Reniform Nematode: Possibly the Most Serious Threat to World Cotton Production Since the Boll Weevil

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ABSTRACT

The reniform nematode Rotylenchulus reniformis (Rr), is world-wide in distribution, has life stages adapted to resist environmental stresses, possesses great fecundity, parasitises over 50 agricultural plant species and is highly pathogenic to cotton. Data from laboratory, field, microplot and greenhouse studies, conducted between 1993 and 1997 with 12 isolates of this nematode from all cotton-producing states in the southern USA and Hawaii, clearly support the hypothesis that biological "races" of Rr exist in cotton agroecosystems of the southern USA and probably elsewhere. Supporting evidence from field studies includes differential cotton cultivar performance (with yield differences as great as 20%) and nematicide efficacy (non-significant to highly significant reductions in population densities of Rr) among locations with nearly identical growing conditions and cotton-related pests. Evidence from microplot cultivar inoculation studies (conducted employing populations of Rr derived from single egg masses) includes highly significant differences among populations in reproduction (47-455 fold increases in population density during a single season) and cotton growth suppression (ranging from 8-63%). Greenhouse data also showed consistent differences in host suitability and preference among Rr isolates. The six plant species ('Deltapine 61' cotton, 'NC 95' tobacco, 'California Wonder' pepper, 'Charleston Grey' watermelon, 'Florunner' peanut, and 'Rutgers' tomato) commonly employed for separation of common species of root-knot nematode, were inoculated with 500-535 eggs and juveniles of Rr and reproductive rates (R=Pf/Pi, where R=the reproductive value and Pf=final population density in soil and Pi=infestation level) were calculated after 90 days. Reproductive values for populations over two trials ranged from: 9.4 to 163.0 on cotton, 8.2-144.9 on tobacco, 0.1 to 31.7 on pepper, 0.1-0.2 on watermelon, 0.1-3.7 on peanut, and from 140.3-232.5 on tomato.

Introduction

The nematode genus *Rotylenchulus* contains ten species of sedentary endoparasites. The common name, reniform nematode, refers to the unique kidney-shape of the body of the mature female. Reniform nematode is highly adaptive, parasitising plant species in over 30 different botanical families. *Rotylenchulus reniformis*, the most economically important and "type species" for this genus, was first identified as a pathogen of cotton in Georgia by Smith and Taylor in 1941 and was first associated with cotton failure in Louisiana by Birchfield and Jones in 1961. Currently, the nematode is known from 187 counties and parishes of the southeastern United States and is particularly widespread in Louisiana, occurring in 47 of 64 parishes. During the last five years, reniform nematode awareness among cotton producers has increased markedly as damage has become more widespread (Figure 1A). This is probably due both to the cultivation of cotton on land previously planted to soybean and to the use of nematode-contaminated farm equipment. Although reniform nematode is a known pathogen of soybean, many cultivars permit the accumulation of high populations of reniform nematode while sustaining relatively little damage. However, with cotton, microplot studies have shown that this nematode causes reduction in growth of 10-66% in cotton cultivars that are common in the southern United States (McGawley *et al.*, unpublished).

In 1962, Birchfield and Brister in Louisiana suggested the possibility that races of reniform nematode exist and in 1971, races designated as "a" and "b" on the basis of their reproduction on cowpea, castor bean and cotton, were described from India. The primary observation that precipitated our revitalization of the "race hypothesis" was the differential yield response of cotton cultivars at various locations within the state (Table 1). Cultivars performed well in reniform-infested soil in one location, did poorly in another and were intermediate at still other locations (Overstreet and McGawley, 1995). Obvious factors such as soil texture, pH, rainfall, and crop rotation might have explained this variation until variations in cultivar response were identified within geographic ranges much smaller than the parish level in which edaphic factors were roughly comparable (Stetina *et al.*, 1997; Overstreet and McGawley, 1997; McGawley and Sankaralingam, 1994).

