PATHOGENICITY AND REPRODUCTION OF *MELOIDOGYNE ARENARIA* RACES 1 AND 2 AND *M. INCOGNITA* RACE 3 ON SOYBEAN†

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ABSTRACT

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Pathogenicity and reproduction of three South Carolina populations of *Meloidogyne arenaria* race 2 (designated Florence, Govan, and Pelion), a Florida population of *M. arenaria* race 1, and a South Carolina population of *M. incognita* race 3 were compared in greenhouse experiments on the soybean cultivars Centennial, Coker 6738, Coker 6847, Kirby, and Perrin. In one experiment, all race 2 populations of *M. arenaria* produced more galls, egg masses, and eggs per gram of fresh root than the race 1 population on all cultivars. The Florence and Govan populations exhibited greater reproduction than the Pelion population on Centennial, Coker 6738, and Kirby, and the Florence population had greater reproduction than the Govan population on Perrin. In another experiment, a series of six inoculum densities between 500 and 20 000 eggs per plant were used. At most inoculum levels, the Florence population of *M. arenaria* race 2 had nearly 10-fold greater reproduction on 'Davis' soybean than either *M. arenaria* race 1 or *M. incognita* race 3.

Key words: soybean, *Meloidogyne arenaria, Meloidogyne incognita,* races, nematode populations, varieties.

RESUMEN

Ibrahim, I. K. A. y S. A. Lewis. 1993. Patogenicidad y reproducción de *Meloidogyne arenaria,* razas 1 y 2, y de *Meloidogyne incognita,* raza 3 en soya. Nematrópica 23:159–166.

La patogenicidad y reproducción de tres poblaciones de la raza 2 de *Meloidogyne arenaria* de Carolina del Sur (designadas como Florence, Govan y Pelion), una población de Florida de *M. arenaria* (raza 1) y otra de *M. incognita* (raza 3) procedente de Carolina del Sur, fueron comparadas en experimentos de invernadero en los cultivares de soya Centennial, Coker 6738, Coker 6847, Kirby y Perrin. En un experimento, todas las poblaciones de la raza 2 de *M. arenaria* produjeron más agallas, masas de huevos y huevos por gramo de raíz que la poblacion de la raza 1 en todos los cultivares. Las poblaciones Florence y Govan manifestaron mayor reproducción que la Govan sobre el cultivar Perrin. En otro experimento inoculaciones de seis densidades de inóculo (entre 500 y 20 000 huevos por planta) fueron estudiadas. A los niveles más altos, la población Florence de la raza 2 de *M. arenaria* tuvo casi 10 veces más reproducción sobre la soya 'Davis' que *M. arenaria* raza 1 o *M. incognita* raza 3.

Palabras clave: cultivares, *Meloidogyne arenaria, Meloidogyne incognita,* nematodo, poblaciones, razas, soya, variedades.

INTRODUCTION

The root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood is widespread, adversely affecting growth and yield of soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), and other crops in the southern United States (8,10,14,15). Host race 2 of *M. arenaria* is increasingly found in many locations in South Carolina (5). The wide-

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spread use of tobacco and soybean cultivars resistant to *M. incognita* (Kofoid & White) Chitwood may have promoted a shift to *M. arenaria* from *M. incognita*, a species commonly associated with the traditional production of cotton (*Gossypium hirsutum*) in earlier years.

Carpenter and Lewis (2) studied the responses of 24 soybean selections to *M. arenaria* in infested fields at Pelion and Govan, South Carolina, and at Govan found significant differences among those selections in gall ratings and root fresh weights. Differences between locations, which differed greatly in soil characteristics, were highly significant for gall and egg mass ratings, but no interaction between location and ratings occurred. Ibrahim and Lewis (9) reported that reproduction of the Govan population of *M. arenaria* race 2 was very high on Centennial, Govan, and Kirby soybeans when compared to *M. incognita* race 1. The latter two cultivars have tolerance to *M. arenaria*; *i.e.*, nematode reproduction occurs but there is limited yield suppression.

