VARIATION IN REPRODUCTION AND PATHOGENICITY OF GEOGRAPHIC ISOLATES OF *ROTYLENCHULUS RENIFORMIS* ON COTTON

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ABSTRACT

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The comparative reproduction and pathogenicity of isolates of *Rotylenchulus reniformis* from Alabama, Arkansas, Hawaii, Louisiana, Mississippi and Texas on cotton was evaluated in microplot trials. Prior to initiation of microplot trials, ten single-egg mass populations were derived from each geographic isolate. Reproduction of the populations of each geographic isolate was evaluated in greenhouse studies with Stoneville LA887 cotton by assessing the numbers of vermiform stages in soil and eggs per gram of root tissue 60 days after inoculation. On the basis of these trials, each repeated once, one population of each of the six isolates was selected for use in microplot trials. Averaged over the two trials, population designations selected for use in microplot trials and their respective reproduction values (R, where R = Pf/Pi) and numbers of eggs per gram of root were: AL-8 (R = 14.9, eggs = 202); AR-3 (R = 30.4, eggs = 525); HI-9 (R = 20.2, eggs = 183); LA-3 (R = 18.2, eggs = 517.); MS-7 (R = 25.7, eggs = 602) and TX-10 (R = 42.8, eggs = 938). Data from full-season (147 days) microplot trials, averaged over 2 years, showed significant differences (Tukey's HSD test ($P \leq 0.05\%$)) among isolates of reniform nematode in both reproduction and pathogenicity. Dry plant weight at harvest averaged 370.6 g for the non-inoculated control. All isolates except the one from HI produced root weights at harvest that were reduced significantly below that of the control. Harvest weights for plants inoculated with LA-3 and MS-7 were significantly lower than those from the other four geographic regions.

Key words: cotton, reniform nematode, reproduction, *Rotylenchulus reniformis*.

RESUMEN

McGawley, E. C., M. J. Pontif, and C. Overstreet. 2010. Variación en la reproducción y patogenicidad de aislamientos geográficos de *Rotylenchulus reniformis* en algodón. Nematropica 40:275-288.

Se evaluó la reproducción y patogenicidad de aislamientos de *Rotylenchulus reniformis* provenientes de Alabama, Arkansas, Hawaii, Louisiana, Mississippi y Texas en algodón en microparcelas. Antes de iniciar las evaluaciones de microparcelas, se generaron 10 poblaciones a partir de masas de huevos individuales para cada aislamiento. Se evaluó la reproducción de las poblaciones en el invernadero en algodón Stoneville LA887, contando la cantidad de estados vermiformes en el suelo y los huevos por gramo de raíz a los 60 días después de la inoculación. Con base en estos estudios de reproducción, se seleccionó una población de cada aislmiento para los ensayos de microparcelas. Los valores de reproducción (R = población final/población inicial) y de huevos por gramo de raíz para las poblaciones seleccionadas fueron los siguientes: AL-8 (R = 14.9, huevos = 202); AR-3 (R = 30.4, huevos = 525); HI-9 (R = 20.2, huevos = 183); LA-3 (R = 18.2, huevos = 517.); MS-7 (R = 25.7, huevos = 602) and TX-10 (R = 42.8, huevos = 938). Los datos de las microparcelas (147 days), en promedios de 2 años, indicaron diferencias significativas (prueba de Tukey HSD ($P \leq 0.05\%$)) entre aislamientos de nematodo reniforme tanto en la reproducción como en la patogenicidad. El peso seco de la planta al momento de la cosecha fue de 370.6 g en promedio para el control no inoculado. Todos los aislamientos, excepto el proveniente de Hawaii, produjeron pesos de raíces al momento de la cosecha que fueron sig-

nificativamente menores que el control. El peso al momento de la cosecha de las plantas inoculadas con LA-3 y MS-7 fue significativamente menor que el de las plantas inoculadas con aislamientos provenientes de las otras regiones geográficas.

Palabras clave: algodón, nematodo reniforme, reproducción, *Rotylenchulus reniformis*.

INTRODUCTION

The reniform nematode, *Rotylenchulus reniformis*, is widespread and damaging in much of the cotton acreage of the Southern U.S.A. (Koenning, *et al.*, 2004). Currently, there are 232 counties or parishes in the cotton producing areas that are infested with this nematode (Anonomyous, 2010a). The value of the U.S. cotton crop, produced on 3.1 million hectares (Anonomyous, 2010b), was estimated to be $3.74 billion (U.S.) dollars during 2009 (Anonomyous, 2010c). Losses caused by the reniform nematode to cotton during 2009 were $60 million (U.S.) and occurred primarily in the states of Alabama, Arkansas, Georgia, Louisiana, Mississippi, Tennessee and Texas (Blassingame *et al.,* 2010). Significantly increased incidence of reniform nematode, especially over the last decade, has been documented in the Southern U.S.A. (Robinson, 2007; Overstreet and McGawley, 1999).

Rotylenchulus reniformis has been documented to be quite variable in both morphology (Ching, 1969; Germani, 1978; Lehman and Inserra, 1990; Soares *et al.*, 2003, 2004; Agudelo *et al.*, 2001, 2005) and pathogenicity (Decker *et al*, 1966; Carter, 1981; Heald and Meridith, 1987; McGawley and Overstreet, 1995; Nakasomo, 2004; Arias *et al*, 2009). There has also been significant variation, and even some direct contradictions (Robinson *et al*, 1997), in reported host associations of the nematode. Even among different cultivars of a single host species, marked variation in reproduction has been documented for a single population of *R. reniformis*: Routary et al. with chili pepper (1988); Birchfield and Brister, Koenning *et al.* and Usery *et al.* with cotton (1963, 2000, 2005, respectively) and Robbins *et al.* with soybean (2001, 2002). Additionally, inoculation studies employing multiple populations of *R. reniformis* document variation in reproduction and pathogenicity across a variety of crops. Heald and Meredith in 1987 found that a population of *R. reniformis* from Venzuela produced significantly greater damage on tobacco than two U.S. populations. Carter, 1981 found that a population of *R. reniformis* from the Rio Grande Valley in Texas reproduced better on some onion cultivars than populations of this nematode from other U.S. locations including Lubbock, Texas or Baton Rouge, Louisiana.

