment[z].

Isolate	Root-associated nematode life stages			
	Vermiform females	Swollen females	Females with egg masses	Total
West Carroll	1 a	13 a	71 a	85 a
Rapides	2 a	13 a	53 b	68 b
Morehouse	2 a	11 ab	47 bc	60 bc
East Carroll	2 a	8 b	36 c	46 c

[w] Genotypes were MT 2468 Ren3, M713 Ren5, and Stoneville 4946GLB2
[x] Isolates were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[y] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[z] Data were combined over two 21-day duration trials and are means of twelve replications. Means followed by a common letter are not significantly different based on Student's t-test.

Table 13. Across four isolates[x] of *Rotylenchulus reniformis* and three cotton genotypes grown in polystyrene centrifuge tubes[y], main effects on fresh root weight and root-associated nematode life stages in a laboratory environment[z].

Genotype	Fresh root weight (g)	Root-associated nematode life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
MT2468 Ren3	1.0 a	3 a	15 a	74 a	92 a
M713 Ren5	1.0 a	2 ab	17 a	78 a	97 a
Stoneville 4946GLB2	0.7 b	0 b	2 b	3 b	5 b

[x] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[y] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[z] Data were combined over two 21-day duration trials and are means of twelve replications. Means followed by a common letter are not significantly different based on Student's t-test.

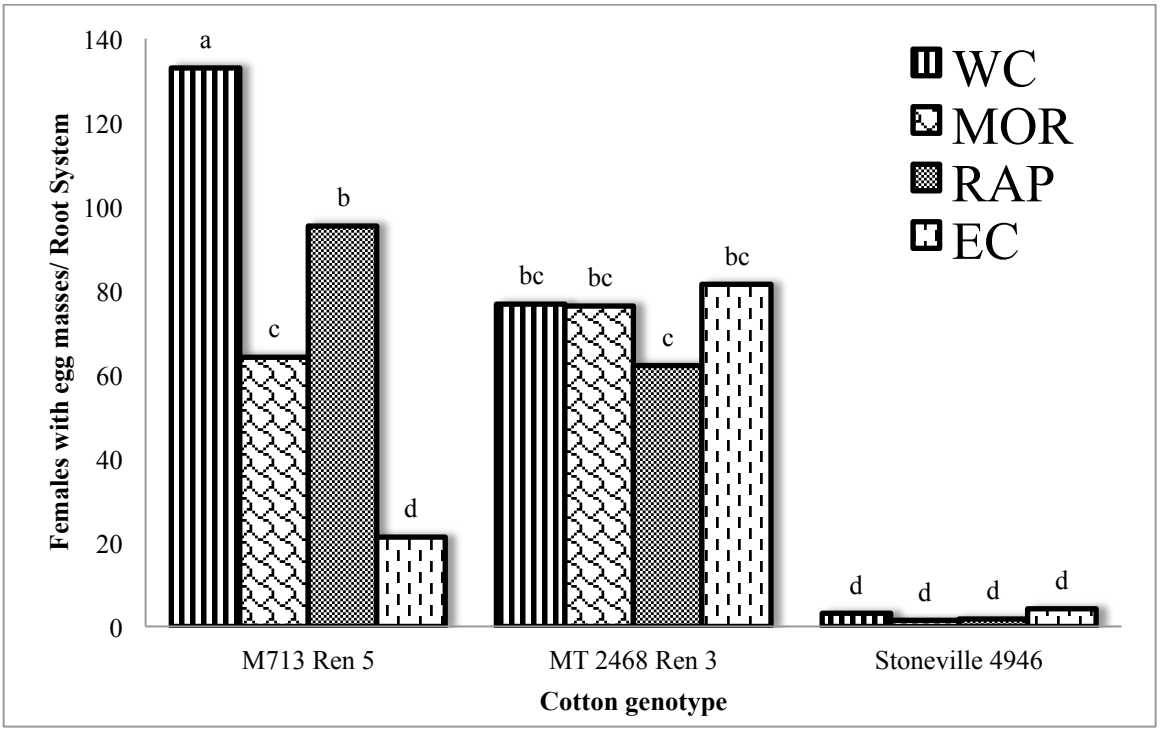

Figure 6. Numbers of females of *Rotylenchulus reniformis* with egg masses from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from root systems of the cotton genotypes M713 Ren5, MT 2468 Ren3, and Stoneville 4946GLB2. Data are means of twelve replications combined over two 21-day duration trials conducted in a laboratory environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

A histogram of totals for root-associated life stages is presented as Figure 7. The pattern for this figure is very similar to that of Figure 6 but takes into account the addition of numbers of vermiform and swollen females. The totals for root associated life stages also show that M7 but not the other two cotton genotypes distinguish the four nematode isolates. There was also a significant and stepwise decrease in root totals on the M7 genotype that averaged 160 for the WC isolate, 117 for the RAP isolate, 82 for the MOR isolate, and 28 for the EC isolate. As was the case with females with egg masses, root totals for MT2 were not significantly different than each other and averaged 104, 93, 90, and for EC, MOR, WC, and RAP respectively. Respectively, root totals of 5, 2, 7, and 6 were obtained for the isolates from WC, MOR, RAP, and EC on STN.