Soybean cultivars which suppress nematode reproduction are needed for effective management of root-knot nematode populations. Soybean cultivars that are highly resistant to *M. incognita* and moderately resistant to some populations of *M. arenaria* are being developed (17).

Information concerning the pathogenicity of different populations of *Meloidogyne* spp. is important in designing a plant breeding program to develop resistant soybean cultivars with improved performance under field conditions. The objectives of this study were to compare the pathogenicity and reproduction of selected populations of *M. arenaria* and *M. incognita* in relation to the growth of soybean cultivars that differ in susceptibility to root-knot nematodes.

MATERIALS AND METHODS

Four populations of *Meloidogyne arenaria* representing host races 1 and 2 and one population of *M. incognita* race 3 were used. *Meloidogyne arenaria* race 1, collected originally from peanut (*Arachis hypogaea*), was obtained from Dr. D. W. Dickson, University of Florida. Three populations of *M. arenaria* race 2 were obtained from infected soybean plants near Florence, Govan, and Pelion, South Carolina. A population of *M. incognita* race 3 was obtained from watermelon (*Citrullus lanatus*) near Blackville, South Carolina. Populations were identified to species by perineal patterns (16) and electrophoresis (4) and to race by host differentials (16), and were increased on tomato cv. Rutgers (*Lycopersicon esculentum*). After 7–8 weeks, eggs were collected from tomato roots using commercial sodium hypochlorite solutions (7).

Pathogenicity and reproduction of races 1 and 2 of M. arenaria *on soybean:* Seven-day-old soybean seedlings of cultivars Centennial, Coker 6738, Coker 6847, Kirby, and Perrin were transplanted into equal volumes of steam-sterilized sand and sandy loam soil in 15-cm-diam plastic pots (two plants per pot). Centennial is considered intolerant to *M. arenaria* while the other four cultivars are considered moderately tolerant. Plants were inoculated with the appropriate nematode populations by pipetting 8 000 eggs/pot around the root system. Non-inoculated checks were included to determine the effect of the nematode populations on plant growth. Pots were arranged in a randomized complete block design with six replications in a greenhouse at 20–26 °C.

The experiment was terminated 6 weeks after transplanting and inoculation. Roots were washed free of soil and stained in 0.1% aqueous phloxine B (3). Galls and egg masses were rated on a 0–5 scale, where 0 = 0, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = > 100 galls and egg masses per root system. Root and shoot fresh weights and shoot dry weights were determined. Eggs were extracted from each root system as described previously and counted. Oostenbrink's (12) reproduction factor (RF = the final egg number/initial egg number), and the numbers of eggs per gram of fresh root (EGR) were determined for each cultivar-nematode combination. Hosts were rated as highly suitable (RF ≥ 5.0), suitable (1.0 < RF < 5.0), or unsuitable (RF ≤ 1.0).

A modified tolerance index (TI) (1), equal to the shoot dry weight of nematode infested soybean divided by shoot dry weight of uninfested soybean, was calculated for each soybean-nematode treatment. Levels of tolerance were defined as high (TI = 96–100), moderate (TI = 68–95), or intolerant (TI = 0–67).

Effect of inoculum level of races 1 and 2 of M. arenaria *and race 3 of* M. incognita*:* This experiment included the *M. incognita* race 3 population, the *M. arenaria* race 1 population, and the *M. arenaria* race 2 population from Florence. Seeds of soybean cv. Davis (8) were sown in 12.5-cm-diam plastic pots filled with the previously described steam-sterilized sand and sandy loam soil mix. Davis is a suitable but intolerant host for *M. incognita* and *M. arenaria*. Seven days after emergence, seedlings were thinned to two per pot and inoculated with the appropriate nematode populations by pipetting 500, 1 000, 2 500, 5 000, 10 000, or 20 000 eggs per pot into the soil around the root system. Non-inoculated plants receiving water without eggs served as controls. Pots were arranged in a randomized complete block design with five replications in a greenhouse maintained at 20–26 °C.