Observations by Birchfield and Brister (1962) led them to postulate the existence of races or pathotypes of *R. reniformis*. Dasgupta and Seshadri (1971a; 1971b) were the first to define races of *R. reniformis*, which they designated as race A and race B. These race assignments were made on the basis of differential reproduction on cotton, castor or cowpea. Vadhera *et al.*, 1999 identified another population of *R. reniformis* that failed to reproduce on castor and considered it as another race. Three distinct biological types of *R. reniformis* have been identified in Japan (Nakasono, 2004) based on the relative abundance or absence of males. Populations within these three biological types also expressed differential pathogenicity on several test crops (Nakasono, 2004). Rao and Ganguly (1996) found four physiological variants among six geographical isolates of *R. reni-*

formis in India based upon reproduction on five hosts (cotton, castor, cowpea, bajra, and mustard).

The objectives of this research were: 1) to document the spread of *R. reniformis* within the cotton producing areas of Louisiana, U.S.A.; 2) to evaluate the reproductive variation on cotton within six geographic isolates of *R. reniformis*; and 3) to evaluate reproduction and pathogenicity among the six geographic isolates on cotton.

MATERIALS AND METHODS

Distribution of reniform nematode

Data for the distribution of reniform nematode on cotton in Louisiana was obtained from soil samples submitted by county agents, consultants, and producers to the LSU AgCenter Nematode Advisory Service during the period of 1979-2010. Samples were processed by elutriation (Byrd *et al.*, 1976) and centrifugal-flotation (Jenkins, 1964). The 1961 report by Birchfield and Jones indicated occurrence of *R. reniformis* in three parishes. 1985 data represents a 24 year interval from the initial report and 2010 data represents an additional interval of 25 years.

Isolates of reniform nematode

Isolates of reniform nematode from Alabama, Arkansas, Hawaii, Louisiana, Mississippi and Texas were supplied/collected by W. S Gazaway, R. T Robbins, E. C. McGawley, E. C. McGawley, E. C. McGawley and A. F. Robinson, respectively. Reniform nematode infested soil from each of the six states is maintained in 35 kg capacity clay pots planted with tomato (cultivars Rutgers PS, Seedway; Hall, New York 14463) and maintained in an isolated greenhouse facility accessible only to nematology personnel. Infested tomato roots from these axenic cultures were collected and transported to the nematology lab for isloation and propagation of single egg mass cultures. Single egg masses were removed from root tissue using a dissecting microscope at 40× and a dental pulp canal tool. Single egg masses were transferred to individual 50 ml capacity plastic centrifuge tubes three-quarters filled with sterile soil and containing a single Rutgers tomato seedling. Single egg mass cultures in tubes were maintained in centrifuge tube racks placed under fluorescent grow-lux bulbs in the laboratory. After 4-5 weeks, cultures were moved to a greenhouse environment where they were maintained and propagated further in clay pots on tomato. For each geographic isolate, a total of 10 single egg mass populations were produced and maintained using appropriate dividers and separate benches to avoid cross-contamination. Plastic liners were placed under each pot and all watering was done by adding water to liners rather than by "spraying" from above. For each of the 60 populations, ten for each of the six geographic isolates, 25 immature females were examined microscopically and stylet length and position of the vulva determined using Openlab™ (Improvision/PerkinElmer) and Spot™ imaging software (Diagnostic Instruments, Inc.). Measurement data plus the abundance of males in all populations confirmed them as *R. reniformis* as delineated by Robinson, *et al.*, 1997.

Preliminary greenhouse studies

A total of 12 trials, one in early spring (April-May) and another in late fall (September-October), were conducted with each of the six geographic isolates of *R. reniformis* during 2004 and 2005. For each trial, 50 terra cotta pots having top diameters of 15 cm, each containing two kg of steam pasteurized soil and representing five replicates of each of 10 single egg mass derived populations from each geographic

isolate, were established in a randomized block design. Each pot contained a single 15-day-old Stoneville LA887 cotton seedling that was inoculated by pipetting a three ml aqueous suspension containing 300-325 vermiform individuals of *R. reniformis* into depressions surrounding the seedling three days after transplanting. Duration of trials was 59 to 63 days. Air and soil temperatures ranged from 18-30C and 21-33C, respectively over the course of these trials. At the completion of each trial, eggs of reniform nematode were extracted from three grams of fresh root tissue (randomly selected after chopping the entire root system into 2.5-3.5 cm segments) by stirring in 0.6% NaOCl for 10 min (Hussey and Barker, 1973) and soil populations were extracted from a 500 g subsample of soil from each pot using the wet-sieving (nested 425 and 38-µm-pore sieves) centrifugal/sugar flotation technique (Jenkins, 1964). Immature life stages of reniform nematode were enumerated at 40X using an inverted microscope. Total population density per pot (Pf) and reproductive values (R, where R = Pf/Pi and Pf is the final population density and Pi is the initial infestation level) were determined.