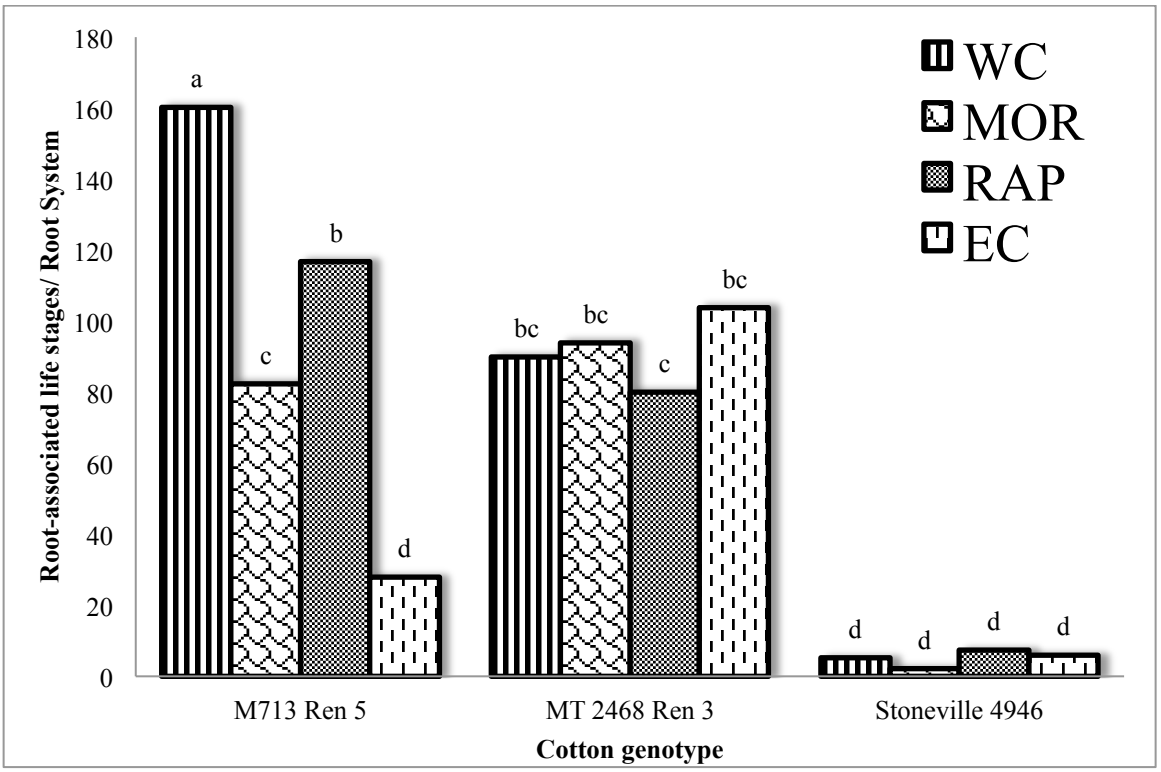

Figure 7. Total numbers of root-associated life stages of *Rotylenchulus reniformis* from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from root systems of the cotton genotypes M713 Ren5, MT 2468 Ren3, and Stoneville 4946GLB2. Data are means of twelve replications combined over two 21-day duration trials conducted in a laboratory environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

The second set of laboratory tests was conducted to determine whether or not a shorter time interval, 14 days, would still differentiate the four isolates of the nematode. In the soybean portion of the test there were significant isolate and genotype main effects and significant isolate by genotype interactions (Table 14). There were significant main effects of both isolate and genotype on fresh root weight at the 1% level. Main effects of isolate impacted numbers of vermiform females, swollen females, and root totals at the 5% level of significance and there was no significant effect on swollen females. There were significant genotype effects on vermiform females and females with egg masses at the 1% level and there was no genotype influence on

swollen females. The effect of genotype was significant on root totals but only at the 5% level. The only significant isolate by genotype interaction occurred with swollen females and was significant at the 5% level.

Table 14. Main and interaction effects (P values) of four isolates of *Rotylenchulus reniformis* endemic in Louisiana and three soybean genotypes grown in polystyrene centrifuge tubes[w] on fresh root weight and root-associated nematode life stages in a laboratory environment[x].

Source	DF	Fresh root weight	Root-associated nematode life stages			
			Vermiform females	Swollen females	Females with egg masses	Total
Isolate (I)[y]	3	<0.0001**	0.0398*	0.0118*	0.2155	0.0272*
Genotype (G)[z]	2	0.0025**	0.0075**	0.5481	<0.0001**	0.0136*
I x G	6	0.4894	0.6937	0.0170*	0.3972	0.0775

[w] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[x] Data were combined over two 14-day duration trials and are means of twelve replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[z] Genotypes were Progeny 4930LL, PI 90763, and PI 548316.

Across the three soybean genotypes, the highest fresh root weight occurred with the EC isolate and averaged 1.5 g (Table 15). Fresh root weights for the WC and MOR isolates both averaged 0.9 g and were significantly greater than that for EC. Across the genotypes, the significantly lowest root weight of 0.6 g was observed for RAP. Numbers of vermiform females was greatest on WC, averaging 8 per root system, and was lowest on EC with 4 per root system. Values for the WC isolate were significantly greater than those for RAP and EC but not MOR. Numbers of swollen females were greatest for WC and EC and WC was significantly greater than RAP and MOR. A similar trend is seen for total root stages with values of 68, 55, 48, and 46 for WC, EC, RAP, and MOR, respectively.