Six weeks following inoculation, plants were harvested, roots were carefully washed free of soil, and egg masses were stained with phloxine B. The fresh weights of roots and shoots, dry weight of shoots, tolerance index, reproduction factor, and number of eggs per gram of fresh root were determined.

RESULTS

Pathogenicity and reproduction of races 1 and 2 of M. arenaria*:* All soybean cultivars supported high levels of reproduction of all three populations of *M. arenaria* race 2. The race 1 population produced significantly less egg masses and had lower RF and EGR values (P = 0.05) than the race 2 population on all cultivars. On the other hand, race 1 did reproduce prolifically on all cultivars. Based on RF values, Centennial and Coker 6847 were highly suitable hosts (RF > 5.0) for race 1 while the other cultivars were suitable hosts (RF = 1.1–5.0) (Table 1).

All race 2 populations caused extensive galling and had egg mass indices of 5 on all cultivars. The Florence and Govan populations exhibited significantly higher RF and EGR values than the Pelion population on Centennial, Coker 6738, and Kirby. The RF and EGR values of the Florence and Govan populations did not differ significantly on any cultivar except Perrin, where the Florence population had a significantly higher RF (Table 1).

Root fresh weights of soybean cultivars were not affected by any of the nematode populations (data not shown), and most populations had no significant effect on the fresh and dry weights of shoots (Table 2). Only the shoot fresh weight of Cen-

Table 1. Egg mass index (EMI), reproduction factor (RF), and number of eggs per gram of fresh root (EGR) for *Meloidogyne arenaria* race 1 and for race 2 populations of *M. arenaria* from Florence, Govan, and Pelion on soybean cultivars Centennial, Coker 6738, Coker 6847, Kirby, and Perrin, 6 weeks after inoculation with 8 000 eggs/plant.

M. arenaria	Centennial			Coker 6738			Coker 6847			Kirby			Perrin		
population	EMI	RF	EGR	EMI	RF	EGR	EMI	RF	EGR	EMI	RF	EGR	EMI	RF	EGR
Race 2															
Florence	5.0a[z]	183.3a	964a	5.0a	103.5a	437a	5.0a	117.6a	712a	5.0a	124.2a	962a	5.0a	145.2a	620a
Govan	5.0a	185.8a	1009a	5.0a	113.7a	603a	5.0a	128.1a	586a	5.0a	133.2a	977a	5.0a	109.5a	494ab
Pelion	5.0a	102.4b	439b	5.0a	72.5b	235b	5.0a	111.3a	440b	5.0a	90.4b	526b	5.0a	93.4b	391b
Race 1	3.8b	9.4c	43c	3.0b	4.4c	17c	3.0b	8.8b	41c	3.4b	4.5c	29c	1.8b	3.0c	11c

[z]Data followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

tennial was significantly suppressed by all race 2 populations. The fresh and dry weights of shoots of Coker 6738 were suppressed by the Govan population and the shoot fresh weight of Coker 6847 was suppressed by the Florence population (Table 2).

All soybean cultivars were highly tolerant to the race 1 population of *M. arenaria*. Coker 6738 and Coker 6847 were also tolerant to the Pelion race 2 population. All cultivars were moderately tolerant to race 2 populations from Florence and Govan, except for Coker 6738, which was intolerant to the Govan population (Table 3).

Effect of inoculum levels of races 1 and 2 of M. arenaria *and race 3 of* M. incognita*:* Davis soybean was a highly suitable host, based on nematode reproduction, for all *Meloidogyne* populations tested. The number of nematode eggs in the roots showed a gradual increase with the increase of the initial inoculum level (Pi) of each population. The number of eggs recovered increased to a maximum at Pi = 20 000, with 105 851, 20 423, and 8 120 EGR respectively for *M. arenaria* race 2, *M. incognita* race 3, and *M. arenaria* race 1, at the end of the study (Table 4).