Microplots

Microplots employed in these studies are autoclaved terra cotta containers having top outside diameters of 35.6 cm and soil capacities of 15 kg. Microplots are placed in preformed depressions in soil with only the rim of the pot exposed. Soil used in microplots is a steam sterilized Commerce silt loam soil (fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) with a pH of 6.9-7.2 and an organic matter content of 1.0-1.4 percent. Microplots are spaced 1-meter apart and arranged as a randomized block in a 6 by 7 pattern. The microplot area is bounded by a 17-meter-long by 9-meter-wide aluminum quonset hut skeletal frame open at both ends and covered with one layer of clear, 6-millimeter thick polyethylene greenhouse film and one layer of 20% reflective foilcloth. Light intensity under the foilcloth was measured as 512 µE/S-1/M-2, about 68% of full sunlight. This cover, necessary to protect plants in microplots from excessive summer rainfalls that are common in southern Louisiana, is equipped with overhead fans and an automated micro-misting irrigation system that prevents splashing during irrigation and allows for the maintenance of near-natural air and soil temperature and moisture conditions. The trial was conducted in 2006 with planting on 28 April and harvest on 22 September and repeated in 2007 with planting on 2 May and harvest on 26 September, an experimental duration of 147 days (full-season) in both years. As in greenhouse studies, a single Stoneville LA887 cotton seedling was initially transplanted to the center of each microplot and inoculated with 500 vermiform individuals of *R. reniformis* 3 days later. At 75 days after planting, nematode population levels were estimated by collecting soil samples, six 1.9 × 20 cm cores, from each microplot. Sampling holes were filled with steam-sterilized soil. At the conclusion of each trial, numbers of bolls per plant were recorded, tops and root systems were removed, a fresh root sample was collected for egg extraction, after which all plant material was dried at 40C. Soil from each microplot was bulked and a 500 g subsample collected for nematode analysis. Nematode data was collected and reproduction evaluated as described for greenhouse studies.

Statistical Analyses

Analysis of variance and Tukey's HSD means separation procedures were performed on nematode and plant data using the "Fit Model" module of SAS JMP, version

5.0 (SAS Institute, Cary, NC). There were no significant season by treatment interactions in greenhouse trials or year by treatment interactions in microplot trials and data in both types of trials was therefore combined over seasons and years, respectively.

RESULTS

Distribution of reniform nematode

Distribution data between 1961 and 2010 is based on a total of 18,416 samples. Between 1979 and 1985, reniform nematode was detected in 497 cotton fields. By 2010, the number of reniform nematode infested cotton fields had increased to 8,879. Figures 1-3 respectively show the know distribution of reniform nematode on cotton in Louisiana in 1961, 1985 and 2010. Reniform nematode infestations were known in 14 parishes in 1985 and now, 2010, are documented in all 24 of the major cotton-producing parishes. Northeastern parishes of Louisiana including

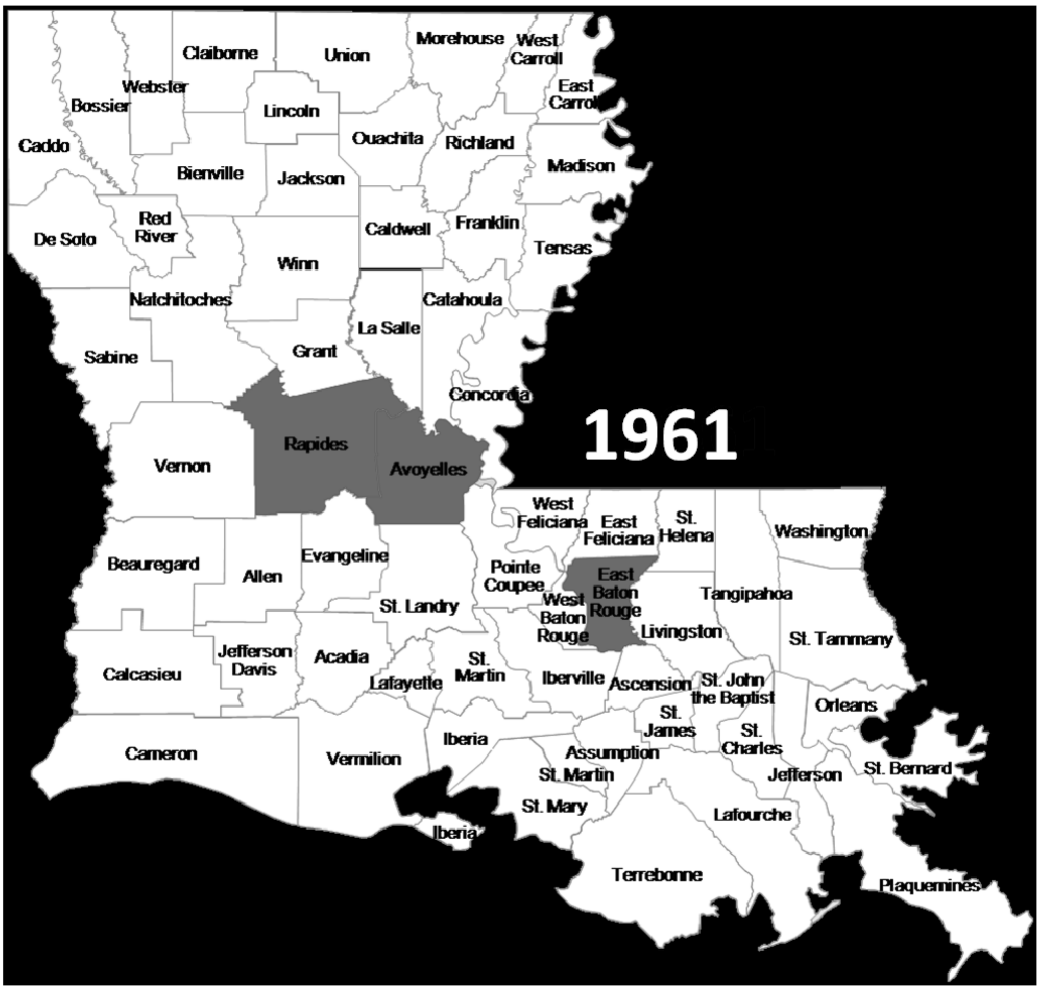

Fig. 1. Distribution of *Rotylenchulus reniformis* on cotton in Louisiana in 1961 (outlined in red).

