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

E.C. McGawley*[1], C. Overstreet[1] and M. J. Pontif[2]

[1]Louisiana State University AgCenter, Department of Plant Pathology and Crop Physiology, Baton Rouge, LA 70803, USA. [2]LSU AgCenter, Sugarcane Research Station, St. Gabriel, LA 70776, USA. Telephone: 225-578-7145, Fax: 225-578-1415. Email: emcgawley@agctr.lsu.edu

ABSTRACT

McGawley, E. C., C. Overstreet and M. J. Pontif. 2011. Variation in reproduction and pathogenicity of geographic isolates of *Rotylenchulus reniformis* on soybean. Nematropica 41:12-22.

Known incidence of *Rotylenchulus reniformis* in Louisiana increased from 3 parishes in 1961 to 11 parishes in 1985 to all 21 of the major soybean producing parishes in 2010. Comparative reproduction and pathogenicity of isolates of *Rotylenchulus reniformis* from Alabama, Arkansas, Hawaii, Louisiana, Mississippi and Texas on soybean was evaluated in microplot trials. Prior to initiation of microplot trials, ten populations of each geographic isolate were derived. Reproduction of the single egg-mass (SEM) populations of each geographic isolate were evaluated in greenhouse studies with Deltapine 4331 soybean 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 SEM population of each of the six isolates was selected for use in microplot trials. Averaged over the two trials, SEM populations 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-7 (R = 3.5, eggs = 1,082); AR-4 (R = 26.7, eggs = 2,186); HI-1 (R = 30.2, eggs = 1,624); LA-3 (R = 30.2, eggs = 1,656); MS-2 (R = 43.9, eggs = 5,215) and TX-5 (R = 55.4, eggs = 4,329). Data from full-season (126 day) 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 273.3 g for the non-inoculated control. All isolates except the ones from HI and TX produced root weights at harvest that were reduced significantly below that of the control. With the exception of the MS-2 isolate, harvest weights for plants inoculated with AR-4 were significantly lower than those from the other four geographic regions. Relative to the control, numbers and dry weights of pods per plant at harvest were reduced significantly by all reniform nematode isolates except those from AL and HI.

Key words: host suitability, pathogenicity, reniform nematode, *Rotylenchulus reniformis,* virulence phenotypes, soybean.

RESUMEN

McGawley, E. C., C. Overstreet and M. J. Pontif. 2011. Variación en la reproducción y patogenicidad de aislamientos geográficos de Rotylenchulus reniformis en soya. Nematropica 41:12-22.

La incidencia de *Rotylenchulus reniformis* en el estado de Louisiana aumentó de 3 parroquias en 1961 a 11 en 1985, y luego a todas las 21 parroquias productoras de soya en 2010. Se evaluó la reproducción y la patogenicidad comparativa de aislamientos de *Rotylenchulus reniformis* provenientes de Alabama, Arkansas, Hawaii, Louisiana, Mississippi y Texas en soya, en microparcelas. Antes de los ensayos de microparcelas, se generaron diez poblaciones a partir de masas de huevos individuales (subpoblaciones) de cada aislamiento geográfico. Se evaluó la reproducción de las subpoblaciones de cada aislamiento geográfico, en el invernadero, en soya Deltapine 4331, midiendo el número de estados vermiformes en el suelo y la cantidad de huevos por gramo de raíz 60 días después de la inoculación. Con base en estos resultados, se seleccionó una subpoblación de cada uno de los seis aislamientos para los ensayos de microparcelas. Los datos de reproducción (R = Pf/Pi) y la cantidad de huevos por gramo de raíz, promedio de dos repeticiones, para las subpoblaciones seleccionadas fueron: AL-7 (R = 3.5, huevos = 1,082); AR-4 (R = 26.7, huevos = 2,186); HI-1 (R = 30.2, huevos = 1,624); LA-3 (R = 30.2, huevos = 1,656); MS-2 (R = 43.9, huevos = 5,215) y TX-5 (R = 55.4, huevos = 4,329). Los datos de los ensayos de microparcelas (126 días), en promedio de 2 años, mostraron diferencias significativas (prueba HSD de Tukey ($P \leq 0.05\%$)) entre aislamientos del nematodo reniforme tanto en la reproducción como en la patogenicidad. El promedio del peso seco de las plantas controles, no inoculadas, al momento de la cosecha fue de 273.3 g. Todos los aislamientos, excepto los de HI y TX, produjeron pesos de raíz que fueron inferiores a los de las plantas control. Con excepción del aislamiento MS-2, el peso de las plantas inoculadas con AR-4 fue significativamente menor al momento de la cosecha que el de las plantas inoculadas con aislamientos de las otras

cuatro regiones. La cantidad y el peso de las vainas al momento de la cosecha se redujeron significativamente con respecto al control con todos los aislamientos del nematodo, excepto con los de AL y HI.