The RF values of *M. arenaria* race 1 showed a significant decrease as inoculum level increased even at the lowest inoculum levels (Pi = 500 and 1 000) with the RF value decreasing to 5.9 at Pi = 20 000 (Table 4).

Each inoculum level of *M. arenaria* race 2 had a higher RF value than the corresponding inoculum level of *M. incognita* race 3 and *M. arenaria* race 1. The low inoculum levels of *M. arenaria* race 2 from Florence (Pi = 500, 1 000, 2 500) had significantly higher RF values than the highest inoculum level (Pi 20 000) (Table 4). Similarly, the RF values of *M. incognita* race 3 at low inoculum levels (Pi 500 and 1 000) were significantly higher than at the two highest inoculum levels.

Root and shoot fresh weights of Davis soybean were not significantly affected by any of the nematode treatments. Shoot dry weight was not affected by *M. arenaria* race 1 at any inoculum level, but it was significantly suppressed by *M. arenaria* race 1 at the two higher inoculum levels (Pi = 10 000 and 20 000 eggs) and by *M.*

Table 2. Effect of *Meloidogyne arenaria* race 1 and race 2 populations on the fresh and dry weights of shoots (in grams) of Centennial, Coker 6738, Coker 6847, Kirby, and Perrin soybeans, 6 weeks after inoculation with 8 000 eggs/plant.

	Centennial		Coker 6738		Coker 6847		Kirby		Perrin	
M. arenaria population	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Race 2										
Florence	33.8b[z]	6.0a	34.6a	5.9ab	27.8b	4.4a	24.3a	3.7a	42.5ab	7.5a
Govan	31.2b	5.5a	25.9b	4.5b	33.3ab	5.1a	25.9a	4.0a	42.6ab	6.8a
Pelion	32.5b	5.9a	37.6a	7.5a	31.6b	5.8a	25.8a	4.7a	38.8b	6.7a
Race 1	41.7a	7.6a	38.4a	7.3a	40.1a	6.4a	33.9a	5.4a	50.3a	9.4a
Control	42.7a	6.9a	39.4a	7.3a	36.6ab	5.9a	32.8a	5.1a	46.6ab	8.4a

[z]Data followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. The tolerance index[z] of soybean cvs. Centennial, Coker 6738, Coker 6847, Kirby, and Perrin infected with *Meloidogyne arenaria* race 1 and three populations of race 2, 6 weeks after inoculation with 8 000 eggs/plant.

M. arenaria population	Centennial	Coker 6738	Coker 6847	Kirby	Perrin
Race 2					
Florence	87	81	75	73	89
Govan	80	62	86	78	81
Pelion	86	103	98	92	80
Race 1	110	100	108	105	112

[z]Tolerance index defined as the shoot dry weight of nematode-infested soybean divided by the shoot dry weight of uninfested soybean, × 100. Levels of tolerance: High = >95; moderate = 68–95; intolerant = 0–67.

incognita race 3 at Pi = 2 500, 5 000, 10 000, and 20 000 eggs.

DISCUSSION

The results indicate that soybean cvs. Centennial, Coker 6738, Coker 6847, Kirby, and Perrin are highly suitable hosts for three populations of *M. arenaria* race 2 from South Carolina. Our previous study (9) also showed that the reproduction of the Govan race 2 population was prolific on Centennial and Kirby soybeans. Previous studies gave some evidence for differences in the reaction of soybean to race 2 populations of *M. arenaria* (2,6). In the present study, race 2 populations from South Carolina were more aggressive than the Florida race 1 population and produced several times more egg masses and EGR on all soybean cultivars tested. Of the race 2 populations, those from Florence and Govan reproduced more than the one from Pelion on most cultivars, and on cv. Perrin the Florence population had greater reproduction than the Govan population. Thus, there is appreciable variation in aggressiveness among populations as well as between races of *M. arenaria* on soybean. Similarly, a Georgia population of *M. arenaria* race 1 produced more eggs on peanut and soybean than did other tested *M. arenaria* race 1 populations (11). Reproductive variability of field populations of *M. incognita* and *M. hapla* also occur (13,18). Such differences in prolificacy in *Meloidogyne* spp. could affect management strategies (11).