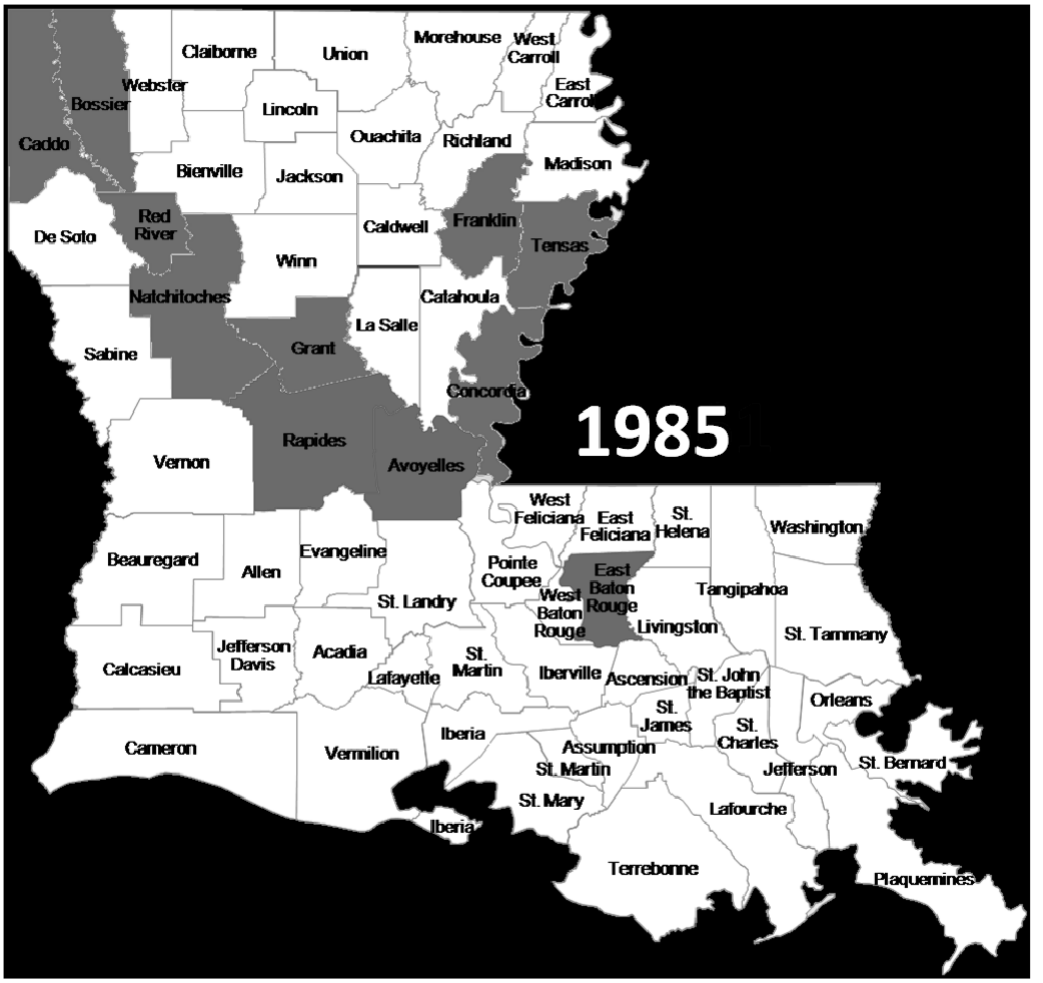

Fig. 2. Distribution of *Rotylenchulus reniformis* on cotton in Louisiana in 1985 (outlined in red).

Rapides, Morehouse, and Franklin are most severely impacted by reniform nematode with incidences in 2,462, 1,786, and 1,476 fields, respectively. East Baton Rouge, East Feliciana, Iberville, Evangeline, Caddo, and Bossier have historically had low production of cotton with less than 25 fields in each parish known to be infested with reniform nematode.

Preliminary greenhouse studies

Greenhouse trials with populations of *R. reniformis* from Alabama, Arkansas, Hawaii, Louisiana, Mississippi and Texas are summarized in Tables 1-6, respectively. For all six isolates of the nematode, data for vermiform and egg life stages within populations was much less variable

