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Interrelationships of *Meloidogyne arenaria* and *M. incognita* on Tolerant Soybean[1]

I. K. A. Ibrahim and S. A. Lewis[2]

Abstract: Reproduction of *Meloidogyne arenaria* race 2 was excellent on Centennial, Govan, and Kirby soybeans, the latter two of which have tolerance to this species. The *M. incognita* race 1 isolate reproduced poorly on Centennial, especially at the higher of two temperature regimes. Numbers of galls and egg masses of *M. arenaria* plus *M. incognita* in simultaneous equivalent infestations on Centennial did not differ from sequential infestations in which *M. arenaria* was added first and *M. incognita* was added to the same pots, 1, 2, or 3 weeks later. However, at both 25 and 30 C, suppression of galls and egg masses occurred when inoculation of *M. incognita* preceded that of *M. arenaria* by 2 weeks. Generally, *M. arenaria* reproduced well at 25 or 30 C, whereas *M. incognita* reproduced better at 30 C. Kirby was tolerant to either nematode species at 25 and 30 C, but in combined infestations of *M. arenaria* and *M. incognita* there was evidence of synergistic growth suppression. Govan was tolerant of *M. arenaria* at 25 C but not at 30 C. Moreover, general plant growth was less vigorous for Govan at the higher temperature, whereas Centennial was much more vigorous at this temperature. Kirby grew equally well at both temperatures.

Key words: host resistance, interaction, *Glycine max* (soybean), *Meloidogyne incognita, Meloidogyne arenaria,* root-knot nematode, soil temperature.

The root-knot nematodes *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. arenaria* (Neal, 1889) Chitwood, 1949 are widespread, adversely affecting soybean (*Glycine max* Merr.) production in the southern United States (7,9). Both *M. arenaria* race 2 and *M. incognita* are increasingly found together in the same tobacco (4) and soybean (N. G. Conrad, unpubl.) root samples, particularly since 1982. The widespread use of *M. incognita*-resistant tobacco and soybean cultivars may have promoted a shift, in some locations, to *M. arenaria* from *M. incognita,* commonly associated with the traditional cotton production of earlier years. Moreover, loss of the cost-effective DBCP and EDB fumigant nematicides, coupled with some evidence that alternative chemicals were less effective against *M. arenaria* than *M. incognita* (1), may have contributed to an incipient change in species dominance.

In 1982 a dramatic increase occurred in

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[2] Visiting Professor and Professor, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29631. Home address of senior author: Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

the incidence of fields with tobacco affected by *M. arenaria*, and the epiphytotic was thought to be environmentally correlated (4). Experiments were initiated to determine the comparative effect temperature has on nematode reproduction and plant growth and to compare inherent virulence of *M. arenaria* and *M. incognita* on tolerant soybean.

Materials and Methods

Meloidogyne arenaria race 2 (Ma) and *M. incognita* race 1 (Mi), identified by perineal patterns and host differentials (10), were isolated from roots of soybean from Barnwell County, South Carolina, and increased on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) for 60 days in a greenhouse. Nematode eggs for infesting the 1:1 (v:v) sand and sandy loam soil were extracted from galled tomato roots (5). Plants were grown in 15-cm-d plastic pots (one plant per pot) and nematode eggs in 10 ml water were used to infest the soil.

Ma and Mi on tolerant soybean: Ten-day-old seedlings of Govan and Kirby soybeans (8) were transplanted into soil and infested with a suspension of nematode eggs to assess nematode reproduction. Treatments included 6,000 eggs of Ma or Mi or 3,000 Ma plus 3,000 Mi eggs per pot. Noninoculated plants served as controls and treatments were randomized in five replicates and grown in the greenhouse at 20–26 C. Roots of plants harvested after 7 weeks were washed free of soil and stained with phloxine B (2). Gall and egg mass ratings were made on a 0–5 scale (0 = 0, 1 = 1–2, 3 = 11–30, 4 = 31–100, and 5 = > 100 galls and egg masses). Root and shoot dry weights of the plants were determined.

In a similar experiment, Govan and Kirby were grown in temperature-controlled water baths at 25 and 30 C in the greenhouse with treatments of 3,000 or 6,000 eggs of Ma or Mi or 6,000 eggs each of Ma and Mi. Roots of harvested plants were treated as before and galls and egg masses were counted. Roots of combined Ma and Mi treatments were stained with hot acid fuchsin in lactoglycerol and 25 mature *Meloidogyne* females were identified, using perineal patterns.