Material and Methods

Greenhouse studies utilized 15 cm diam. clay pots that contained 1.6 kg of a soil mixture composed of 3 parts fumigated (67% methyl bromide, 33% chloropicrin) Convent silt loam soil (Aeric Fluvaquent, coarse-silty, mixed, nonacid, thermic) and two parts autoclaved sand. Seeds of soybean ('Bragg' or 'Hartz 6686') treated with a commercial preparation of *Bradyrhizobium japonicum* and cotton ('Stoneville LA887', 'Deltapine 90', and 'Deltapine 41') were sown in flats of fumigated soil and uniform seedlings were selected and transplanted singly to the center of test pots when they were 10 and 15 days old, respectively. Studies were terminated after a period of 80-92 days. All populations of *Rr* were derived from single egg masses and maintained as monoxenic cultures on 'Rutgers' tomato. Inoculum consisted of vermiform nematodes obtained from greenhouse cultures and removed from soil by wet sieving (Cobb, 1918) and centrifugal-flotation (Jenkins, 1964). Soil in pots in each experiment was infested by pipetting nematodes suspended in water into depressions (1 cm diameter x 4 cm deep) made around the base of stem on opposite sides of the plant. Following infestation, depressions were filled with fumigated soil. Greenhouse temperatures ranged from 22 to 35ºC. Supplemental lighting from fluorescent (ca. 260 µE/s-1/m-2) and incandescent sources provided a minimum of 14 hours of continuous light daily.

Each microplot consisted of a 30 cm diam. clay pot that contained 15 kg of fumigated (67% methyl bromide, 33% chloropicrin or 32.7% sodium methyldithiocarbamate, 67.3% inert ingredients) Mhoon silt loam soil (Tiypic Fluvaquent, fine silty, mixed, nonacid, thermic). Microplots were set into the ground to the depth of the pot rim and spaced 1m apart. The entire microplot area (37 m long by 14 m wide) was covered by a 6 ml polyethelene-covered quonset hut frame, open at both ends and covered with shadecloth which provided plants with approximately 40% of full sunlight. Plants in microplots were grown for 14-16 weeks and fertilization and insect control were in accordance with recommendations provided by the Louisiana Co-operative Extension Service.

At the conclusion of each experiment, six soil cores (2.5 cm diam.) from the soil surface to the bottom of the pot were collected, bulked, sub-sampled (250g), and extracted for nematodes as described above. Nematode population levels per pot were estimated and reproductive ("R") values were calculated on the basis of the formula R=Pf/Pi, where Pf is the final population density and Pi is the initial population density (inoculum level). Plant stems were cut at the soil surface and the root-soil mass was removed from damaging than those evaluated to date. The Mississippi isolate was intermediate in both reproduction and damage potential. As was observed under greenhouse conditions, the isolate from Hawaii reached very high population levels but caused no detectable plant damage.

each pot. Root systems were freed from soil by gentle washing with tap water. Nematode egg production was estimated by subjecting a 5.0g, randomly collected, root subsample to the extraction method of Hussey and Barker (1973). Weights for stems and roots were recorded after drying for 72 hours at 60ºC.

Egg hatch studies were conducted under laboratory conditions using controlled temperature incubators. Incubation media were either sterile distilled water, adjusted to pH 7.0 using monobasic sodium phosphate, or fumigated soil at a pH level (6.8) suitable for the production of soybeans and cotton.

All experiments were repeated at least once. Data were subjected to analysis using ANOVA and Tukey's HSD means so that values could be compared with those of the control.

Results and Discussion

On both cotton and soybean, there were significant differences among populations in both reproduction and pathogenicity (Table 2). Reproductive values on cotton ranged from lows of 8.2 and 9.3 to highs of 68.5 and 28.2 on Deltapine 90 and 41, respectively. Pathogenicity among isolates of *Rr*, as evidenced by reductions below noninoculated controls in root dry weights, ranged from 6% to 30% on Deltapine 90 and from 5% to 41% on Deltapine 40. On soybean, reductions in root weights were from 6%-59% on Bragg and from 3% to 39% on Hartz 6686. On both crops, reniform isolates from Louisiana (LA) and Mississippi (MS) increased to the highest population levels and caused the greatest amount of damage. In contrast, populations from Hawaii (HI) reproduced least and caused relatively little root damage. Populations from Texas (TX) and Arkansas were intermediate.