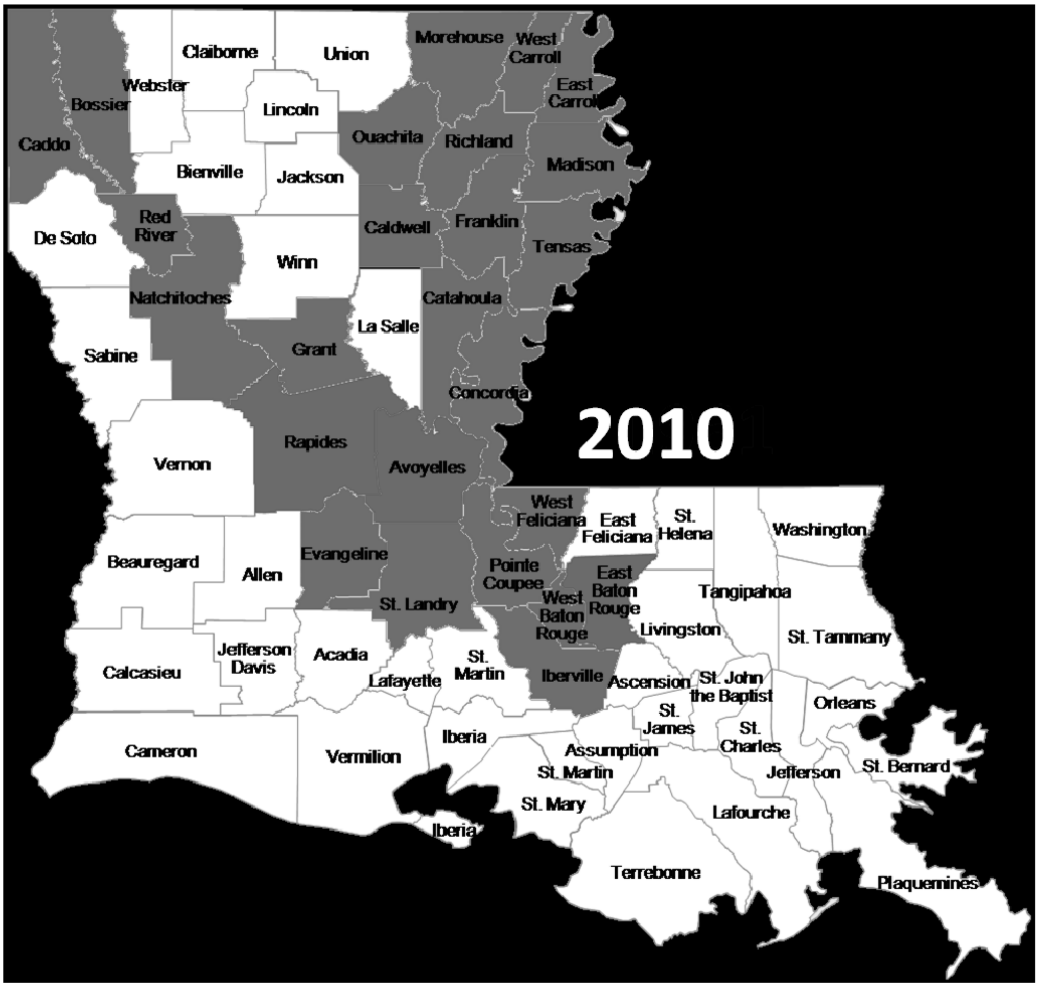

Fig. 3. Distribution of *Rotylenchulus reniformis* on cotton in Louisiana in 2010 (outlined in red).

than that observed between geographic populations. Additionally, data evaluating egg production within populations was less variable than that for vermiform stages. Only two of the six groups of populations, those from AL and HI, produced statistical differences in the numbers of egg per three grams of root at 60 to 62 days.

Within the 10 populations from AL (Table 1), numbers of vermiform individuals in soil ranged from 3,175 to 5,311 producing reproductive (R) values of 10-17. Number of eggs per three grams of root ranged from 606 to1,143 for this population. Mean numbers of vermiform and egg stages for the 10 populations was calculated and on this basis, population AL8 was

Table 1. Reproduction of populations of *Rotylenchulus reniformis* from Alabama on Stoneville LA887 cotton[W].

Population	Vermiform stages per 2 kg soil[X]	Reproductive value[Y]	Eggs per 3 g of root
AL 1	4,009 b	12.8	768 ab
AL 2	5,311 a	17.0	985 a
AL 3	5,150 a	16.5	622 b
AL 4	3,875 b	12.4	1,050 a
AL 5	4,672 a	14.8	673 b
AL 6	3,770 b	12.1	1,143 a
AL 7	5,275 a	16.8	849 a
AL 8[Z]	4,460 ab	14.9	606 b
AL 9	3,175 b	10.3	951 a
AL 10	3,994 b	12.6	720 b

[W]Data combined over two 60-62 day duration trials with five replications per trial.
[X]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05\%$). Means followed by a common letter in a column are not significantly different.
[Y]Reproductive value was calculated by dividing the numbers of juveniles per 2 kg of soil at 60-62 days by the inoculum level of 300-325 vermiform life stages.
[Z]population selected for use in microplot trials.

Table 2. Reproduction of populations of *Rotylenchulus reniformis* from Arkansas on Stoneville LA887 cotton[W].

Population	Vermiform stages per 2 kg soil[X]	Reproductive value[Y]	Eggs per 3 g of root
AR 1	10,133 a	32.4	1,675 a
AR 2	9,294 ab	29.7	1,737 a
AR 3[Z]	9,125 b	30.4	1,575 a
AR 4	7,352 c	23.9	1,486 a
AR 5	13,520 a	43.2	1,299 a
AR 6	9,754 a	31.1	1,374 a
AR 7	8,571 b	27.4	1,627 a
AR 8	7,450 c	24.0	1,857 a
AR 9	8,810 b	28.2	1,425 a
AR 10	7,481 c	24.0	1,557 a

[W]Data combined over two 60-62 day duration trials with five replications per trial.
[X]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05\%$). Means followed by a common letter in a column are not significantly different.
[Y]Reproductive value was calculated by dividing the numbers of juveniles per 2 kg of soil at 60-62 days by the inoculum level of 300-325 vermiform life stages.
[Z]population selected for use in microplot trials.

selected for use in microplot trials. Numbers of vermiform stages in soil and resultant R values ranged from 7,352 to 13,520 and 23.9 to 43.2 respectively, among the populations from AR (Table 2). Egg production by the populations from AR

Table 3. Reproduction of populations of *Rotylenchulus reniformis* from Hawaii on Stoneville LA887 cotton[W].

Population	Vermiform stages per 2 kg soil[X]	Reproductive value[Y]	Eggs per 3 g of root
HI 1	6,982 a	22.3	569 a
HI 2	6,260 a	20.1	637 a
HI 3	5,842 a	18.7	414 b
HI 4	5,435 a	17.4	474 a
HI 5	5,973 a	19.2	486 a
HI 6	5,588 a	17.7	425 ab
HI 7	6,030 a	19.4	710 a
HI 8	5,702 a	18.3	531 a
HI 9[Z]	6,056 a	20.2	549 a
HI 10	6,621 a	21.3	377 b

[W]Data combined over two 60-62 day duration trials with five replications per trial.
[X]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05\%$). Means followed by a common letter in a column are not significantly different.
[Y]Reproductive value was calculated by dividing the numbers of juveniles per 2 kg of soil at 60-62 days by the inoculum level of 300-325 vermiform life stages.
[Z]Population selected for use in microplot trials.

Table 4. Reproduction of populations of *Rotylenchulus reniformis* from Louisiana on Stoneville LA887 cotton[W].