Palabras clave: fenotipo de virulencia, nematodo reniforme, patogenicidad, *Rotylenchulus reniformis*, soya

INTRODUCTION

Soybean is an important crop in the U.S.A., produced on 30.9 million ha (Anonymous, 2010a) with a value of 31.7 billion (U.S.) dollars during 2009 (Anonymous, 2010b). The reniform nematode, *Rotylenchulus reniformis,* is known to be a serious pathogen of soybean in several states in the U.S.A., including Alabama, Arkansas, Georgia, Louisiana, and North Carolina (Wrather and Koenning, 2006; Heald and Robinson, 1990; Koenning *et al.*, 1999). In 1961, Birchfield and Jones reported the occurrence of *R. reniformis* in three parishes in Louisiana on cotton. To date, there have been no reports detailing the distribution of *R. reniformis* on soybean in Louisiana. Loss estimates to soybean due to the reniform nematode were combined with those for either *Meloidogyne* spp. or Columbia lance, *Hoplolaimus columbus* for these five states in 2005 with losses of 77,206 tonnes (Wrather and Koenning, 2006). Although the reniform nematode has been more closely associated with cotton production in the U.S.A. (Robinson, 2007), significant increases in incidence have been documented in the Southern states over the past decade (Overstreet and McGawley, 1999; Robinson, 2007; Sikora *et al.*, 2009). Additionally, low prices for cotton during the past several years have resulted in an increase in soybean production in areas where cotton has historically been grown in response to increased prices for soybeans.

Damage to soybean caused by reniform nematode within fields or on plants growing in greenhouse environments can be high, and losses of 31% (Lawrence and McLean, 1999), 33% (Rebois, 1971), 45% (Castillo *et al.*, 1978), 44-55% (Singh, 1975), and 50-60% (Prasad, 2007) have been reported. Davis *et al.* (1996) found that the reniform nematode caused more root necrosis than either *Meloidogyne incognita, M. arenaria, M. javanica*, or *Heterodera glycines*. Root necrosis was also strongly correlated (r = 0.89) with resistance (Lim and Castillo, 1979).

Variability in the resistance of soybean cultivars to *Heterodera glycines* has been well documented. Also well documented is the fact that resistance to *H. glycines* is linked with some degree of resistance to *R. reniformis* (Rebois *et al.*, 1970; Robbins *et al.*, 1994; Pipolo, 1994; Ha *et al.,* 2007). Most reports indicate that only a few soybean cultivars are currently considered resistant to the reniform nematode (Asmus, 2008; Asmus and Schirmann, 2004; Shekhar *et al.*, 1996; Lim and Castillo, 1979; Robbins *et al.*, 2000; 2001; 2009; 2010). However, Agu (2006) did report that only one cultivar out of 23 tested in Ethiopia was susceptible to the reniform nematode.

Rotation with a crop such as corn or a resistant soybean is usually effective in suppressing populations of reniform nematode (Davis *et al.*, 2003). Westphal and Scott, in 2005, found a resistant soybean cultivar as effective in increasing cotton yields as grain sorghum. Corn and grain sorghum were more effective in reducing reniform nematode than soybean in a 20-year rotation study in Louisiana (Hague *et al*., 2002). Williams *et al.* (1983) found two consecutive years of growing a resistant soybean in a reniform infested field was as good as fumigation for a susceptible soybean or cotton cultivar the following year.

Rotylenchulus reniformis has been reported to vary morphologically among different populations (Germani, 1978b; Lehman and Inserra, 1990; Nakasono, 2004; Soares *et al*., 2003; Agudelo *et al*., 2005). Plant host has also been reported to influence the morphology of reniform nematode (Ganguly and Ramesh, 1996; 1997; Ramesh and Ganguly, 1994). Numerous reports document considerable variability in reproduction of *R. reniformis* on different host species (Ayala, 1962; Nakasono, 2004; Rao and Ganguly, 1996; Ramesh and Ganguly, 1994; Robinson *et al.*, 1997). Robinson *et al.* (1997) did report that 22 plant species had conflicting host reports from a literature search. Although some of these differences were explained as being the result of resistant cultivars, high levels of resistance or contamination from weed hosts, other differences may be more related to the occurrence of races (virulence phenotypes) of the reniform nematode. Dasgupta and Seshadri (1971) were the first to propose two different races to distinguish the differences observed in reproduction on cowpea, cotton and castor bean. Germani (1978a) also identified two populations of reniform nematode that could be differentiated based on pathogenicity on tomato cultivars 'Rossol' and 'Rama' as well as peanut cultivar '28-206'. Other researchers have shown differing host status by various populations of the reniform nematode (Rao and Ganguly, 1996; Nakasono, 2004; Vadhera *et al.*, 1999).