Under microplot conditions, which more closely simulate a production environment but permit precise control of numbers and kinds soil micro-organisms, the geographic isolates of *Rr* also fell into several different categories of reproduction and pathogenicity (Table 3 and Figure 1B). The Louisiana isolate, LA-8, reached the highest populations level with an "R" value of 186 representing an average density of 271,920 individuals per microplot and caused approximately a 70% reduction in plant weight. Reproduction by the Arkansas population was less than half that of the Louisiana population, although the amount of damage caused was roughly equivalent. This observation, if confirmed in subsequent studies, suggests the existence in nature of isolates even more

Egg production among isolates ranged from a low of 47 eggs per gram of root for the Hawaii isolate to a ten-fold higher rate, 464, for the Arkansas population.

Studies of reniform egg hatch under controlled temperature conditions, in both water and soil, also showed marked differences among the isolates (Table

4). Across all isolates, egg hatch was greater in soil than in water. Additionally, as the soil temperature was increased from 23ºC to 28ºC hatch of all isolates increased. Egg hatch among isolates varied with incubation medium and temperature. In water, hatch ranged from approximately 42-85%. Hatch in soil at 23, 28, and 32ºC, respectively, ranged from 44-93%, from 51-90%, and from 61-93%.

Conclusions

Data collected over 5 years of laboratory, greenhouse, microplot and field study with isolates of *Rotylenchulus reniformis* from the Southern United States and Hawaii strongly suggest the existence of distinct "races" or "biotypes" of this important nematode pathogen. A method for distinguishing between such populations must be developed before durable resistant crop cultivars can be developed. Research is currently underway to determine whether or not an assay, similar to the host differential assay employed for separation of the common species of *Meloidogyne* can be devised to accommodate races of *R. reniformis*.

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References

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.

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

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

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.

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

Overstreet, C. and E.C. McGawley. (1995): Varietal reaction to *Rotylenchulus reniformis* in a multi-site study. Journal of Nematology 27:58.

Overstreet, C. and E.C. McGawley. (1997): Reniform nematode and its influence on the cotton industry in the United States. Proceedings of the Beltwide Cotton Conferences, P. Dugger and D.A. Richter (Ed). Natl. Cotton Council, Memphis TN. Pp. 92-94.

Smith, A.L. and A.L.Taylor. (1941): Nematode distribution in the 1940 regional cotton-wilt plots. Phytopathology 31:771 (Abstract).

Stetina, S.R., E.C. McGawley, and J.S. Russin. (1997): Relationship between *Meloidogyne_incognita* and *Rotylenchulus reniformis* as influenced by soybean genotype. Journal of Nematology 29:395-403.

Table 1. Performance of cotton cultivars on silt loam soils in five parishes in Louisiana during the 1994 growing season[1].

Cotton Cultivar	Cotton Yield in lbs. seed cotton. (Numerical Ranking Among Cultivars)				
	Franklin	Morehouse	West Carroll	Rapides	Caldwell
STN 132	2158 (6)	2320 (1)	2440 (1)	2657 (3)	2723 (1)
DPL 5690	2390 (4)	2244 (2)	2137 (5)	2781 (1)	2572 (5)
DPL 51	2375 (5)	2087 (3)	2100 (6)	2159 (6)	2546 (6)
STN 887	2527 (1)	1907 (4)	2374 (3)	2208 (5)	2712 (2)
STN 311	2408 (3)	1881 (5)	2320 (4)	2637 (4)	2659 (4)
HS 46	2442 (2)	1667 (6)	2434 (2)	2700 (2)	2562 (3)
LSD 5%	NS	409	NS	453	NS

[1]At all locations reniform infestation level averaged 6,255 individuals per 500 cm^3 soil. STN = Stoneville, DPL=Deltapine, HS=Hyperformer. Data are means of 4 replications. Plot size was 0.5 ha

Table 2. 1995-1996 Greenhouse inoculation studies with isolates of the reniform nematode, *Rotylenchulus reniformis*, from the Southern United States and Hawaii.