Population	Vermiform stages per 2 kg soil[X]	Reproductive value[Y]	Eggs per 3 g of root
LA 1	7,375 a	23.6	1,413 a
LA 2	5,650 b	18.0	1,744 a
LA 3[Z]	5,908 ab	18.2	1,551 a
LA 4	7,485 a	23.9	1,131 a
LA 5	6,670 a	21.3	1,445 a
LA 6	4,897 b	15.8	1,172 a
LA 7	6,050 a	19.3	1,227 a
LA 8	5,294 b	17.0	1,050 a
LA 9	6,410 a	20.5	1,374 a
LA 10	6,195 a	19.8	1,026 a

[W]Data combined over two 60-62 day duration trials with five replications per trial.
[X]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05\%$). Means followed by a common letter in a column are not significantly different.
[Y]Reproductive value was calculated by dividing the numbers of juveniles per 2 kg of soil at 60-62 days by the inoculum level of 300-325 vermiform life stages.
[Z]Population selected for use in microplot trials.

ranged from 7,352 to 13,520 per three grams of root. Population AR3 was selected for use in microplot trials. There were no significant differences in the numbers of individuals recovered from soil among the 10 populations from HI (Table 3). Subse-

Table 5. Reproduction of populations of *Rotylenchulus reniformis* from Mississippi on Stoneville LA887 cotton[W].

Population	Vermiform stages per 2 kg soil[X]	Reproductive value[Y]	Eggs per 3 g of root
MS 1	9,275 a	29.6	2,065 a
MS 2	8,955 a	28.7	1,794 a
MS 3	9,568 a	30.6	1,710 a
MS 4	8,770 a	28.0	1,926 a
MS 5	6,845 b	21.9	2,136 a
MS 6	7,851 b	25.1	1,745 a
MS 7[Z]	8,364 b	25.7	1,806 a
MS 8	7,285 b	23.3	1,844 a
MS 9	9,477 a	30.3	2,022 a
MS 10	8,505 ab	27.2	1,715 a

[W]Data combined over two 60-62 day duration trials with five replications per trial.
[X]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05\%$). Means followed by a common letter in a column are not significantly different.
[Y]Reproductive value was calculated by dividing the numbers of juveniles per 2 kg of soil at 60-62 days by the inoculum level of 300-325 vermiform life stages.
[Z]population selected for use in microplot trials.

Table 6. Reproduction of clonal populations of *Rotylenchulus reniformis* from Texas on Stoneville LA887 cotton[W].

Population	Vermiform stages per 2 kg soil[X]	Reproductive value[Y]	Eggs per 3 g of root
TX 1	13,452 a	43.1	2,874 a
TX 2	10,590 b	33.8	2,599 a
TX 3	12,561 a	40.1	2,936 a
TX 4	12,415 ab	39.7	3,064 a
TX 5	11,245 b	36.1	2,617 a
TX 6	13,300 a	42.5	2,403 a
TX 7	13,675 a	43.7	2,547 a
TX 8	11,653 b	37.3	2,492 a
TX 9	14,280 a	45.7	2,556 a
TX 10[Z]	12,850 a	42.8	2,813 a

[W]Data combined over two 60-62 day duration trials with five replications per trial.
[X]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05\%$). Means followed by a common letter in a column are not significantly different.
[Y]Reproductive value was calculated by dividing the numbers of juveniles per 2 kg of soil at 60-62 days by the inoculum level of 300-325 vermiform life stages.
[Z]Population selected for use in microplot trials.

quent R values were tightly grouped and ranged from 17.4 to 22.3. Egg production by derivatives of the HI population was low, ranging from 377 to 710 per three grams of root. Population HI9 was selected for use in microplot trials. A final population den-

sity per two kg of soil of 4,897 to 7,485 individuals resulted from the 10 populations from LA with corresponding R values of 15.8 to 23.9 and egg production that averaged 1,026 to 1,744 per sample. Population LA3 was selected for use in microplot trials. The soil density of vermiform stages of populations from MS ranged from 6,845 to 9,568 individuals. Reproductive values were 21.9 to 30.6. Numbers of eggs produced among the populations averaged 1,710 to 2,136. The population MS7 was selected for microplot trials. The geographic population from TX produced isolates which exhibited the greatest overall level of reproduction. Vermiform stages reached population levels of 10,590 to 14,280 over the duration of the trials. This resulted in reproductive values of 33.8 to 45.7. Egg production was uniform among the populations that varied from 2,403 to 3,064 per sample. Population TX10 was selected for use in microplot trials.

Microplots

Data from microplot trials is summarized as Table 7. At 75 days, approximately mid-way through the trials, there were significant differences in reproduction observed among the six populations. The densities of populations from AR, LA and MS in soil were significantly greater than those from AL, HI and TX. At the end of the trials, 147 days, population densities of the isolates representing LA and MS and averaging 80,971 and 86,485 per 15 kg of soil were significantly higher than those representing the other four states. Reproductive values ranged from a low of 109.9 for the Alabama population to a high of 173.0 for the Mississippi population. Cotton plant dry weights at harvest were, relative to the non-inoculated control, reduced significantly by all isolates except the one from HI. Moreover, plant damage, caused by LA and MS isolates was greater than that

Table 7. Reproduction and influence of six geographic isolates of *Rotylenchulus reniformis* on dry weights and numbers of bolls per Stoneville LA 887 cotton plant[W].

Isolate source	Vermiform stages/15 kg of soil		Reproductive value[Z]	147 day plant dry weight (g)	Bolls per plant
	75 days[XY]	147 days			
AL	19,626 b	54,942 c	109.9	297.4 c	35 a
AR	27,795 a	71,873 b	143.7	309.5 bc	23 bc
HI	20,600 b	62,783 bc	125.6	352.8 a	46 a
LA	22,739 a	80,971 a	161.9	227.4 d	28 b
MS	35,036 a	86,485 a	173.0	271.8 d	19 c
TX	13,575 b	64,904 bc	129.8	328.0 bc	35 a
Control	0 c	0 d	0	370.6 a	43 a

[W]Data combined over two 147 day duration microplot trials conducted in 2006 and 2007 with six replications per treatment in each trial.