McGawley and Overstreet (1995) reported variation in reproduction and damage to a single cultivar of susceptible soybean from 17 populations of reniform nematode from Arkansas, Hawaii, Louisiana, Mississippi, and Texas. Davis *et al.* (1996) evaluated reniform nematode from two different populations (North Carolina and Georgia), but found similar reactions with both populations to a susceptible and resistant soybean cultivars. Agudelo *et al.* (2005) found considerable variation in reproduction on soybean among a number of populations of reniform nematode

from 13 states in the U.S.A.

In a companion manuscript, we reported on the variation within and among six geographical isolates of *R. reniformis* on cotton (McGawley *et al*. 2010). This paper reports on similar reactions to soybean.

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

MATERIALS AND METHODS

Distribution of reniform nematode

Data for the distribution of reniform nematode on soybean in Louisiana was obtained from soil samples submitted by county agents, consultants, and producers during the 31-year period from 1979-2010. Samples submitted to the LSU AgCenter Nematode Advisory Service were processed by elutriation (Byrd *et al.*, 1976) and centrifugal-flotation (Jenkins, 1964). Data collected from these samples is used to chart the distribution and density of nematode genera parasitic on soybean.

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 (cultivar Rutgers PS, Seedway LLC; 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 isolation and propagation of single egg mass cultures. Single egg masses were removed from root tissue using a dissecting microscope at 40X 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 SEM 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 SEM 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 every trial, 50 terra cotta pots having top diameters of 15 cm were established in a randomized block design. All pots contained two kg of steam pasteurized soil and represented five replicates of each of 10 single egg mass derived populations from each geographic isolate. Each pot contained a single 10-day-old Deltapine 4331 (Maturity group IV) soybean 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. The duration of the trials was 59 to 63 days. Air and soil temperatures ranged from 18-30°C and 21-33°C, 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 soil-associated 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 were autoclaved terra cotta containers having a top outside diameter of 35.6 cm and a soil capacity of 15 kg. Microplots were placed in preformed depressions in soil with only the rim of the pot exposed. The soil used in microplots was 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 were spaced 1-meter apart and arranged in a 6 by 7 pattern. The microplot area was bounded by a 17-meter-long by 9-meter-wide aluminum quonset

Table 1. Reproduction of single egg mass (SEM) populations of *Rotylenchulus reniformis* from Alabama on 'Deltapine 4331' soybean[w].

Single egg mass population	Vermiform stages per 2 kg soil[x]	Reproductive value[y]	Eggs per 3 g of root
AL-1	1,902 a	6.1	495 b
AL-2	2,481 a	7.9	689 b
AL-3	1,443 b	4.6	934 a
AL-4	2,624 a	8.4	1,255 a
AL-5	2,169 a	6.9	477 b
AL-6	1,180 b	3.8	910 ab
AL-7[z]	2,033 a	3.5	1,082 a
AL-8	1,836 ab	5.9	1,427 a
AL-9	2,755 a	8.8	591 b
AL-10	1,377 b	4.4	1,279 a

[w] Data combined over two 58-64 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]SEM population selected for use in microplot trials.

Table 2. Reproduction of single egg mass (SEM) populations of *Rotylenchulus reniformis* from Arkansas on 'Deltapine 4331' soybean[w].

Single egg mass population	Vermiform stages per 2 kg soil[x]	Reproductive value[y]	Eggs per 3 g of root
AR-1	7,281 a	23.3	2,927 a
AR-2	9,355 a	29.9	1,451 a
AR-3	8,790 a	28.1	2,642 a
AR-4[z]	8,331 a	26.7	2,186 a
AR-5	9,118 a	29.2	1,993 a
AR-6	7,150 a	22.9	2,730 a
AR-7	8,985 a	28.8	2,164 a
AR-8	9,315 a	29.8	1,791 a
AR-9	8,003 a	25.6	2,460 a
AR-10	7,465 a	23.9	2,042 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 58-64 days by the inoculum level of 300-325 vermiform life stages.
[z]SEM population selected for use in microplot trials.

