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INTERACTION BETWEEN *MELOIDOGYNE ARENARIA* AND *M. INCOGNITA* ON TOBACCO

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Extensive studies have been conducted on pathogenicity, population development and control of root-knot nematodes on tobacco (Arnes and Rich, 1981, Arnes *et al.* 1981; Barker, 1978; Barker *et al.*, 1981; Fortnum, 1984; Lopez *et al.*, 1977). Barker (1977) observed differences in pathogenicity of various *Meloidogyne* spp. on tobacco and demonstrated that *M. javanica* was the most damaging species, followed in decreasing order by *M. arenaria, M. incognita* and *M. hapla*. Arnes *et al.* (1981) indicated that differences in aggressiveness and population development of *M. javanica, M. arenaria* and *M. incognita* on tobacco may be primarily caused by differences in the rates of root invasion and the sizes of galls induced by these nematodes. On the other hand, studies comparing the effects of two or more root-knot nematode species on tobacco have received less attention. Eisenback (1983) studied the interactions among *Meloidogyne* species on tobacco cv. NC95, resistant to *M. incognita* race 1, utilizing the split-root technique. He reported that plants infected with *M. arenaria* or *M. hapla* lost their resistance to *M. incognita* race 1, but plants infected with *M. javanica* or *M. incognita* race 4 remained resistant to *M. incognita* race 1.

The objective of the present work was to study the interactions between *M. arenaria* (Neal) Chitw. race 2 and *M. incognita* (Kofoid *et* White) Chitw. race 1 on tobacco (*Nicotiana tabacum* L.) cv. NC95.

Materials and Methods

Meloidogyne arenaria race 2 (Ma) and *M. incognita* race 1 (Mi) used in this study were identified by a host plant differential test (Taylor and Sasser, 1978) and propagated on tomato (*Lycopersicon esculentum* Mill) cv. Rutgers for 60 days in the greenhouse. Nematode eggs for inocula were extracted from tomato roots by the NaOCl method (Hussey and Barker, 1973).

In the first test, the effect of inoculum densities of Ma on the interaction of Ma and Mi was studied. Sixty-day-old tobacco NC95 seedlings were transplanted into 15 cm diam plastic pots containing a steam sterilized mixture of 1:1 sand and sand loam soil. Three days after transplanting, the soil of the treated pots was infested with nematode eggs suspended in 10 ml water. Nematode treatments included inoculation with 0, 2,000, 4,000 or 6,000 eggs of Ma, singly and combined with 20,000 eggs of Mi. Noninoculated plants served as controls. Treatments were replicated five times. Plants were placed in a randomized complete block design and grown in a greenhouse at 20-26°C.

The experiment was terminated 9 weeks after nematode inoculation. Roots of harvested plants were carefully washed free of soil and stained with phloxine B. Root and shoot fresh weights of tobacco plants and number of galls and egg masses were determined. Plant roots from combined Ma-Mi treatments were stained with hot acid fuchsin-lactoglycerol (0.05% acid fuchsin in a solution of 1:1:1, v:v:v, lactic acid, glycerol and distilled water) and cleared in 50% glycerol solution. About 25 mature females were removed from each root system for species identification by perineal patterns.

In a second test the effect of the time of nematode inoculation on the interaction of Ma and Mi was determined. Two-month-old tobacco seedlings were transplanted into steam sterilized 1:1 sand and sand loam soil in 15 cm diam plastic pots. Three days after transplanting, plants were inoculated with 1) 10,000 Ma or Mi eggs; 2) 5,000 Ma eggs plus 5,000 Mi eggs, or 3) 5,000 Ma eggs first and 5,000 Mi eggs 1 or 2 weeks later. Noninoculated plants served as control. Plants were placed in a randomized complete block design in six replicates and grown in a greenhouse. The experiment was terminated 9 weeks after the first nematode inoculation. Plants were harvested and roots were processed as described earlier.

Results and Discussion

Data of the first test showed that tobacco cv. NC 95 was susceptible to Ma alone and resistant to Mi alone (Table I). Galling and egg mass production of Ma significantly increased as initial infestation level (Pi) increased from 2,000 to 6,000 eggs. Mi alone induced no galls or egg masses. Treatments of combined Ma (Pi 4,000 or 6,000 eggs) and Mi (Pi 20,000 eggs) showed significant interaction of both species as numbers of galls and egg masses were significantly lower than treatments with Ma alone. On the other hand, galls and egg masses of Ma at lower inoculum density (Pi 2,000 eggs) did not significantly differ from the treatment of combined Ma+Mi (Pi 2,000+20,000 eggs).