[X]Population density estimated on the basis of 6 soil cores (1.9 × 20 cm) collected around the base of each plant stem. Sampling holes at 75 days were filled with steam-sterilized soil.

[Y]Data analyzed with ANOVA and Tukey's HSD test ($P \leq 0.05\%$). Means followed by a common letter in a column are not significantly different.

[Z]Reproductive value was calculated by dividing the numbers of juveniles per 15 kg of soil at 147 days by the inoculum level of 500 vermiform life stages.

caused by the AL, AR and TX isolates. Only the reniform isolates from AR, LA and MS significantly reduced the numbers of bolls per plant, 23, 28 and 19, respectively compared with 43 for the control. The isolate from MS had the most pronounced negative effect on numbers of bolls per plant at harvest.

DISCUSSION

Outside of the United States, studies reported in 1971 (Dasgupta and Seshadri) and 1999 (Vadhera et al.) from India proposed the existence of discrete "races" of *R. reniformis*. A study by Nakasomo in Japan, published in 1983 in Japanese and translated and published in English in 2004, evaluated morphological and physiological variation in populations of *R. reniformis*. A primary conclusion from the extensive research summarized in this paper, which employed populations of *R. reniformis* from Japan and also ones from Hawaii and Texas, was that polymorphism in populations "does not simply seem to be a case of a nematode with highly varied phenotype, but rather the polymorphism seems to reflect basic physiological and ecological differences in populations of *R. reniformis*." Data of 2005 from Agudelo *et al.*, in which they also conducted 60-day-duration greenhouse tests with geographic populations of *R. reniformis* on cotton and soybean, showed extensive overlapping in reproduction among populations. Only the population from Texas had significantly greater reproductive indices than other populations included in the trial. Numerically, however, reproductive indices among the populations ranged from 2.8 to 62.1 and 0.5 to 8.4 on 'Braxton' and 'Forrest' soybean, respectively and from 0.3 to 55.7 on 'Deltapine 50' cotton. Research of Agudelo et al. also included an examination of the DNA sequences of the nuclear ribosomal first internal transcribed spacer region (ITSI) from the populations in their studies that represented 20 locations. They found no polymorphisms present in the ITSI among the populations. In similar work with nuclear ribosomal DNA, Tilahun et al. (2003, 2008) found that there was significant variation in the ITSI among populations of *R. reniformis* from Alabama.

Only a few reports in the United States (Overstreet and McGawley, 1994, McGawley and Overstreet, 1995 and Augedelo *et al*, 1995) have described results of inoculation studies with multiple geographic isolates of *R. reniformis*. The few that have were short duration, greenhouse based experiments and some were not repeated. The trials described in this report provide an indication of the amount of variation within geographic populations from a single cotton field having a known history of damage from *R. reniformis*. Overall, the variation within populations from the six states was minimal when compared with that between geographic populations. Within populations, egg production data was less variable than was that for vermiform stages in soil, with four of the six populations having no significant differences in the numbers of eggs produced among the 10 populations.

These microplot trials are the first, to our knowledge, that have compared geographic populations of this nematode over an entire season in an environment free of any other soil inhabiting, and potentially confounding, microorganisms. Data of these microplot trials documents both reproductive and pathogenic variation in populations of this nematode. The existence of significant variation in reproduction and pathogenicity among geographic isolates of *R. reniformis* has marked influence on the utility of using one or only a few isolates in studies designed to identify new sources of resistance.

In a manuscript currently in press (Nematropica 41:1) we describe identical greenhouse and microplot trials with these same geographic populations of *R. reniformis* and soybean. Results of these studies parallel closely those described herein with cotton.

LITERATURE CITED

Agudelo, R. T. Robbins, and J. M. Stewart. 2001. Morphometric variation of reniform nematode geographic populations from cotton-growing regions in the United States. Arkansas Agricultural Experiment Station, Research Series 497:87-91. http://arkansasagnews.uark.edu/1292.htm

Agudelo, P., R. T. Robbins, J. M. Stewart, and A. Szalznski. 2005. Intraspecific variability of *Rotylenchulus reniformis* from cotton-growing regions in the United States. Journal of Nematology 37:105-114.

Anonymous. 2010a. Population Distributions and Densities Maps- reniform nematode. National Cotton Council of America website. http://www.cotton.org/tech/pest/nematode/distributions.cfm?bw=1&type=Reniform

Anonymous. 2010b. Crop production 2009 Summary-January 2010. United States Department of Agriculture, National Agricultural Statistics Service website. http://usda.mannlib.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2010.pdf

Anonymous. 2010c. Crop values 2009 Summary- February 2010. United States Department of Agriculture, National Agricultural Statistics Service website. http://usda.mannlib.cornell.edu/usda/current/CropValuSu/CropValuSu-02-19-2010.pdf

Arias, R. S., S. R. Stetina, J. L. Tonos, J. A. Scheffler, and B. E. Scheffler. 2009. Microsatellites reveal genetic diversity in *Rotylenchulus reniformis* populations. Journal of Nematology 41:146-156.

Birchfield, W., and L. R. Brister. 1962. New hosts and nonhosts of reniform nematode. Plant Disease Reporter 46:683-685.

Birchfield, W., and L. R. Brister. 1963. Susceptibility of cotton and relatives to reniform nematode in Louisiana. Plant Disease Reporter 47:990-992.

Birchfield, W., and J. E. Jones. 1961. Distribution of the reniform nematode in relation to crop failure of cotton in Louisiana. Plant Disease Reporter 45:671-673.

Blassingame, D., M. V. Patel, K. Lawrence, W. Gazaway, M. Olsen, T. Kirkpatrick, S. Monfort, M. Davis, J. Marios, B. Kemerait, P. Colyer, G. Scuimbato, G. Lawrence, A. Wrather, N. Goldberg, S. Koenning, J. T. Pitts, J. Muller, M. Newman, J. Woodard, T. Wheeler, and P. Phipps. 2010. 2009 Cotton disease loss estímate committee report. Proceedings of the Beltwide Cotton Conferences 1:237-240.