The soybean genotypes PI 548316 and PROG had fresh root weights that averaged 1.0 g and 1.1 g, respectively, and were significantly greater than the 0.7 g weight of roots for PI 90763 (Table 16). Numbers of vermiform females ranged from a high of 8 per root system for PI 90763

to a low of 4 for PI 548316. Numbers of vermiform females for PROG averaged 5 per root system and was not significantly different than numbers for PI 548316 but were significantly greater than numbers for PI 90763. There were stepwise and significant decreases in numbers of females with egg masses that averaged 33 for PROG, 24 for PI 548316, and 13 for PI 90763. The highest life stage root totals averaged 65 for PROG and numbers from PI 548316 were not significantly different, averaging 53 per root system. Root totals for PI 90763 averaged 45 and were significantly different from those for PROG but not PI 548316.

Table 15. Across three soybean genotypes[w] grown in polystyrene centrifuge tubes[x] and four isolates[x] of *Rotylenchulus reniformis,* main effects on fresh root weight and root-associated nematode life stages in a laboratory environment[z].

Isolate	Fresh root weight (g)	Root-associated nematode life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
West Carroll	0.9 b	8 a	32 a	28 a	68 a
Rapides	0.6 c	5 b	20 b	23 ab	48 b
Morehouse	0.9 b	6 ab	22 b	18 b	46 b
East Carroll	1.5 a	4 b	27 ab	24 ab	55 ab

[w] Genotypes were PI 548316, Progeny 4930LL, and PI 90763.
[x] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[z] Data were combined over two 14-day duration trials and are means of twelve replications. Means followed by a common letter are not significantly different based on Student's t-test.

A histogram illustrating numbers of swollen females for the twelve possible isolate and genotype combinations is presented as Figure 8. Only the WC isolate-PROG combination resulted in numbers of swollen females, 45 per root system, that were significantly greater than all other nematode isolate-genotype combinations. With PI 548316 there were no differences between isolates in the numbers of swollen females per root system that ranged from 22 for WC to 27 for MOR. On PROG, 45 swollen females per root system were produced by the WC isolate. Numbers from RAP, MOR, and EC did not differ significantly and averaged respectively 14, 24, and 27. With PI 90763 numbers of swollen females did not differ among the WC, RAP,

and EC isolates and ranged from 30 to 16. The MOR isolate had an average number of swollen females per root system of 16 and it was not significantly different than the 23 per root system for the RAP isolate.

Table 16. Across four isolates[x] of *Rotylenchulus reniformis* endemic in Louisiana and three soybean genotypes grown in polystyrene centrifuge tubes[y], main effects on fresh root weight and root-associated nematode life stages in a laboratory environment[z].

Genotype	Fresh root weight (g)	Root-associated nematode life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
PI 548316	1.0 a	4 b	25 a	24 b	53 ab
Progeny 4930LL	1.1 a	5 b	27 a	33 a	65 a
PI 90763	0.7 b	8 a	24 a	13 c	45 b

[x] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[y] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[z] Data were combined over two 14-day duration trials and are means of twelve replications. Means followed by a common letter are not significantly different based on Student's t-test.

In the cotton portion of the second laboratory test there were significant isolate and genotype main effects that influenced every parameter except vermiform females. Root weight was affected both by isolate and genotype, with significance at the 1% and 5% levels, respectively (Table 17). Isolate main effects influenced swollen females and totals for root-associated life stages with significance at the 1% level and females with egg masses at the 5% level. The genotype main effect was significant at the 1% level for swollen females, females with egg masses, and root totals. Isolate by genotype interactions influenced all parameters with significance at the 1% level except for the females with egg masses that was significant at the 5% level.