hut skeletal frame. The frame was open at both ends and covered with one layer of clear, 6-millimeter thick polyethylene greenhouse film and one layer of 20% reflective foil-cloth. Light intensity under the foil-cloth was measured as 512µE/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, was equipped with overhead fans and an automated micro-misting irrigation system that prevented splashing during irrigation and allowed for the maintenance of near-natural air and soil temperature and moisture conditions. The trial was conducted in 2006 with planting on 08 May and harvest on 11 September and repeated in 2007 with planting on 14 May and harvest on 17 September, an experimental duration of 126 days (full-season) in both years. As in greenhouse studies, a single Deltapine 4331 soybean seedling was initially transplanted to the center of each microplot and inoculated with 500 vermiform individuals of *R. reniformis* 3 days later. At 63 days after planting, nematode population levels were estimated by collecting soil samples, six 1.9 X 20 cm cores, from each microplot. Sampling holes were filled with steam-sterilized soil. At the conclusion of

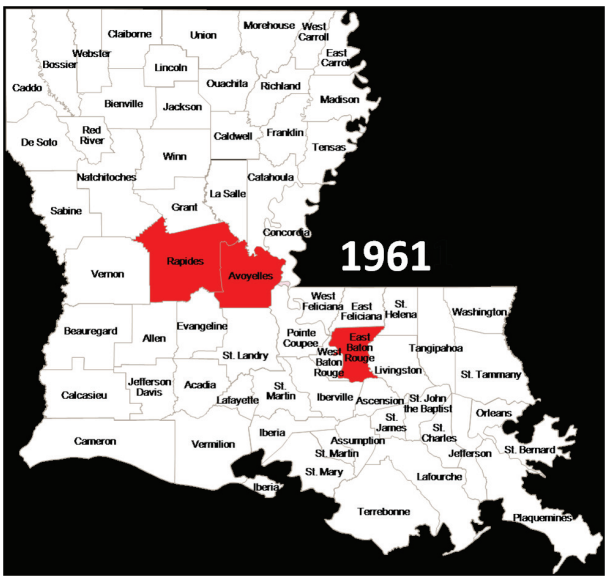

Figure 1. Distribution of *Rotylenchulus reniformis* on soybean in Louisiana in 1961 (shaded parishes) Data from Birchfield and Jones, 1961.

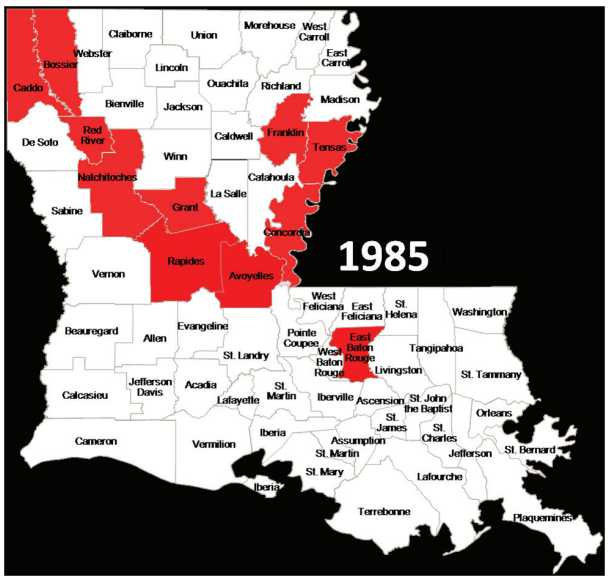

Figure 2. Distribution of *Rotylenchulus reniformis* on soybean in Louisiana in 1985 (shaded parishes).

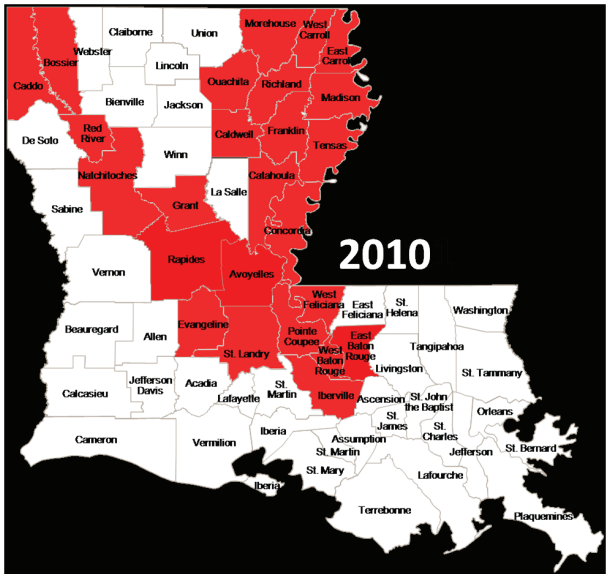

Figure 3. Distribution of *Rotylenchulus reniformis* on soybean in Louisiana in 2010 (shaded parishes).

each trial, numbers and weights of pods per plant were recorded, tops and root systems were removed, and a fresh root sample was collected for egg extraction. All plant material was dried at 40°C for 96 hours. Soil from each microplot was bulked and a 500 g subsample collected for nematode analysis. Nematode data were 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 were therefore combined over seasons and years, respectively.