Byrd, D. W., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, and C. A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206-212.

Carter, W. W. 1981. Onion as a host of *Rotylenchulus reniformis*. Journal of Nematology 13:433-434.

Ching, T. H. 1969. Morphometric variability of *Rotylenchulus reniformis*, the reniform nematode. Journal of the Alabama Academy of Science 40:136-137.

Dasgupta, D. R., and A. R. Seshadri. 1971a. Races of the reniform nematode, *Rotylenchulus reniformis* Lindford and Oliveira, 1940. Indian Journal of Nematology 1:21-24.

Dasgupta, D. R., and A. R. Seshadri. 1971b. Reproduction, hybridization and host adaptation in physiological races of the reniform nematode. Indian Journal of Nematology 1:128-144.

Decker, H., G. R. Casamayor, and P. Gandoy. 1966. Investigaciones sobre la aparición del nemátodo sedentario de las raíces *Rotylenchulus reniformis* en las provincia de Las Villas (Cuba). Memorias Centro de Investigaciones Agropecuairas Universidad Central Las Villas 1966:169-175.

Germani, G. 1978. Caractères morpho-biométriques de trios espèces ouest-africanies de *Rotylenchulus* Linford & Oliveira, 1940 (Nematoda: Tylenchida). Revue de Nématologie 1:241-250.

Heald, C. M., and J. A. Meredith. 1987. Response of three tobacco cultivars to three *Rotylenchulus reniformis* populations. Nematropica 17:95-98.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods for collecting inocula for *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025-1028.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Koenning, S. R., K. R. Barker, and D. T. Bowman. 2000. Tolerance of selected cotton lines to *Rotylenchulus reniformis*. Journal of Nematology 32:519-523.

Koenning, S. R., T. L. Kirkpatrick, J. L. Starr, J. A. Wrather, N. R. Walker, and J. D. Mueller. 2004. Plant-parasitic nematodes attacking cotton in the United States: old and emerging challenges. Plant Disease 88:100-113.

Lehman, P. S., and R. N. Inserra. 1990. Morphometric variation of *Rotylenchulus parvus* and *Rotylenchulus reniformis* populations in the southern United States. Proceedings Soil and Crop Science Society of Florida 49:220-226.

McGawley, E. C., and C. Overstreet. 1995. Reproductive and pathological variation in populations of the reniform nematode *Rotylenchulus reniformis*. Journal of Nematology 27:508.

Nakasono, K. 2004. Studies on morphological and physio-ecological variations of the reniform nematode, *Rotylenchulus reniformis* Lindford and Oliveira, 1940 with an emphasis on differential geographical distribution of amphimictic and parthenogenetic populations in Japan. Journal of Nematology 36: 356-420.

Overstreet, C., and E. C. McGawley. 1999. Evidence for spread by reniform nematode in Louisiana during the past 20 years. Nematropica 29:127-128.

Rao, G. M. V. P., and S. Ganguly. 1996. Host preference of six geographical isolates of reniform nematode, *Rotylenchulus reniformis*. Indian Journal of Nematology 26:19-22.

Robbins, R. T., E. R. Shipe, L. Rakes, L. E. Jackson, E. E. Grur, and D. G. Dombek. 2002. Host suitability of soybean cultivars and breeding lines to reniform nematode in tests conducted in 2001. Journal of Nematology 34:378-383.

Robbins, R. T., L. Rakes, L. E. Jackson, E. E. Gbur, and D. G. Dombek. 2001. Host Suitability in Soybean Cultivars for the Reniform Nematode, 2000 Tests. Journal of Nematology 33:314-317.

Robinson, A. F. 2007. Reniform in U.S. cotton: when, where, why, and some remedies. Annual Review of Phytopathology 45:263-288.

Robinson, A. F., R. N. Inserra, E. P. Caswell-Chen, N. Vovlas, and A. Troccoli. 1997. *Rotylenchulus* species: identification, distribution, host ranges, and crop plant resistance. Nematropica 27:127-180.

Routary, B. N., H. Sahoo, and S. N. Das. 1988. Evaluation of some chilli cultivars against *Meloidogyne incognita* and *Rotylenchulus reniformis*. Journal of Indian Botanical Society Journal 67:220-221.

Soares, P. L. M., J. M. Santos, and P. S. Lehman. 2003. Estudo morfométrico comparativo de popula?ões de *Rotylenchulus reniformis* (Nemata: Rotylenchulinae) do Brazil. Fitopatologia Brasileira 28:292-297.

Soares, P. L. M., J. M. Santos, and A. S. Ferraudo. 2004. Estudo morfométrico comparativo de 58 popula?ões brasileiras de *Rotylenchulus reniformis* (Nemata: Rotylenchulinae). Fitopatologia Brasileira 29:419-424.

Tilahun, Y., A. Zipf, D. Deng, G. Sharma, K. Soliman, K. McLean, and J. Jenkins. 2003. Variation at the molecular level of reniform nematode populations in Alabama. Proceedings of the Beltwide Cotton Conferences 1:A13-18.

Tilahun. Y., K. Soliman, K. S. Lawrence, L. J. Cseke, and J. W. Ochieng. 2008. Nuclear ribosomal DNA diversity of a cotton pest (*Rotylenchulus reniformis*) in the United states. African Journal of Biotechnology 7: 3217-3224.

Usery, S. R., Jr., K. S. Lawrence, G. W. Lawrence, and C. H. Burmester. 2005. Evaluation of cotton cultivars for resistance and tolerance to *Rotylenchulus reniformis*. Nematropica 35:121-133.

Vadhera, I., J. Bhatt, and B. N. Shukla. 1999. Prevalence of a new race of *Rotylenchulus reniformis* in Madhya Pradesh. Journal of Mycological Plant Pathology 29:132-134.

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