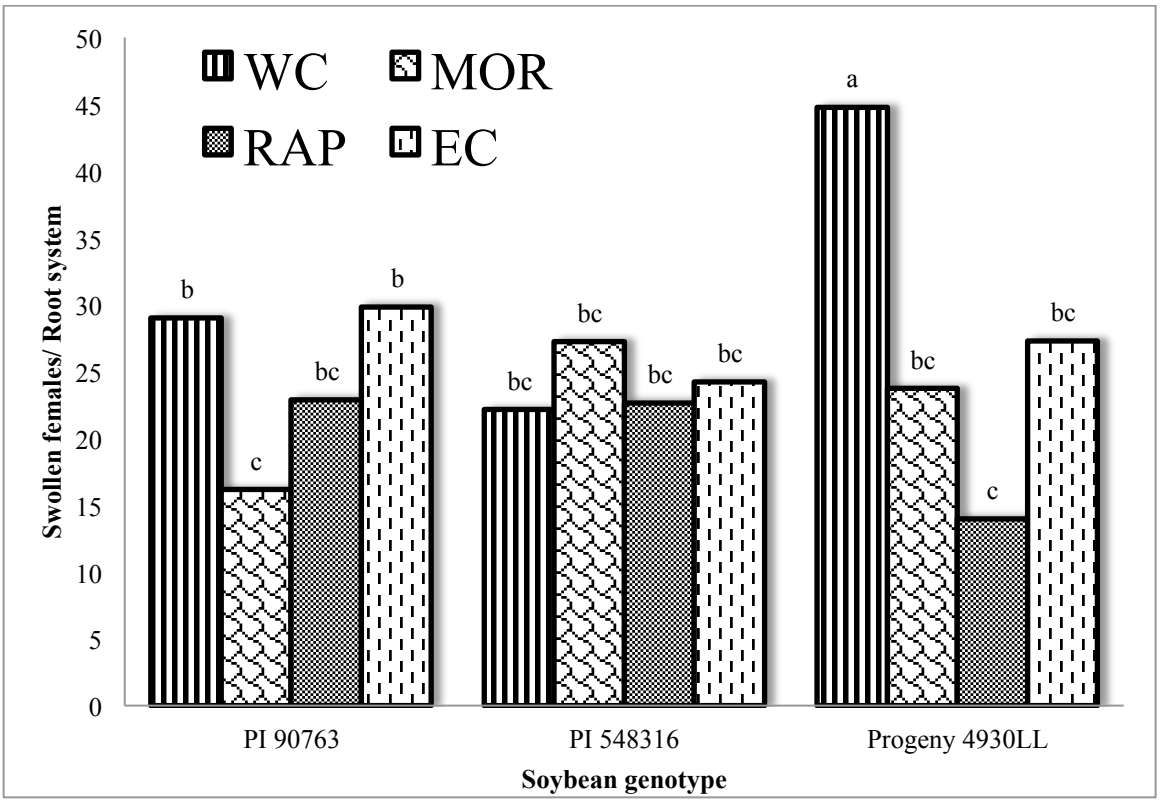

Figure 8. Numbers of swollen females of *Rotylenchulus reniformis* from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from root systems of soybean genotypes PI 90763, PI 548316, and Progeny 4930LL. Data are means of twelve replications combined over two 14-day duration trials conducted in a greenhouse environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

Across the three cotton genotypes, the highest fresh root weight occurred with the EC isolate, which averaged 0.4 g, and was significantly greater than WC, RAP, and MOR isolates, which averaged 0.2 g, 0.3 g, and 0.2 g, respectively (Table 18). Numbers of swollen females ranged from a high of 18 for WC to a low of 7 for MOR. Numbers of swollen females averaged 9 for RAP and 7 for MOR and these were significantly lower than the numbers from WC but not from those for EC. Numbers of females with egg masses per root system averaged 23 for WC, 17 for EC, and 15 for RAP and were not significantly different. The 9 females with egg masses recovered from MOR were significantly low than those recovered from WC. The total life stages

per root system averaged 45 for WC and 33 for EC and were not significantly different. Totals for RAP averaged 26 and those for MOR averaged 17. Totals for RAP, MOR, and EC did not differ significantly.

Table 17. Main and interaction effects (P values) of four isolates of *Rotylenchulus reniformis* endemic in Louisiana and three cotton genotypes grown in polystyrene centrifuge tubes[w] on fresh root weight and root-associated nematode life stages in a laboratory environment[x].

Source	DF	Fresh root weight	Root-associated nematode life stages			
			Vermiform females	Swollen females	Females with egg masses	Total
Isolate (I)[y]	3	<0.0001**	0.0578	0.0020**	0.0222*	0.0021**
Genotype (G)[z]	2	0.0439*	0.1062	<0.0001**	<0.0001**	<0.0001**
I x G	6	0.0044**	0.0023**	0.0028**	0.0126*	0.0006**

[w] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[x] Data were combined over two 14-day duration trials and are means of twelve replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[z] Genotypes were MT2468 Ren3, M713 Ren5, and Stoneville 4946GLB2.

Table 18. Across three cotton genotypes[w] grown in polystyrene centrifuge tubes[y] and four isolates[x] of *Rotylenchulus reniformis* endemic in Louisiana, main effects on fresh root weight and root-associated nematode life stages in a laboratory environment[z].

Isolate	Fresh root weight (g)	Root-associated nematode life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
West Carroll	0.2 b	4 a	18 a	23 a	45 a
Rapides	0.3 b	2 ab	9 b	15 ab	26 bc
Morehouse	0.2 b	1 b	7 b	9 b	17 c
East Carroll	0.4 a	3 ab	13 ab	17 ab	33 ab

[w] Genotypes were MT 2468 Ren3, M713 Ren5, and Stoneville 4946GLB2.
[x] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[z] Data were combined over two 14-day duration trials and are means of twelve replications. Means followed by a common letter are not significantly different based on Student's t-test.

Fresh root weights for M7 and STN each averaged 0.3 g and were significantly greater than the 0.2 g fresh root weight for MT2 (Table 19). Numbers of swollen females were 18 and 13 for MT2 and M7, respectively, and were significantly greater than the 5 per root system

recovered for STN. An average of 27 females with egg masses were collected from MT2 and this was significantly greater than the 18 per root system found for M7 and the 3 per root system found for STN. The totals for root-associated life stages decreased in a step-wise and significant manner from 48 per root system for MT2 to 33 for M7 and to 9 per root system for STN.

Table 19. Across four isolates[x] of *Rotylenchulus reniformis* endemic in Louisiana and three cotton genotypes grown in polystyrene centrifuge tubes[y], main effects on fresh root weight and root-associated nematode life stages in a laboratory environment[z].