Ten-day-old Kirby seedlings were transplanted into steam-sterilized soil for an experiment in which infestation level effects of the two species were assessed. After 3 days, plants and soil were infested with either 0, 2,500, 5,000 or 10,000 eggs of Ma or Mi or combined Ma and Mi treatments of 1,250 + 1,250, 2,500 + 2,500, or 5,000 + 5,000 eggs per plant. Plants were randomized in six replicates and grown in the greenhouse for 7 weeks at 24–32 C. Numbers of galls and egg masses, plant weights, and identity of about 25 *Meloidogyne* females per plant were assessed as described above.

Ma and Mi on Mi-resistant soybean: The effect of first access to roots on developmental success of Ma and Mi was studied in two different experiments. One-week-old seedlings of Centennial, incompatible to Mi (6), were transplanted into soil in 15-cm-d plastic pots. Eggs were added to the soil in the following treatments: 0, 10,000 Ma or Mi, or combinations of 5,000 eggs each of Ma and Mi, which included Ma and Mi at the same time or Ma first and Mi 1, 2, or 3 weeks later. Plant-treatments were replicated six times in a randomized complete block design and grown in a greenhouse at 24–30 C for 9 weeks. Plants were harvested and handled as before and 25 *Meloidogyne* females were picked from each root system and identified as previously described. The experiment was repeated, except 2-week-old soybean seedlings were used and the greenhouse temperature was 20–26 C.

In a similar test, but in a growth chamber, 1-week-old Centennial soybean seedlings were transplanted as before. One week later the soil around each plant was infested, as described, with 0, 10,000 eggs of Ma or Mi or one of the following combinations: (a) 5,000 Ma + 5,000 Mi eggs simultaneously, (b) 5,000 Ma followed by 5,000 Mi 2 weeks later, or (c) 5,000 Mi followed by 5,000 Ma 2 weeks later. Treatments were replicated six times, and plants were grown in growth chambers (14-hour day and 2,600 lux) at 25 or 30 C for 9 weeks. Data were recorded as described.

Results

Ma and Mi on tolerant soybeans: Both Ma and Mi reproduced on Govan and Kirby soybean grown in the greenhouse. The average gall/egg mass rating for Ma on Kirby

TABLE 1. Effect of single and combined infestations of *Meloidogyne arenaria* (Ma) and *M. incognita* (Mi) eggs and juveniles on Kirby soybean at 25 C soil temperature.

Treatment	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)
Control	0 d	0 c	0	10.05 a	13.34 a
Ma 3,000	134.3 ab	183.8 a		5.38 c	11.96 ab
Ma 6,000	174.5 a	191.3 a		5.73 c	9.69 abc
Mi 3,000	12.3 c	17.0		9.31 a	14.23a
Mi 6,000	22.8 c	19.0 b		8.38 ab	11.69 abc
Ma 3,000 + Mi 3,000	73.5 bc	67.8 b	81:19	3.79 c	6.56 c
Ma 6,000 + Mi 6,000	175.8 a	182.0 b	84:16	6.38 bc	7.02 bc

Data are average of five replicates. Numbers followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.

* Identity of 20–30 females per root system based on perineal patterns, expressed as percentage of each species.

was 3.8/3.8 and for Mi 2.8/2.8. On Govan, the gall/egg mass index was 4.3/4.8 for Ma and 4.3/4.3 for Mi. The indices for combined Ma and Mi were similar to the separate treatments. Shoot and root dry weights were generally unaffected by either species in the first greenhouse experiment.

The two tolerant soybean varieties reacted differently to the two temperature regimes, however. Kirby grew equally well at both temperatures while Govan grew vigorously only at 25 C (Tables 1–4). Reproduction of Ma exceeded that of Mi. Reproduction of either nematode species increased at the higher temperature, except for the higher infestation level of Ma. However, root weight was greatly suppressed in this treatment. At 30 C, plant weight was suppressed by Ma, singly and combined with Mi. Temperature effects on

TABLE 2. Effect of single and combined infestations of *Meloidogyne arenaria* (Ma) and *M. incognita* (Mi) on Kirby soybean at 30 C soil temperature.

Treatment	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)
Control	0 e	0 e	0	7.42 ab	12.94 a
Ma 3,000	154.3 c	192.0 c		7.17 ab	12.59 a
Ma 6,000	253.3 b	253.3 b		6.81 b	9.36 ab
Mi 3,000	60.8 d	55.8 d		8.01 ab	14.67 a
Mi 6,000	110.8 cd	102.5 d		8.43 ab	14.65 a
Ma 3,000 + Mi 3,000	280.8 ab	263.8 b	62:38	10.90 a	14.58 a
Ma 6,000 + Mi 6,000	341.3 a	336.3 a	28:72	8.49 ab	6.16 b

Data are average of five replicates. Numbers followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.

* Identity of 20–30 females per root system based on perineal patterns, expressed as percentage of each species.

TABLE 3. Effect of single and combined infestations of *Meloidogyne arenaria* (Ma) and *M. incognita* (Mi) on Govan soybean at 25 C soil temperature.