Combined inoculations with Ma+Mi resulted in infection of tobacco roots by Mi. The proportion of Mi females recovered from infected roots was 15 to 28%. The greatest percentage of Mi females (28%) occurred in combined inoculation with Mi (Pi 20,000 eggs) and Ma at Pi 4,000 eggs, followed by 22% with Ma at Pi 2,000 eggs and 15% with Ma at Pi 6,000 eggs.

In general, root and shoot fresh weights of tobacco plants were not affected by the various nematode treatments.

Results of the second test indicated that no galls or egg masses were observed on plants inoculated only with Mi (Pi 10,000 eggs) (Table II). Inoculation with Ma alone or Ma combined with Mi produced large numbers of galls and egg masses. Treatment with Ma alone (Pi 10,000 eggs) gave significantly more galls and egg masses than combined Ma+Mi treatments. Numbers of galls and egg masses with inoculation of Ma+Mi simultaneously did not significantly differ ($P=0.05$) from sequential inoculation of Ma followed by Mi 1 or 2 weeks later.

Females of Mi were recovered from tobacco roots inoculated with combinations of Ma+Mi. The greatest percentage of Mi females (33%) occurred when both Ma and Mi were inoculated simultaneously whereas only 12% were recovered when Mi was inoculated 1 or 2 weeks after Ma.

Root and shoot fresh weights were suppressed by inoculation with Ma either alone or in combination with Mi, whereas treatment with Mi alone had no effect.

The present study confirms that Ma either alone or in combination with Mi parasitizes and reproduces on tobacco NC95. Tobacco plants inoculated with both species together lost their resistance to *M. incognita* race 1. Numbers of Mi females recovered from infected roots varied with the Pi of Ma and the time of inoculation of Mi. Maximum numbers of Mi females were recovered when both nematode species were inoculated simultaneously, whereas the least were recovered when Mi was inoculated 1 or 2 weeks after Ma.

Table I - *Effect of different inoculum levels (Pi) of* Meloidogyne arenaria *(Ma) and* M. incognita *(Mi), alone and combined, on numbers of root-knot nematode galls and egg masses, and fresh root and shoot weights of tobacco NC 95.*

Treatment	Pi eggs	No. galls	Egg masses	% Females Ma:Mi	Root wt (g)	Shoot wt (g)
Ma	2,000	14.6d	13.6d	—	31.48a	59.56a
Ma	4,000	46.6b	44.0b	—	29.57ab	59.91a
Ma	6,000	58.8a	56.6a	—	26.76ab	49.56b
Ma+ Mi	2,000 20,000	13.4d	12.4d	78:22	29.14ab	58.47ab
Ma+ Mi	4,000 20,000	20.2c	18.8c	72:28	28.79ab	59.82a
Ma+ Mi	6,000 20,000	45.0b	42.4b	85:15	24.38b	49.43b
Mi	20,000	0 e	0 e	—	29.66a	55.33ab
Noninoculated	0	0 e	0 e	—	29.56ab	54.61ab

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

Table II - *Effect of the time of inoculation of* Meloidogyne arenaria *(Ma) and* M. incognita *(Mi) on numbers of galls and egg masses, and root and shoot fresh weights of tobacco NC 95.*

Treatment	Galls	Egg masses	% Females Ma:Mi	Root wt (g)	Shoot wt (g)
Ma	143.2a	132.5a	—	13.0b	19.8b
Mi	0 c	0 c	—	19.8a	26.1a
Ma+ Mi	75.3b	63.0b	67:33	13.9b	19.2b
Ma+ Mi 1 week later	55.7b	38.0b	88:12	13.6b	19.9b
Ma+ Mi 2 weeks later	71.0b	55.8b	88:12	13.4b	16.1b
Noninoculated	0 c	0 c	—	21.9a	27.9a

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

The results obtained provide evidence that Ma infection predisposes NC95 tobacco plants to *M. incognita* race 1 and confirm previous findings of Eisenback (1983) and Tedford *et al.* (1986).

SUMMARY

Meloidogyne arenaria race 2 at infestation levels of 6,000 and 10,000 eggs per plant produced great numbers of galls and egg masses on NC95 tobacco roots in the greenhouse, whereas *M. incognita* race 1 at infestation levels up to 20,000 eggs per plant induced no galls or egg masses on NC95 tobacco roots. Females of *M. incognita* were observed in tobacco roots inoculated with a mixture of *M. arenaria* plus *M. incognita*. The maximum number of *M. incognita* females occurred when both nematode species were inoculated simultaneously while fewer were noted when *M. arenaria* was inoculated first followed by *M. incognita* 1 or 2 weeks later.

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