Genotype	Fresh root weight (g)	Root-associated nematode life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
MT2468 Ren3	0.2 b	3 a	18 a	27 a	48 a
M713 Ren5	0.3 a	2 ab	13 a	18 b	33 b
Stoneville 4946GLB2	0.3 a	1 b	5 b	3 b	9 c

[x] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.
[y] Each centrifuge tube had a capacity of 50 ml and contained 45 g of steam sterilized soil.
[z] Data were combined over two 14-day duration trials and are means of twelve replications. Means followed by a common letter are not significantly different based on Student's t-test.

There were significant isolate by genotype interactions that influenced fresh root weight as well as all root-associated life stages of the nematode at 14 days. As was the case for the interaction influence on cotton genotypes at 21 days histograms describing individual treatment means for each root-associated life stage are very similar and only those for females with egg masses and nematode totals for the root systems are presented in Figures 9 and 10, respectively. On MT2 at 14 days, an average of 40 females with egg masses were recovered with the isolate of reniform nematode from EC parish. The numbers recovered on this genotype with both the WC and RAP isolates were significantly lower and each averaged 25. The lowest number recovered for this genotype, 17 per root system was with the MOR isolate but it was not significantly different than numbers for the WC and RAP isolates. Averages of 32 and 20 females with egg masses for the WC and RAP isolates, respectively, that did not differ significantly were counted on roots of M7 after 14 days. With the WC and RAP isolates totals of 11 and 8 females with egg

masses were observed on M7, respectively. For the EC and MOR isolates there were no statistical differences compared with each other or to the RAP isolate. The genotype STN had roots containing 10 females with egg masses of the WC isolate and 3 of the EC isolate and less than 1 per root system for the MOR and RAP isolates with no statistical differences among isolates.

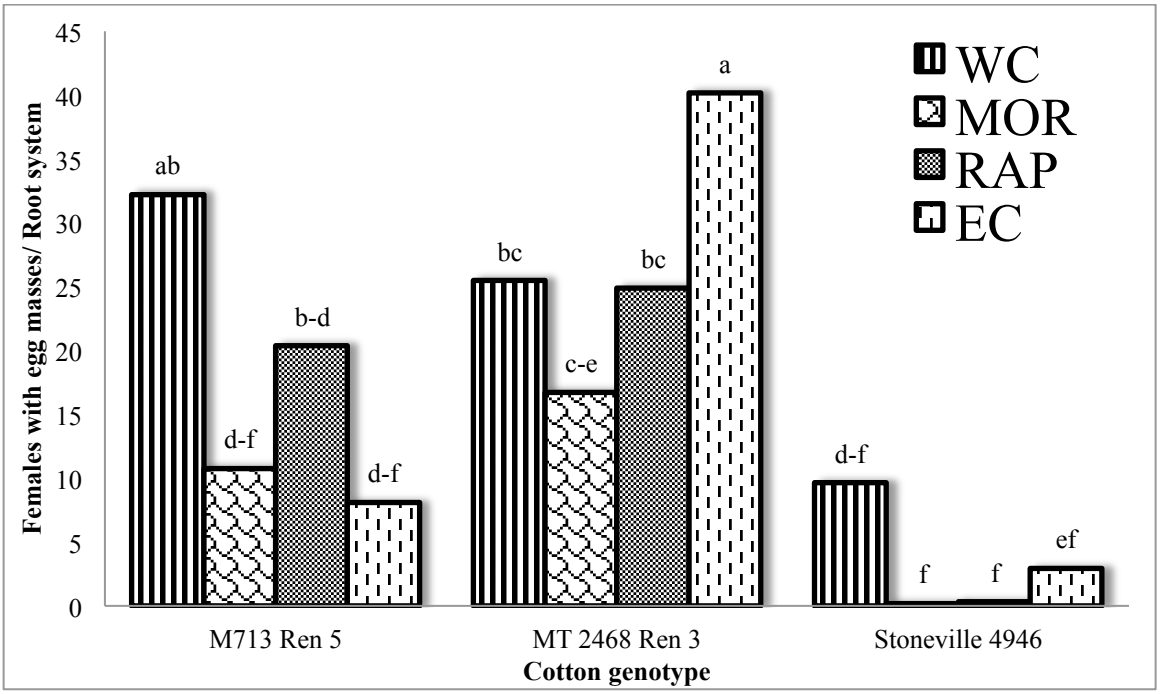

Figure 9. Number of females of *Rotylenchulus reniformis* with egg masses from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from entire root systems of cotton genotypes M713 Ren5, MT 2468 Ren3, and Stoneville 4946GLB2. Data are means of twelve replications combined over two 14-day duration trials conducted in a laboratory environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

Counts of the total numbers for root-associated stages of reniform nematode at 14 days on the cotton genotype MT2 averaged a high of 77 per root system for EC (Figure 10). Averages on this genotype ranged from 45 for WC, 40 for RAP, and 31 for MOR with no significant differences among these totals. A life stage total of 66 individuals per root system was observed for the WC isolate on M7 and this was significantly greater than the numbers recovered from the MOR and EC that averaged 19 and 14, respectively. There were no differences in root totals in

any of the isolates for STN. Numerically the highest total, 23, was for the WC isolate followed by averages of 10 per root system for EC and 2 and 3 respectively for MOR and RAP parishes.