Treatment	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)
Control	0	0	0	10.53 a	15.28 a
Ma 3,000	31.0 bc	26.8 bc		10.04 a	17.22 a
Ma 6,000	139.5 a	140.8 a		8.40 a	16.52 a
Mi 3,000	13.0 c	12.0 c		9.96 a	17.90 a
Mi 6,000	28.8 bc	27.5 bc		8.94 a	15.58 a
Ma 3,000 + Mi 3,000	61.5 b	59.0 b	57:43	8.34 a	15.14 a
Ma 6,000 + Mi 6,000	117.5 a	105.3 a	81:19	8.95 a	17.78 a

Data are average of five replicates. Numbers followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.

* Identity of 20–30 females per root system based on perineal patterns, expressed as percentage of each species.

TABLE 4. Effect of single and combined infestations of *Meloidogyne arenaria* (Ma) and *M. incognita* (Mi) on Govan soybean at 30 C soil temperature.

Treatment	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)
Control	0 d	0 e	0	7.58 a	10.76 a
Ma 3,000	66.0 b	70.8 bc		4.84 c	8.53 b
Ma 6,000	82.0 b	95.0 b		3.13 d	5.13 c
Mi 3,000	31.3 c	25.8 d		6.42 ab	10.00 a
Mi 6,000	67.0 b	59.5 c		5.57 bc	9.64 ab
Ma 3,000 + Mi 3,000	18.5 c	16.3 d	54:46	4.62 c	6.04 c
Ma 6,000 + Mi 6,000	152.5 a	130.0 a	66:34	3.05 d	4.63 c

Data are average of five replicates. Numbers followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.
* Identity of 20–30 females per root system based on perineal patterns, expressed as percentage of each species.

TABLE 5. Effect of different egg and juvenile inoculum levels (Pi) of *Meloidogyne arenaria* (Ma) and *M. incognita* (Mi), singly and combined, on number of root galls and egg masses and on plant weights of Kirby soybean.

Pi (eggs)	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)
Ma					
2,500	183.3 d	176.0 d		7.14 bc	8.46 bc
5,000	299.0 b	289.7 b		6.76 c	7.77 c
10,000	445.8 a	429.2 a		6.61 c	7.65 c
Mi					
2,500	7.3 g	6.2 g		8.48 ab	11.03 a
5,000	14.5 g	12.3 g		8.57 ab	9.78 ab
10,000	17.8 g	15.3 g		8.38 ab	9.84 ab
Ma + Mi					
1,250 + 1,250	84.2 f	78.0 f	81:19	7.18 bc	8.53 bc
2,500 + 2,500	146.0 e	139.0 e	80:20	6.78 c	8.21 c
5,000 + 5,000	216.8 c	210.8 c	90:10	6.80 c	7.76 c
Control	0	0		8.90 a	11.19 a

Data are average of five replicates. Numbers followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.
* Identity of 20–30 females per root system based on perineal patterns, expressed as percentage of each species.

TABLE 6. Effect of single and combined infestations (Pi) with *Meloidogyne arenaria* (Ma) and *M. incognita* (Mi) on numbers of nematode galls and egg masses and fresh root and shoot weights of Centennial soybean in the greenhouse.

Treatment	Pi (eggs)	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)
Control		0 d	0 d		5.33 a	8.49 a
Ma	10,000	626.2 a	590.2 a		4.33 a	4.59 c
Mi	10,000	71.5 c	44.7 c		5.23 a	6.51 abc
Ma + Mi	5,000 5,000	556.2 b	485.0 b	90:10	3.72 a	4.85 c
Ma 1st then Mi 1 wk later	5,000 5,000	491.0 b	450.5 b	78:22	4.10 a	5.99 bc
Ma 1st then Mi 2 wk later	5,000 5,000	494.2 b	463.8 b	84:16	4.31 a	7.20 ab
Ma 1st then Mi 3 wk later	5,000 5,000	521.8 b	459.3 b	92:8	4.38 a	7.70 ab

Data are average of six replicates. Numbers followed by the same letter in columns are not significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.
* Identity of 20–30 females per root system based on perineal patterns, expressed as a percentage of each species.

reproduction of Ma on Kirby were minimal, whereas Mi reproduction increased markedly at the higher temperature (Tables 1, 2).

Increasing infestation levels of all treatments with Ma, but not Mi alone, resulted in corresponding increases in egg masses and galls on Kirby (Table 5). Reproduction and galling by Mi were minimal. Combined infestations of Ma and Mi resulted in gall and egg mass numbers similar to, but slightly less than, the number corresponding to the treatments with similar levels of Ma by itself. All treatments with Ma, but none with Mi alone, resulted in suppressed plant growth.