RESULTS

Distribution of reniform nematode

Distribution data between 1961 and 2010 are based on a total of 4,164 samples where soybean was grown during the previous three years. Between 1979 and 1985, reniform nematode was detected in 180 soybean fields. Between 1986 and 2010, the number of soybean fields infested by reniform nematode had increased by 824. Figures 1-3, respectively, show the known distribution of reniform nematode on soybean in Louisiana in 1961, 1985 and 2010. Reniform nematode infestations were known in 11 parishes in 1985 and now, in 2010, are documented in 21 of the major soybean-producing parishes. Rapides parish has the greatest incidence of reniform nematode with 591 fields reported since 1986.

Preliminary greenhouse studies

Greenhouse trials with SEM 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 SEM populations were much less variable than that observed

Table 3. Reproduction of single egg mass (SEM) populations of *Rotylenchulus reniformis* from Hawaii on 'Deltapine 4331' soybean[w].

Single egg mass population	Vermiform stages per 2 kg soil[x]	Reproductive value[y]	Eggs per 3 g of root
HI-1[z]	9,446 a	30.2	1,624 a
HI-2	8,856 a	28.3	2,484 a
HI-3	9,168 a	29.3	1,943 a
HI-4	10,890 a	34.8	1,845 a
HI-5	10,496 a	33.6	2,029 a
HI-6	9,381 a	30.0	1,796 a
HI-7	8,528 a	27.3	2,140 a
HI-8	9,643 a	30.9	1,673 a
HI-9	8,762 a	28.0	1,480 a
HI-10	9,971 a	31.9	2,294 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 58-64 days by the inoculum level of 300-325 vermiform life stages.
[z]SEM population selected for use in microplot trials.

Table 4. Reproduction of single egg mass (SEM) populations of *Rotylenchulus reniformis* from Louisiana on 'Deltapine 4331' soybean[w].

Single egg mass population	Vermiform stages per 2 kg soil[x]	Reproductive value[y]	Eggs per 3 g of root
LA-1	9,119 b	29.2	1,795 a
LA-2	12,338 a	39.5	2,091 a
LA-3[z]	9,446 a	30.2	1,656 a
LA-4	8,856 ab	28.3	2,140 a
LA-5	9,052 b	29.0	1,115 a
LA-6	11,349 a	36.3	1,706 a
LA-7	10,758 a	34.4	2,189 a
LA-8	8,003 b	25.6	1,631 a
LA-9	9,643 a	30.9	1,460 a
LA-10	10,955 a	35.1	1,558 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 58-64 days by the inoculum level of 300-325 vermiform life stages.
[z]SEM population selected for use in microplot trials.

between geographic populations. Data evaluating egg production within SEM populations were slightly less variable than that for vermiform stages. Within three of the six groups of SEM populations, those from AL, MS and TX, there were statistical differences in the numbers of eggs per three grams of root at 58 to 64 days.

Within the 10 SEM populations from AL (Table 1), numbers of vermiform individuals in soil ranged from 1,180 to 2,755, producing reproductive (R) values of 3.5 -8.8. Number of eggs per three grams of root ranged from 477 to 1,427 for the populations representing AL. Mean numbers of vermiform nematodes and egg stages for the 10 SEM populations were calculated and, on this basis, SEM population AL-7, which most closely approximated the mean, was selected for use in microplot trials. Among the ten SEM populations from AR (Table 2) and HI (Table 3), there were no statistical differences in either the numbers of vermiform life stages in soil or the numbers of eggs per three grams

Table 5. Reproduction of single egg mass (SEM) populations of *Rotylenchulus reniformis* from Mississippi on 'Deltapine 4331' soybean[w].

Single egg mass population	Vermiform stages per 2 kg soil[x]	Reproductive value[y]	Eggs per 3 g of root
MS-1	16,006 ab	51.2	7,798 a
MS-2[z]	13,710 b	43.9	5,215 b
MS-3	11,874 b	38.0	2,681 c
MS-4	9,774 c	31.3	6,790 b
MS-5	14,957 b	47.9	9,446 a
MS-6	15,022 b	48.1	6,322 b
MS-7	18,565 a	59.4	4,600 b
MS-8	12,792 b	40.9	5,854 b
MS-9	10,496 bc	33.6	3,788 c
MS-10	11,742 b	37.6	5,609 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 58-64 days by the inoculum level of 300-325 vermiform life stages.
[z]SEM population selected for use in microplot trials.

Table 6. Reproduction of single egg mass (SEM) populations of *Rotylenchulus reniformis* from Texas on 'Deltapine 4331' soybean[w].