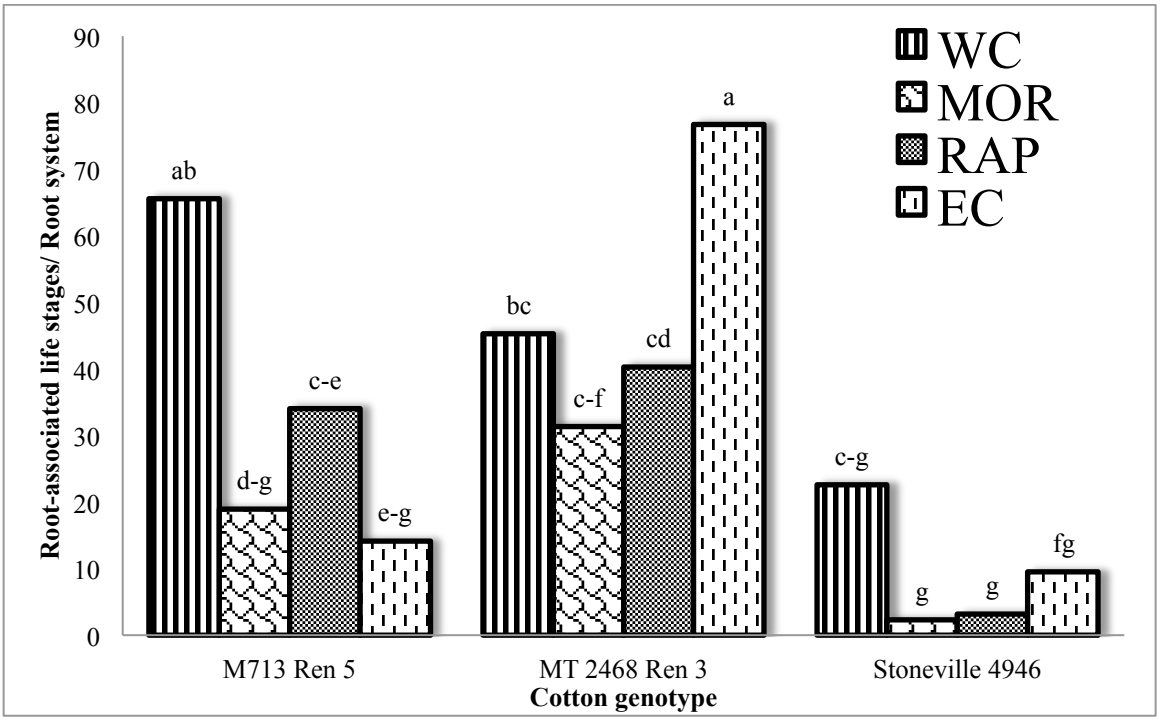

Figure 10. Total numbers of root-associated life stages of *Rotylenchulus reniformis* from West Carroll (WC), Morehouse (MOR), Rapides (RAP), and East Carroll (EC) parishes recovered from entire root systems of cotton genotypes M713 Ren5, MT 2468 Ren3, and Stoneville 4946GLB2. Data are means of twelve replications combined over two 14-day duration trials conducted in a laboratory environment. Bars with common letters are not significantly different based on Student's t-test ($P \leq 0.05$).

At 18 days after inoculation, tomato plants grown in soilless germination pouches that showed significant effects of isolate on swollen females and total root-associated nematode life stages at the 1% level and on females with egg masses at the 5% level (Table 20). Numbers of vermiform females did not differ significantly among the four isolates of the nematodes with values ranging from 0 to 2 females per root system (Table 21). Numbers of swollen females that averaged 19 and 15 for WC and RAP were both significantly greater than numbers of 4 per plant that were observed for both the MOR and EC isolates. The highest numbers of females with egg masses averaged 19 per root system for the RAP isolate and 12 per root system for the WC

isolate. Numbers of females with egg masses for MOR and EC isolates averaged 1 and 5 per root system and were not significantly different then the numbers recovered from the WC isolate. The total numbers of life stages per tomato root system averaged 35 and 31 for WC and RAP isolates, respectively, Both of these totals were significantly higher than the 11 and 6 per root system observed for the MOR and EC isolates.

Table 20. Main effects (P values) of isolate for four isolates of *Rotylenchulus reniformis* endemic in Louisiana and Rutgers tomato in germination pouches on fresh plant weight and root-associated nematode life stages in a laboratory environment[y].

Source	DF	Fresh plant weight	Root-associated nematode life stages			
			Vermiform females	Swollen females	Females with egg masses	Total
Isolate (I)[z]	3	0.3547	0.5522	0.0051**	0.0277*	0.0087**

[y] Data were combined over two 18-day duration trials and are means of eight replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[z] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.

Table 21. Numbers of root-associated life stages of *Rotylenchulus reniformis* on roots of Rutgers tomato and fresh root weights of plants grown in soilless growth pouches in a laboratory environment[y].

Isolate[z]	Fresh plant weight (g)	Root-associated life stages			
		Vermiform females	Swollen females	Females with egg masses	Total
West Carroll	0.2 a	0 a	19 a	12 ab	35 a
Rapides	0.3 a	1 a	15 a	19 a	31 a
Morehouse	0.3 a	0 a	4 b	1 b	11 b
East Carroll	0.2 a	2 a	4 b	5 b	6 b

[y] Data were combined over two 18-d duration trials and are means of eight replications. Means followed by a common letter are not significantly different based on Student's t-test.
[z] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.