Ma and Mi on Mi-incompatible soybean: Reproduction and galling by Mi on the Mi-resistant Centennial was approximately 10% of that of Ma (Table 6). A slightly greater percentage of Mi adult females were found in roots of the 1-week and 2-week sequential infestations, with Ma added first, than in those receiving both species simultaneously or Mi after 3 weeks. Plant shoot weight was suppressed most by Ma in single or simultaneous infection with Mi.

In controlled temperature tests, reproduction of Mi was 7.1% and 0.7% of that of Ma at 25 and 30 C, respectively (Table 7). Root galling followed similar trends. Reproduction of Mi was somewhat greater at 25 than at 30 C in contrast to the temperature effect observed with the tolerant varieties (Tables 1–4). In addition, growth of Centennial was much better at the higher temperature. There were fewer egg masses when Mi was added first and followed by Ma 2 weeks later than when Ma was added first.

Discussion

Centennial soybean was reported to be highly resistant to Mi but susceptible to Ma, which caused drastic yield suppressions (8). We confirm this report and add that the level of incompatibility to Mi increased somewhat at the higher temperature.

Our greenhouse tests demonstrate that numbers of galls and egg masses resulting from simultaneous infestations of the two species were similar to sequential infestations in which Ma preceded Mi by 1, 2, or

TABLE 7. Effects of single and combined infestations (Pi) of *Meloidogyne arenaria* (Ma) or *M. incognita* (Mi) on numbers of nematode galls and egg masses and fresh root and shoot weights of Centennial soybean at 25 and 30 C in a growth chamber.

		25 C					30 C				
Treatment	Pi (eggs)	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)	Galls	Egg masses	Ma:Mi*	Root fresh wt (g)	Shoot fresh wt (g)
Control		0 e	0 f		3.64 bc	7.06 a	0	0		15.44 b	14.51 a
Ma	10,000	533.5 a	522.0 a		2.67 d	4.35 b	697.5 a	642.3 a		11.73 c	5.99 d
Mi	10,000	74.0 d	37.2 e		3.24 cd	5.50 ab	7.2 e	4.3 e		14.99 b	13.51 ab
Ma + Mi	5,000 5,000	297.5 b	281.5 b	95:5	3.75 bc	5.68 ab	295.8 c	277.8 c	96:4	17.34 ab	9.91 c
Ma 1st + Mi 2 wk later	5,000 5,000	221.3 c	208.8 c	90:10	4.33 ab	6.20 ab	415.3 b	389.3 b	92:8	15.37 b	10.67 cb
Mi 1st + Ma 2 wk later	5,000 5,000	97.5 d	87.7 d	90:10	4.79 a	7.29 a	137.7 d	123.2 d	80:20	18.10 a	15.50 a

Data are average of six replicates. Numbers followed by the same letter in columns are not significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.
* Identity of 20–30 females per root system based on perineal patterns, expressed as a percentage of each species.

3 weeks. It was our hypothesis that Ma may alter the level of resistance, allowing reproduction of Mi, as was reported in tobacco (3). There is only very limited evidence to support this hypothesis; that is, the percentage of sampled females identified as Ma or Mi by the subjective perineal pattern analysis. Reproduction of combined populations should have been greater than that attributable to Ma alone for the hypothesis to be accepted.

Suppression of galling and egg mass production occurring when *M. incognita* was inoculated prior to *M. arenaria* may have been the result of the plant's defense against *M. incognita*. The phytoalexin produced in Centennial in response to *M. incognita* invasion was not active against *M. javanica* in single species infections (6), but if the chemical was already mobilized by *M. incognita* it possibly could be detrimental to *M. arenaria.*

Temperature is known to affect nematode reproduction, dating from the excellent work of Tyler in 1933 (11). Correlation of the root-knot disease on tobacco and soybean in 1982 with above-normal early season temperatures (4) prompted research on this subject. Our work has demonstrated that *M. arenaria* reproduces well at both temperature regimes but *M. incognita* does not. This response may give *M. arenaria* a selective early season advantage, which can be further exploited by its fecundity.

Reproduction of Ma is much greater than that of Mi on Kirby and Govan soybean. However, Mi reproduction increased 3–5-fold at the higher temperature on Kirby and 2-fold on Govan, which was not vigorous at the higher temperatures. Kirby looks especially promising for use where tolerance to several common *Meloidogyne* spp. is needed. In view of the fact that an increasing number of fields have both Ma and Mi, the apparent synergistic growth suppression of this cultivar when infested with mixed populations of these two nematode species suggests a need for further study. Kirby's generally excellent tolerance at higher temperatures under greenhouse conditions contrasts with the poor growth of Govan under these conditions and suggests a nematode tolerance–temperature interaction.

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