SEM population	Vermiform stages per 2 kg soil[x]	Reproductive value[y]	Eggs per 3 g of root
TX-1	20,205 a	64.7	7,158 a
TX-2	12,398 b	39.7	4,577 b
TX-3	19,811 a	63.4	5,753 ab
TX-4	16,859 a	53.9	3,813 c
TX-5[z]	17,318 a	55.4	4,329 b
TX-6	15,678 a	50.2	3,665 c
TX-7	18,827 a	60.2	4,183 bc
TX-8	16,334 a	52.3	2,993 c
TX-9	18,630 a	59.6	5,215 b
TX-10	17,450 a	55.8	3,301 c

[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 58-64 days by the inoculum level of 300-325 vermiform life stages.
[z]SEM population selected for use in microplot trials.

of root. The numbers of vermiform stages ranged from 7,150 to 9,355 and from 8,528 to 10,890 individuals per two kg of soil among the SEM populations from AR and HI, respectively. Similarly, egg counts ranged from 1,451 to 2,927 for the AR SEM populations and from 1,480 to 2,484 for the HI SEM populations. At the conclusion of the trials, reproductive values for the SEM populations from AR ranged from 23.3 to 29.9 while those for the SEM populations from HI averaged 27.3 to 33.6. SEM populations AR-4 and HI-1 were selected for use in microplot trials. A final population density per two kg of soil ranging from 8,003 to 12,338 vermiform individuals were observed within the 10 SEM populations from LA (Table 4). Reproductive values were from 25.6 to 36.3 and egg production estimates were from 1,115 to 2,189 per sample. SEM population LA-3 was selected for use in microplot trials. The soil density of vermiform stages of SEM populations from MS ranged from 9,774 to 18,565 individuals (Table 5). Reproductive values were 31.3 to 59.4. Number of

Table 7. Reproduction and influence of six geographic isolates of *Rotylenchulus reniformis* on dry weights and numbers of pods per 'Deltapine 4331' soybean plant[w].

Isolate source	Vermiforms/15 kg soil		Reproductive Value[z]	126 day plant dry weight (g)	Pods per plant	Pod dry weight (g)
	63 days[xy]	126 days				
AL	11,572 b	52,251 cd	104.5	200.7 c	182 a	83.5 a
AR	19,463 a	138,261 a	276.3	137.5 d	147 b	60.8 b
HI	9,500 c	44,583 d	89.2	260.0 a	204 a	80.2 a
LA	17,395 a	93,672 b	187.7	196.8 b	151 b	60.0 b
MS	15,009 a	118,568 a	237.4	154.9 cd	134 b	62.5 b
TX	10,804 b	71,590 bc	143.3	241.6 a	119 c	55.7 b
Control	0	0	0	273.3 a	197 a	87.3 a

[w]Data combined over two 126 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 X 20 cm) collected around the base of each plant stem. Sampling holes at 63 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 126 days by the inoculum level of 500 vermiform life stages.

eggs produced among the SEM populations averaged 2,681 to 9,446. SEM population MS-2 was selected for microplot trials. The soil density of vermiform stages of SEM populations from TX (Table 6) exhibited the greatest overall level of reproduction among the six geographic populations and ranged from 12,398 to 20,205 individuals per pot. Reproductive values varied from a low of 39.7 to a high of 64.7. Numbers of eggs produced among the SEM populations were from 2,993 to 7,158 per 3 g of root tissue. SEM population TX-5 was selected for microplot trials.

Microplots

Data from microplot trials are summarized as Table 7. At 63 days, approximately mid-way through the trials, there were significant differences in reproduction observed among the six geographic populations of *R. reniformis.* The soil population densities from AR, LA, and MS were significantly greater than those from AL, HI, and TX. At the end of the trials, 126 days, population densities of the isolates representing AR and MS and averaging 138,261 and 118,568, respectively, per 15 kg of soil were significantly higher than those representing the other four states. Reproductive values ranged from a low of 89.2 for the Hawaii population to a high of 276.3 for the Arkansas population. Soybean plant dry weights at harvest were, relative to the non-inoculated control, reduced significantly by all isolates, except the ones from HI and TX. The greatest reductions in soybean plant dry weights were observed with reniform nematode populations from AR and MS. Both the numbers and weights of soybean pods were reduced significantly by all populations, except those from AL and HI.

DISCUSSION

Research 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, 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 from Agudelo *et al*. (2005), in which they also conducted 60-day-duration greenhouse tests with geographic populations of *R. reniformis* on soybean and cotton, 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 of *R. reniformis* 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.