The dissection and examination of egg masses of each of the four isolates of reniform nematode from infected roots of Rutgers tomato showed that the effect of isolate was significant at the 1% level for hatched juveniles, eggs containing fully developed juveniles, eggs without fully developed juveniles as well as totals for the entire egg mass content (Table 22). The WC isolate had significantly greater numbers of hatched juveniles, eggs containing fully developed

juveniles, eggs without fully developed juveniles as well as egg mass totals than did the isolates from MOR and EC parishes (Table 23). For the WC isolate the numbers of eggs without a fully developed juvenile averaged 16 per egg mass, the number of eggs containing a fully developed juvenile averaged 11, hatched juveniles averaged 4, and totals per egg mass averaged 31. Similarly values for the isolate from RAP parish averaged 15 eggs without fully developed juveniles, 5 that contained a fully developed juvenile and 2 that contained a hatched juvenile resulting in a total of 22 eggs and juveniles per egg mass. In the same order, stages for the MOR isolate averaged 9, 0, 1 and 10 and for the EC isolate values averaged 9, 3, 1, and 14.

For egg viability, there were significant effects of isolate at 48 hours and on cumulative totals (Table 24). At 24 hours there were no significant differences in numbers of juveniles recovered from funnels for the WC, RAP, and MOR isolates (Table 25). Totals for isolates from these three parishes averaged 323, 319, and 230, respectively. The numbers of juveniles for the EC isolate were significantly lower, averaging 101, than numbers for WC and RAP but not the MOR isolate. After 48 hours an additional 44 juveniles were recovered from the WC isolate and an additional 41 and 27 for the MOR and EC isolates. Numbers of juveniles for these three isolates were not significantly different but all except the MOR isolate were significantly lower than the 94 recovered for the RAP isolate. At 72 hours there were no significant differences in numbers of additional juveniles that hatched from eggs. Numbers for the RAP, WC, MOR, and EC isolates averaged 47, 44, 33, and 16. There was also no difference in the additional numbers of juveniles that hatched from the four isolates after 96 hours. Additional numbers of juveniles were 16 for EC, 14 for WC, 13 for RAP, and 8 for MOR. The cumulative totals at 96 hours were

not significantly different for the RAP, WC, and MOR isolates, averaging 473, 424, and 312, respectively. The cumulative total for the isolate from EC averaged 161 and was not significantly different than the cumulative total for MOR.

Table 22. Effect of isolate (P values) on four isolates of *Rotylenchulus reniformis* endemic in Louisiana on egg mass contents from Rutgers tomato in a greenhouse environment[y].

Source	DF	Egg mass contents			
		Eggs without a fully developed juvenile	Eggs containing a developed juvenile	Hatched juvenile	Total
Isolate (I)[z]	3	0.0036**	<0.0001**	0.0008**	<0.0001**

[y] Data were combined over two 18-day duration trials and are means of eight replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[z] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.

Table 23. Numbers of eggs and juveniles contained within egg masses of four isolates[y] of *Rotylenchulus reniformis*[z] on Rutgers tomato.

Isolate	Egg mass contents			
	Eggs without a fully developed juvenile	Eggs containing a developed juvenile	Hatched juvenile	Total
West Carroll	16 a	11 a	4 a	31 a
Rapides	15 a	5 b	2 b	22 b
Morehouse	9 b	0 c	1 b	10 c
East Carroll	9 b	3 bc	1 b	14 c

[y] Isolates were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[z] Data were combined over two trials and are means of thirty replications. Means followed by a common letter are not significantly different based on Student's t-test.

In the laboratory portion of this research the isolates employed in all studies were those from WC, RAP, MOR, and EC parishes that were selected in the greenhouse based portion of this project. At 21 days the cotton test in centrifuge tubes distinguished the WC isolate as the one with the greatest amount of reproduction, the RAP and MOR isolates as the ones with intermediate levels of reproduction and the EC isolate as the one with the least amount of reproduction. In the centrifuge tube environment at 21 days there were no differences in populations densities of Reniform nematode among the three soybean genotypes. When the duration of the test was reduced to 14 days, the order of reproduction by the isolates changed

somewhat but remained greatest by the WC isolate. However, in this shorter duration test the isolates responded similarly on both soybean and cotton.

Table 24. Isolate effect (P values) of four isolates of *Rotylenchulus reniformis* endemic in Louisiana on egg viability from Rutgers tomato in a greenhouse environment[y].

Source	DF	Hours of Incubation				Cumulative total
		24	48	72	96	
Isolate (I)[z]	3	0.0782	0.0410*	0.2675	0.7824	0.0166*

[y] Data were combined over two 18-day duration trials and are means of eight replications; * and ** indicates P values significant at 0.05 and 0.01 levels, respectively.
[z] Isolates were each derived from a single egg mass and were collected from cotton fields in West Carroll, East Carroll, Morehouse, and Rapides parishes.

Table 25. Estimated hatch of juveniles from eggs[x] of four isolates[y] of *Rotylenchulus reniformis* employing a Baermann funnel apparatus[z].

Isolate	Hours of Incubation				Cumulative total
	24	48	72	96	
West Carroll	323 a	44 b	44 a	14 a	425 a
Rapides	319 a	95 a	47 a	13 a	474 a
Morehouse	230 ab	41 ab	33 a	8 a	312 ab
East Carroll	101 b	27 b	16 a	16 a	160 b

[x] At time zero, 1,000 freshly collected eggs from Rutgers tomato were pipetted onto each funnel.
[y] Isolates were each derived from a single egg mass and were collected from cotton fields in Louisiana.
[z] Data were combined over two trials and are means of eight replications. Means followed by a common letter are not significantly different based on Student's t-test.

Growth of soybean and cotton in the soilless pouches was erratic and inconsistent for both cotton and soybean. With tomato, differences among isolates could be observed at 18 days. Growth pouches produced results similar to those seen across the three cotton genotypes in centrifuge tubes at 21 days for root-associated life nematode stage totals but especially for numbers of females with egg masses.

Studies with egg masses show that total egg mass contents on tomato could also be used to differentiate three of the four isolates. Results from egg hatch studies with Baermann funnels showed only that fewer numbers of juveniles hatched from the EC isolate than from the WC and RAP isolates of Reniform nematode.

DISCUSSION

The research described herein was divided into greenhouse and laboratory environments. The objectives were 1) to determine whether or not a greenhouse assay can distinguish endemic populations of *Rotylenchulus reniformis* and 2) to determine if root-associated life stages and a laboratory environment can also distinguish among endemic populations of *Rotylenchulus reniformis.* Data of the 30-day duration greenhouse tests from this research, as well as that from full-season, 150-day duration, trials conducted concurrently by Khanal et al. (2016) and Kularathna et al. (2016) using the same nematode isolates and plant species showed that there were significant differences in reproduction among endemic isolates. In the greenhouse environment, reproduction by the isolate from West Carroll Parish was greatest, that by the isolate from East Carroll was least and that by the isolates from Rapides and Morehouse parishes were intermediate. Greenhouse tests further demonstrated that the cotton genotype M713 Ren5 and the soybean genotype PI 548316 were best able to discriminate among the four endemic isolates of Reniform nematode.

When this research was moved to the laboratory environment employing the same cotton and soybean genotypes and the same isolates of Reniform nematode, the objective was to determine whether or not results from this environment would mirror those of the greenhouse and allow separation of the isolates on the basis of shorter duration studies with only root-

associated stages of the nematode. Additionally, the utility of conducting these studies with host plant growth media involving the use of steam sterilized soil or a soilless pouch system was compared.

Results from the 21-day duration laboratory tests with cotton in the centrifuge tube system mirrored those obtained in the greenhouse system. However, results obtained for soybean grown in the centrifuge tubes for 21-day did not distinguish levels of reproduction among the four isolates of reniform nematode.

When the experimental duration was shortened from 21 to 14 days, both soybean and cotton distinguished the WC isolate as the one having the greatest level of reproduction. However, reproduction by the EC isolate in this shorter time period changed its reproductive ranking among the four isolates from last to second greatest but reduced the four isolates into only two statistically distinguishable groups. Because 14 days is only slightly greater than half the normal life cycle duration of this species of reniform nematode (Nakasono, 1966) it is probably not useful to reduce the soil-based experimental duration to less than the 21 days.

Evaluation of whether or not a soilless environment would be effective in distinguishing reproductive classes among the four isolates of the nematode was a difficult and time-consuming endeavor. Numerous tests using soybean and cotton produced erratic and non-reproducible results that were in large part based on unsatisfactory growth of these two plant species in a soilless environment. Despite effects to control temperature, humidity, fertilizer rates, and nematode infestation levels, reproducible results were not obtained until the test plant was changed from soybean or cotton to tomato. Even though this system only generated two statistically distinguishable reproduction groupings, the results more closely resembled those from both the centrifuge tube results at 21 days in the laboratory as well as the greenhouse data.

Notable conclusions from the research described herein include: 1) virulence phenotypes of the nematode can probably be separated on the basis of the examination and enumeration of only life stages of the nematode associated with host roots; 2) a laboratory environment may be used in lieu of greenhouse based tests to distinguish among root-associated life stages of isolates of *R. reniformis* in 18-21 days; and 3) cotton and tomato are more effective than soybean in distinguishing among endemic isolates of *R. reniformis*.

The research of Khanal et al. (2016) and Kularathna et al. (2016) clearly demonstrated that these same endemic isolates of *R. reniformis* display differences in both reproduction and pathogenicity on cotton and soybean. Population data from their full-season microplot studies, which focused only on the second and third stage juveniles and males that occur in soil, showed that populations can be distinguished on the basis of these life stages. Results from the greenhouse and laboratory work with these same isolates of the nematode focused on the vermiform and swollen female life stages of Reniform nematode with and without attached egg masses. Research summarized herein, concludes that these root-associated life stages of the nematode can also be used to separate the nematode into reproduction classes. The significance of this observation is that it suggests that, if verified by studies with an expanded range of populations from other southern states, virulence phenotypes of the nematode may be identified on the basis of only the examination and enumeration of stages of the nematode that are associated with host roots. Moreover, if a direct assessment, under laboratory conditions, of only the female life stages on roots is possible and distinguishes among virulence phenotypes of this nematode, a tremendous reduction in soil-processing time, greenhouse experimentation and maintenance requirements and labor may be realized.

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