Reniform Isolate	Cotton				Soybean			
	Deltapine 90		Deltapine 41		Bragg		Hartz 6686	
	R Value	Dry wt. (g)	R Value	Dry wt. (g)	R Value	Dry wt. (g)	R Value	Dry wt. (g)
LA-1	53.8b	79.6bc	25.1a	70.4e	88.3c	106.4b	153.7a	117.9a
LA-4	40.2c	73.9c	20.4b	95.0c	137.8b	83.4d	90.1bc	95.0b
LA-5	63.5a	80.1b	22.7ab	93.5cd	150.4b	57.3f	117.4b	78.0c
LA-8	68.5a	67.4d	28.2a	106.1b	185.0a	70.5e	131.5b	107.6a
AR-1	45.2c	83.7b	29.0a	80.6e	63.5d	91.7cd	83.2c	98.3a
HI-1	8.2d	88.1a	9.3c	115.7a	41.9e	124.6a	43.9d	114.5a
HI-2	17.6d	86.5ab	14.5c	103.1bc	30.2e	133.0a	58.5d	93.2b
MS-2	66.1a	79.4c	25.5a	75.8e	90.6c	92.8c	147.0a	122.7a
TX-1	35.6c	83.9b	26.3a	90.2d	75.3d	102.5b	97.3b	127.9a
Control	0	94.6a	0	121.5a	0	140.7a	0	126.8a

Within columns, values with common letters are not significantly different at the 5% level according to Tukey's HSD test.

Table 3. 1997 Microplot study with isolates of the reniform nematode, *Rotylenchulus reniformis*, from the Southern United States and Hawaii.

Reniform	*Rotylenchulus reniformis* Life Stage		Cotton Dry Weights (g)		
Isolate	Juveniles/250cc[2]	Eggs/g root	Above ground	Root	Plant
HI-2	3,328b (137)	47c	402.2a	66.0a	468.2a
TX-1	3,768b (155)	285b	277.8b	47.0b	324.8b
MS-2	2,899bc (119)	184c	164.9bc	30.4c	195.3c
AK-1	2, 634d (88)	464a	137.1c	23.1c	160.2d
LA-8	4,532a (186)	377a	138.4c	28.5c	166.9d
Control	0	0	439.6a	70.2a	509.8a

[1]For all isolates, Pi was ca. 1400 Juveniles and eggs.
[2] Numbers in parentheses are "R" values. Within columns, values with common letters are not significantly different at the 5% level according to Tukey's HSD test.

Table 4. Hatch of eggs of the reniform nematode, *Rotylenchulus reniformis*, from the Southern United States and Hawaii in water and soil.[1]

Isolate	Water (28ºC)	Soil (23ºC)	Soil (28ºC)	Soil (32ºC)
LA-1	58.2d	85.3ab	82.5b	87.2ab
LA-4	62.6c	78.9b	85.3b	89.5a
LA-5	84.3a	89.2a	86.6b	91.4a
LA-8	61.7cd	82.4b	85.0b	86.1b
AR-1	44.3e	45.8c	51.2c	61.4d
HI-1	73.6b	81.0b	85.2b	84.6b
HI-2	85.2a	92.6a	90.4a	92.5a
MS-2	68.3b	74.9b	78.5b	76.3c
TX-1	41.7e	44.5c	60.4c	62.0d

[1] Sterile distilled water adjusted to pH 7.0. Soil fumigated with 67% methyl bromide, 33% chloropicrin (pH 6.8). Within columns, values with common letters are not significantly different at the 5% level according to Tukey's HSD test

Figure 1. A) Cotton field in Rapides parish showing plants damaged by reniform nematode and B) Representative microplots showing difference in damage potential of various isolates of *R. reniformis* in 1997 study (left-right; noninoculated control, Mississippi isolate, Louisiana isolate, Texas isolate, and Arkansas isolate).