Only a few reports from the United States (McGawley and Sankaralingam, 1994, McGawley and Overstreet, 1995 and Augedelo *et al.*, 2005) 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 greenhouse trials described in this study provide an indication of the amount of variation within geographic populations from a single field having a known history of damage from *R. reniformis*. Overall, the variation within SEM populations from the six states was minimal when compared with that observed between geographic populations. In these trials with soybean there was no statistically significant variation in data for either juveniles or eggs in the populations from Arkansas and Hawaii. There was slight and roughly equivalent variation in juvenile and egg data within SEM populations from Alabama and Mississippi. Among SEM populations from Louisiana, there was more variation in data for juveniles than that for eggs. The opposite was true for the SEM populations from Texas. In contrast, on cotton (McGawley *et al.,* 2010) egg production data within SEM populations was less variable than that for densities of vermiform stages in soil.

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 from these microplot trials with soybean, as was reported for cotton, further documents both reproductive and pathogenic variation in populations of this nematode on a second major crop host. Inspection and comparison of plant dry weight data for soybean in this work and for cotton described in McGawley *et al.*, 2010 indicates that overall, in a microplot environment, the negative impact of *R. reniformis* on plant growth and yield was greater on soybean than on cotton. Averaged across all six geographic isolates of the nematode, the percent reduction in harvest dry weight, relative to those of the non-inoculated soybean and cotton controls, were 27.4% for soybean and 19.7% for cotton.

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 local or regional isolates of the nematode in studies designed to identify new sources of resistance. Such studies should include as many isolates of the nematode as possible, or perhaps use inocula composed of a blend of isolates and/or have such studies carried out in multiple locations employing a range of local isolates.

LITERATURE CITED

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.

Agu, C. M. 2006. Susceptibility of soyabean cultivars to root nematodes in southwestern Ethiopia. Tropical Science 46:143-146.

Anonymous. 2010a. 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. 2010b. 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

Asumus, G. L. 2008. Reação de genótipos de soja ao nematóide reniforme. Tropical Plant Pathology 33:69-71.

Asmus, G.L. and M. R. Schirmann. 2004. Reação de cultivares de soja recomendadas no Mato Grosso do Sul ao nematóide reniforme. Nematologia Brasileira 28:239-240.

Ayala, A. 1962. Pathogenicity of the reniform nematode on various hosts. Journal of Agriculture of the University of Puerto Rico 46:73-83.

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.

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.

Castillo, M. B., M. B. Arceo and J. A. Litsinger. 1978. Population dynamics of plant parasitic nematodes I. *Rotylenchulus reniformis* in a poorly-drained soil and its effect on yield of field legumes. Philippine Agriculturist 61:238-252.

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

Davis, E. L., S. R. Koenning, J. W. Burton and K. R. Barker. 1996. Greenhouse evaluation of selected soybean germplasm for resistance to North Carolina populations of *Heterodera glycines, Rotylenchulus reniformis,* and *Meloidogyne* species. Journal of Nematology 28:590-598.

Davis, R.F., S.R. Koenning, R.C. Kemerait, T.D. Cummings and W.D. Shurley. 2003. *Rotylenchulus reniformis* management in cotton with crop rotation. Journal of Nematology 35:58-64.

Ganguly, S. and V. Ramesh. 1996. Crop-induced morphobiometrical variability in reniform nematode, *Rotylenchulus reniformis*. I. Mature females and eggs. Annals of Plant Protection Sciences 4:22-28.

Ganguly, S. and V. Ramesh. 1997. Crop-induced morphobiometrical variability in reniform nematode, *Rotylenchulus reniformis*. II. Young

females, males and juveniles. Annals of Plant Protection Sciences 5:75-89.

Germani, G. 1978a. Tests preliminaires de sensibilite de deux cultivars de tomate et d'un cultivar d'arachide a deux souches de *Rotylenchulus reniformis* (Nematoda: Tylenchida). Revue de Nématologie 1:111-112.

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

Ha, B., R. T. Robbins, F. Han, R. S. Hussey, J. F. Soper and H. R. Boerma. 2007. SSR mapping and confirmation of soybean QTL from PI 43654 conditioning resistance to reniform nematode. Crop Science 47:1336-1343.

Hague, S. S., C. Overstreet and E. van Santen. 2002. Crop rotation effects on nematode populations. Proceedings of 25th Annual Southern Conservation Tillage Conference for Sustainable Agriculture, Auburn, AL, USA, 24-26 June, 2002, pp 156-160.

Heald, C. M. and A. F. Robinson. 1990. Survey of current distribution of *Rotylenchulus reniformis* in the United States. Journal of Nematology 22:695-699.