This study confirms that Davis soybean is a suitable host for race 3 of *M. incognita* as well as both races of *M. arenaria* (8,10, 14). However, our data indicate that at 20–26 °C, race 2 of *M. arenaria* produces far more eggs and causes more plant growth suppression than *M. arenaria* race 1 or *M. incognita* race 3 on Davis, especially at high inoculum levels.

Monocropping of soybean cultivars with given levels of resistance sufficient to limit reproduction of *M. incognita* may have allowed *M. arenaria* populations to increase and become the dominant species in some areas. Nematicide usage also can apparently select for *M. arenaria* due to the greater sensitivity of *M. incognita* to fumigant nematicides (B. A. Fortnum, Clemson University, unpublished). Selection for a more aggressive nematode pest, by whatever means, has placed an increased management burden on growers and advisors. The evidence that *M. arenaria* is increasing in distribution would not affect cotton growers directly

Table 4. Effect of initial infestation levels of *Meloidogyne arenaria* race 1, *Meloidogyne arenaria* race 2, and *Meloidogyne incognita* race 3 on the shoot dry weight, tolerance index, reproduction factor (RF) and number of eggs per gram of fresh root (EGR) of Davis soybean.

Initial infestation level (eggs/plant)	*M. arenaria* race 1				*M. arenaria* race 2				*M. incognita* race 3			
	Shoot dry wt. (g)	Tolerance index[y]	RF	EGR	Shoot dry wt. (g)	Tolerance index	RF	EGR	Shoot dry wt. (g)	Tolerance index	RF	EGR
0	5.82a[z]	—	0e	0c	5.82ab	—	0e	0e	5.82a	—	0c	0e
500	5.56a	96	20.5a	475c	5.92a	102	152abc	3 390d	5.92a	102	21a	504c
1 000	5.36a	92	12.9b	735c	5.46abc	94	166ab	9 374d	5.88a	101	22a	1 206c
2 500	5.14a	88	8.2c	1 520c	4.76bc	82	198a	32 367c	4.40b	76	20ab	3 163c
5 000	4.82a	83	8.2c	3 269c	4.94bc	76	113bcd	39 551c	4.80b	82	18ab	5 255bc
10 000	4.78a	82	5.7d	4 509b	4.56c	78	106cd	59 964b	4.83b	83	14b	8 481b
20 000	5.00a	86	5.9d	8 120a	3.28d	56	83d	105 851a	4.08b	70	14b	20 423a

[y]Tolerance Index defined as the shoot dry weight of nematode-infested soybean divided by the shoot dry weight of uninfested soybean × 100. Levels of tolerance: High = >95, moderate = 68–95, intolerant = 0–67.

[z]Data followed by the same letter within columns are not significantly different (P = 0.05) according to Duncan's multiple range test.

since cotton is not a host for *M. arenaria*. In soybean production, however, the use of cultivars resistant to *M. incognita* and tolerant to *M. arenaria*, allowing for reproduction of *M. arenaria*, is increasing the management problems caused by *M. arenaria* on tobacco, corn, and soybean. The differences in aggressiveness of *M. arenaria* populations, as pointed out here, could pose a problem even on tolerant cultivars (9), where interactions of the two *Meloidogyne* species could occur as well as on selected rotations (11). Where nematicides are used, as on tobacco, there could be differences in the amount of nematicide needed on different varieties with different nematode populations. A high level of soybean resistance to *M. arenaria* is needed for effective management of this species.

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