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., C. Overstreet, J. W. Noling, P. A. Donald, J. O. Becker and B. A. Fortnum. 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. Supplement to the Journal of Nematology 31:587-618.

Lawrence, G. W. and K. S. McLean. 1999. Plant-parasitic nematode pests of soybean. Pp 291-310 in L.G. Heatherly and H. F. Hodges (eds.), Soybean Production in the Midsouth, CRC Press, Boca Raton, Florida. 394 pp.

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.

Lim, B. K. and M. B. Castillo. 1979. Screening soybeans for resistance to reniform nematode disease in the Philippines. Journal of Nematology 11:275-282.

McGawley, E.C. and A. Sankaralingam. 1994. Reproductive and parasitic variation in populations of the reniform nematode, *Rotylenchulus reniformis.* Journal of Nematology 26:109.

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

McGawley, E. C., M. J. Pontif and C. Overstreet. 2010. Variation in reproduction and pathogenicity of geographic isolates of *Rotylenchulus reniformis* on cotton. Nematropica 40: 275-288.

Nakasono, K. 2004. Studies on morphological and physio-ecological variations of the reniform nematode, *Rotylenchulus reniformis* Linford 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.

Pipolo, V. C. 1994. Fontes de resistência ao nematóide reniforme *Rotylenchulus reniformis* Linford & Oliveira, 1940. Semina (Londrina) 15:100-103.

Prasad, D. 2007. Management of *Rotylenchulus reniformis* on soybean through integrated approach. Annals Plant Protection Science 15:202-205.

Ramesh, V. and S. Ganguly. 1994. Crop-induced bio-ecological variations in *Rotylenchulus reniformis*. Indian Journal of Nematology 24:45-49.

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.

Rebois, R. V. 1971. The effect of *Rotylenchulus reniformis* inoculum levels on yield, nitrogen, potassium, phosphorus and amino acids of seed of resistant and susceptible soybean (*Glycine max*). Journal of Nematology 3:326-327.

Rebois, R.V., J. M. Epps and E. E. Hartwig. 1970. Correlation of resistance in soybeans to *Heterodera glycines* and *Rotylenchulus reniformis*. Phytopathology 60:695-700.

Robbins, R. T., L. Rakes and C. R. Elkins. 1994. Reproduction of the reniform nematode on thirty soybean cultivars. Supplement to Journal of Nematology 26:659-664.

Robbins, R. T., L. Rakes, L. E. Jackson, E. E. Gbur and D. G. Dombek. 2000. Host suitability in soybean cultivars for the reniform nematode, 1999 tests. Journal of Nematology 32:614-621.

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.

Robbins, R. T., E. Shipe, P. Arelli, P. Chen, G. Shannon, S. Koenning, L. Rakes, L. E. Jackson, E. E. Gbur and D. G. Dombek. 2009. Reniform nematode reproduction on soybean cultivars and breeding lines in 2008. p 104-114. *in* Proceedings Beltwide Cotton Conference, San Antonio, TX January 2010. National Cotton Council of America, Cordova, TN.

Robbins, R. T., P. Chen, L. E. Jackson, E. E. Gbur, D. G. Dombek, J. T. Velie, E. Shipe, P. Arelli, G.

Shannon, and C. Overstreet. 2010. Reniform nematode reproduction on soybean cultivars and breeding lines in 2009. p 190-199. *in* Proceedings Beltwide Cotton Conference, New Orleans, LA January 2010. National Cotton Council of America, Cordova, TN.

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.

Shekhar, C., A.U. Siddiqui and A. Parihar. 1996. Observations on pathogenicity and life cycle of *Rotylenchulus reniformis* on soybean and reaction of some varieties. Indian Journal of Nematology 26:98-101.

Sikora, E. J., K. S. Lawrence and J. Murphy. 2009. Survey of plant-parasitic nematodes in soybean fields in Alabama. Alabama Agricultural Experiment Station Research Report No. 37, 1 p.

Singh, N.D. 1975. Studies on selected hosts of *Rotylenchulus reniformis* and its pathogenicity to soybean (*Glycine max*). Nematropica 5:46-51.

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

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.

Westphal, A., and A. W. Scott, Jr. 2005. Implementation of soybean in cotton cropping sequences for management of reniform nematode in South Texas. Crop Science 45:233-239.

Williams, C., D. F. Gilman, J. E. Jones, and W. Birchfield. 1983. Cotton-soyabean rotation for reniform nematode control. Louisiana Agricultural Experiment Station Bulletin No. 741, 22 pages.

Wrather, J. A. and S. R. Koenning. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. Journal of Nematology 38:173-180.

Received: *21/X/2010*
Recibido:

Accepted for publication: *26/I/2011*
Aceptado para publicación: