

# Relationship between *Meloidogyne incognita* and *Rotylenchulus reniformis* as Influenced by Soybean Genotype<sup>1</sup>

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**Abstract:** The effect of soybean genotype on competition between *Meloidogyne incognita* race 2 (Mi) and *Rotylenchulus reniformis* (Rr) was evaluated in greenhouse and microplot replacement series experiments. Soil in pots containing seedlings of 'Davis' (susceptible to Mi) or 'Buckshot 66' (resistant to Mi) was infested with 1,000 vermiform individuals in the following Mi:Rr ratios: 0:0, 100:0, 75:25, 50:50, 25:75, or 0:100. After 91 days, the relative nematode yields (number of nematodes in mixed culture divided by the number in nonmixed culture) of each species were calculated based on soil and root nematode populations expressed as nematodes per gram of dry root tissue. To define the relationship between the two species, calculated relative nematode yields were compared with a theoretical noncompetition model using lack-of-fit regression. In the greenhouse, Mi populations on 'Davis' were stimulated in the presence of Rr. In microplots, low Mi and Rr population densities likely resulted from severe galling and destruction of feeder roots that probably occurred early in the season. Enhanced susceptibility to Mi was not observed on 'Buckshot 66', which remained resistant to Mi even when colonized by Rr. Host resistance is a key factor in determining the nature of the relationship between Mi and Rr.

**Key words:** competition, concomitant populations, *Glycine max*, *Meloidogyne incognita*, nematode, reniform nematode, replacement series, root-knot nematode, *Rotylenchulus reniformis*, soybean.

Root-knot (*Meloidogyne incognita* (Kofoid & White) Chitwood) and reniform (*Rotylenchulus reniformis* Linford & Oliviera) nematodes are pathogenic to soybean (*Glycine max* (L.) Merr.) (Sinclair and Backman, 1989). These species share the same geographic and host ranges in Louisiana, where nematode damage has reduced soybean yield 4% to 8% annually during 1988-1993 (Sciumbato, 1993; Wrather and Sciumbato, 1995).

The replacement series approach, originally proposed by plant ecologists (De Wit, 1960; De Wit et al., 1966) for use in competition studies, has been used successfully in several subdisciplines of plant pathology (Adee et al., 1990; Janisiewicz, 1996; Wilson and Lindow, 1994a, 1994b; Zitko and Timmer, 1994). This approach recently has been adapted for use in competition studies involving phytoparasitic nematodes (Erwin et al., 1995; Stetina et al., 1997b). Replace-

ment series experiments are designed to quantitatively assess the relative impact of inter- and intraspecific competition between two species at a single community density. Target species are introduced alone or together in various ratios. At the end of the experiment, relative nematode yields (number of each species in mixed culture divided by number in nonmixed culture) are calculated for each species. Inhibition or stimulation of a species can be visualized by plotting the relative nematode yields against the input proportion of that species (Stetina et al., 1997b). If inter- and intraspecific competition are equal, final nematode population sizes for each species should be directly proportional to the percentage of that species initially introduced.

In replacement series experiments, Erwin et al. (1995) and Stetina et al. (1997b) showed increased *M. incognita* reproduction in the presence of *R. reniformis*. This was evidenced by relative yields for *M. incognita* populations in soil that were significantly higher than predicted at all ratios at which this species occurred together with *R. reniformis*. Relative nematode yields for *R. reniformis* populations in soil did not differ from predicted yields, which indicated no effect of *M. incognita* on reproduction by *R. reniformis*. These experiments, however, were

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limited to the soybean cultivar Davis, which is susceptible to *M. incognita*. The objective of this research was to determine if the relationship between *M. incognita* and *R. reniformis* documented on a susceptible soybean cultivar was similar to that found on a soybean cultivar resistant to *M. incognita*.

#### MATERIALS AND METHODS

*General procedures:* Two experiments were conducted in a greenhouse where temperatures ranged from 22 to 35 °C. Supplemental incandescent and fluorescent lighting (ca. 260  $\mu\text{E} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ ) provided a minimum of 14 hours of continuous light daily. These studies utilized 15-cm-diam. clay pots that contained approximately 1.6 kg of a soil mixture composed of three parts fumigated (67% methyl bromide, 33% chloropicrin) Convent silt loam soil (Aeric Fluvaquent, coarse-silty, mixed, nonacid, thermic) and two parts autoclaved sand.

Two experiments were conducted in microplots. Each microplot consisted of a 30-cm-diam. clay pot that contained approximately 15 kg of fumigated (32.7% sodium methylthiocarbamate, 67.3% inert ingredients; 18.8 ml fumigant in 882 ml water per pot) Mhoon silt loam soil (Typic Fluvaquent, fine-silty, mixed, nonacid, thermic). Microplots were set into the ground to the depth of the pot rim and spaced 1 m apart. The entire microplot area was sheltered by a polyethylene-covered quonset hut frame, open at both ends, and covered with black shade cloth. Plants in microplots received 516  $\mu\text{E} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$  of light (approximately 30% of full sunlight). Supplemental lighting was not used in the microplot area.

Seeds of Davis (susceptible to both nematode species) or Buckshot 66 (resistant to *M. incognita*, susceptible to *R. reniformis*) soybean were treated with a commercial preparation of *Bradyrhizobium japonicum* (Kirchner) Jordan (Nitragin; Nitragin, Milwaukee, WI) and sown in flats. Seedlings of uniform size were selected when plants were at growth stage VI (Fehr et al., 1971), and a single seedling was transplanted to the center of each test pot for greenhouse tests or to

a 10-cm-square multi-pot (Hummert International, Earth City, MO) for microplot tests. Plants were fertilized with 120 ml of a 23-19-17 N-P-K fertilizer solution (RapidGro; Chevron, San Ramon, CA) 3 days after transplanting. Plants received approximately 26 ppm N, 20 ppm P, and 33 ppm K.

Populations of *M. incognita* race 2 and *R. reniformis* were derived from single egg masses and maintained on tomato (*Lycopersicon esculentum* L. 'Rutgers') in a greenhouse. Inoculum consisted of vermiform nematodes obtained from soil by wet-sieving (Cobb, 1918) and centrifugal-flotation (Jenkins, 1964). Soil in each pot was infested with the required number of each species by pipetting nematodes suspended in tap water into two depressions made in the soil. Tap water was pipetted into depressions in control pots. Each depression was 1 cm in diam., 4 cm deep, and 5 cm from the base of the stem on opposite sides of the plant. After infestation, the depressions were filled with additional fumigated soil.

In greenhouse tests, pots remained undisturbed until harvest. In microplot tests, the plant and soil from the multi-pot were transferred 10 days after infestation into a depression of comparable size made in the microplot soil. Pots then remained undisturbed until harvest.

At the end of each experiment, five soil cores (2.5-cm-diam.) from the soil surface to the bottom of the pot were collected from each pot, mixed thoroughly, and subsampled (150 g). Nematodes were extracted with wet-sieving and centrifugal-flotation. Numbers of juveniles, males, vermiform females, and swollen females collected on a 38- $\mu\text{m}$ -pore sieve were recorded for each species.

Plant stems were cut at the soil surface, and the root-soil mass was removed from each pot. Root systems were freed from soil by washing gently in tap water. Severity of galling caused by *M. incognita* was rated according to the following scale: 0 = no galls, 1 = galls < 3 mm in diam. with no reduction in the number of feeder roots, 2 = galls 3 to 10 mm in diam. with no reduction in the number of feeder roots, 3 = galls 11 to 20 mm in

diam. with no or slight reduction in the number of feeder roots, 4 = galls > 20 mm diam. with moderate reduction in the number of feeder roots, and 5 = galls > 20 mm diam. with major reduction in the number of feeder roots. Incidence of galling caused by *M. incognita* was rated according to the following scale: 0 = no galls, 1 = galls confined to 25% or less of the root system, 2 = galls appearing on 26% to 50% of the root system, 3 = galls appearing on 51% to 75% of the root system, and 4 = galls appearing on 76% or more of the root system.

Nematodes were extracted from a subsample (2 g) removed at random from each root system. Root tissue was combined with 60 ml of 0.5% NaOCl and ground for 10 seconds at maximum speed in a blender fitted with a 500-ml stainless steel container (Stetina et al., 1997a). The slurry was poured onto nested 75- and 25- $\mu$ m-pore sieves, and vermiform and swollen individuals of each nematode species were counted. Eggs collected on the 25- $\mu$ m-pore sieve could not be identified to species, so egg counts were not included in population totals.

*Replacement series experiments:* The relationship between *M. incognita* and *R. reniformis* was examined on Davis and Buckshot 66 soybean. Experiments on each cultivar were conducted twice, i.e., once in the greenhouse and once in microplots. Numerous test-by-treatment interactions were detected in the initial analyses, so each test was analyzed independently. All four tests were established in randomized complete block designs with five (microplot) or 10 (greenhouse) replications. *Meloidogyne incognita* and *R. reniformis* were introduced alone or in combination at an initial community density of 1,000 individuals/pot when plants reached growth stages V2 to V3. Soil was infested with nematodes at one of the following *M. incognita*:*R. reniformis* ratios: 0:0, 100:0, 75:25, 50:50, 25:75, or 0:100. Experiments were terminated 91 to 93 days after nematodes were introduced, when soybeans were at growth stages R4 or R5 in greenhouse tests, or R6 in microplot tests. At harvest, plants were divided into root and shoot por-

tions by cutting the stem at the soil line. Soybean roots and shoots were dried at 70 °C for 4 days, and weighed after galling assessment and collection of tissue samples for nematode extraction. Soil samples were processed and nematodes were counted. Relative nematode yields were based on the total number of nematodes of each species extracted from soil and roots, expressed per gram of dry root tissue. For these experiments, relative nematode yield was calculated by dividing the number of nematodes of a species extracted from mixed culture by the number of nematodes of the same species recovered from unmixed culture (Stetina et al., 1997b).

*Data presentation and analyses:* To examine the relationship between *M. incognita* and *R. reniformis*, differences between the predicted relative nematode yield lines (representing equal interspecific and intraspecific competition) as defined by the replacement series (De Wit, 1960; De Wit et al., 1966), and the relative nematode yield lines plotted using calculated relative nematode yield values, were determined by lack-of-fit regression with the "Fit Model" module of SAS JMP version 3.0 (SAS Institute, Cary, NC). Paired *t*-tests using the "Fit Y by X" module of SAS JMP version 3.0 were used to determine at which ratio(s) the predicted and calculated relative nematode yield values differed. Plant weights were subjected to analysis of variance, Fisher's protected LSD, and orthogonal polynomial contrasts with the "Fit Model" and "Fit Y by X" modules of SAS JMP version 3.0. Galling indices for plants inoculated with *M. incognita* were examined by orthogonal polynomial contrasts with the "Fit Model" module of SAS JMP version 3.0.

## RESULTS

*Meloidogyne incognita* and *R. reniformis*, separately or concomitantly, did not impact shoot or root weight of Davis or Buckshot 66 soybean in greenhouse tests (Fig. 1A-D). In microplot tests, 'Davis' shoot weights were lowest on plants inoculated with high levels (100:0, 75:25) of *M. incognita* (Fig. 1A).

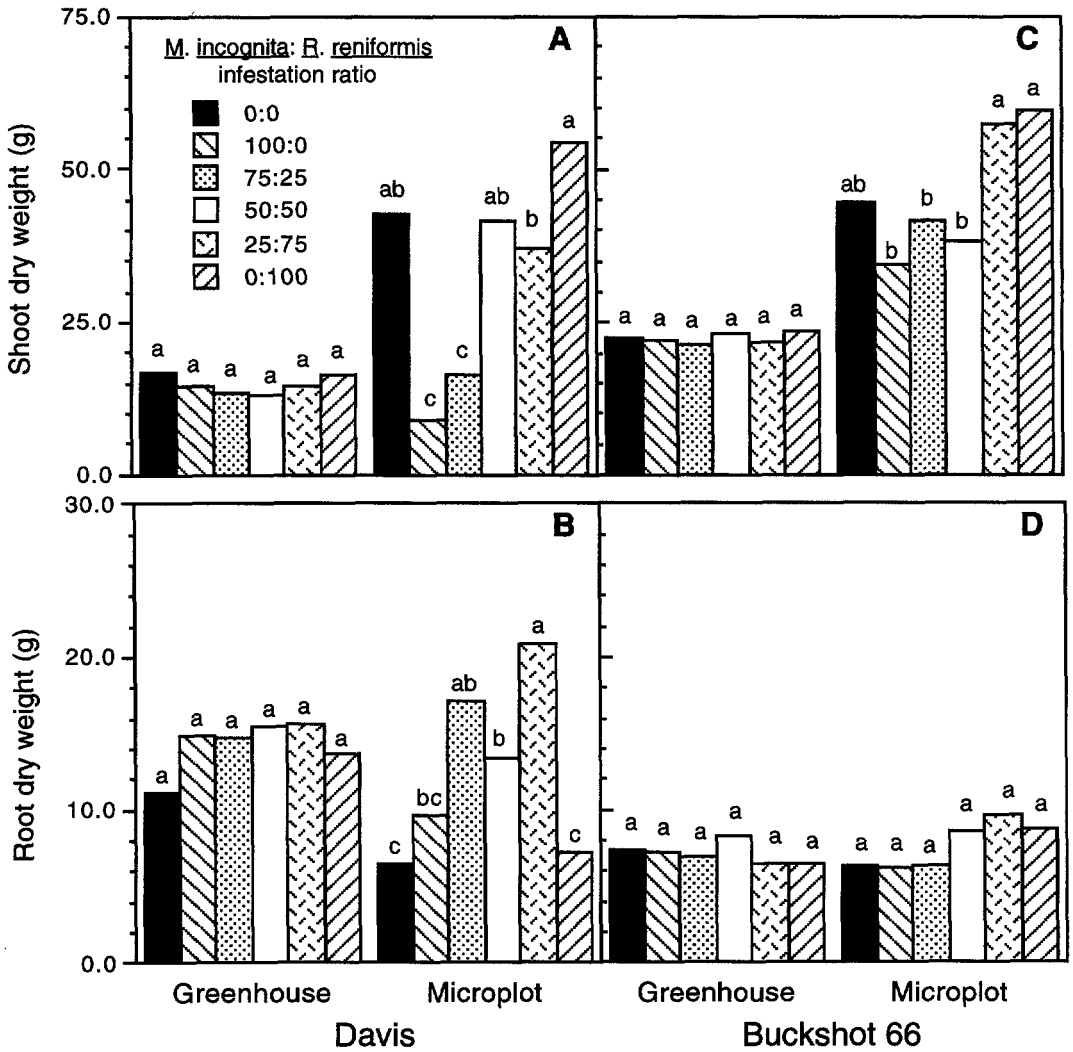


FIG. 1. Effect of *Meloidogyne incognita* race 2 and *Rotylenchulus reniformis* on dry weight of the soybean cultivars Davis (susceptible to both species) and Buckshot 66 (resistant to *M. incognita*, susceptible to *R. reniformis*) in greenhouse (10 replications) and microplot (5 replications) tests. Within each parameter, cultivar, and location, means with the same letter do not differ (Fisher's protected LSD,  $P \leq 0.05$ ). A) Shoot dry weight on 'Davis'. B) Root dry weight on 'Davis'. C) Shoot dry weight on 'Buckshot 66'. D) Root dry weight on 'Buckshot 66'.

Shoot weights increased in a linear fashion ( $t = 4.35, P > |t| = 0.0008$ ) as the proportion of *M. incognita* in the inoculum decreased. Plants inoculated with mixtures of *M. incognita* and *R. reniformis* had heavier roots than the uninoculated control (Fig. 1B) in microplot tests. A quadratic relationship ( $t = -3.06, P > |t| = 0.0090$ ) was detected among all inoculated treatments, with heavier root systems on plants infected by mixtures of nematodes. Where *M. incognita* was included

in the inoculum, root weight increased in a linear manner ( $t = -2.47, P > |t| = 0.0357$ ) as the level of *M. incognita* decreased. In the microplot test, 'Buckshot 66' plants inoculated with low levels (25:75, 0:100) of *M. incognita* had heavier shoots than plants inoculated with moderate to high levels of *M. incognita*, though weights in both groups did not differ from uninoculated controls (Fig. 1C). Root dry weights were not influenced by the nematodes at any ratio on 'Buckshot

66' in the microplot test (Fig. 1D). Orthogonal polynomial contrasts did not reveal any trends in shoot or root weight related to nematode infestation on 'Buckshot 66'.

Incidence and severity of galling were generally greater on 'Davis' than on 'Buckshot 66' in both greenhouse and microplot

tests (Fig. 2A-D). Galling was so severe on 'Davis' that feeder roots were almost completely absent in the microplot test. On 'Davis', orthogonal polynomial contrasts revealed a cubic relationship between the proportion of *M. incognita* in the inoculum and gall incidence ( $t = -2.33, P > |t| = 0.0378$ )

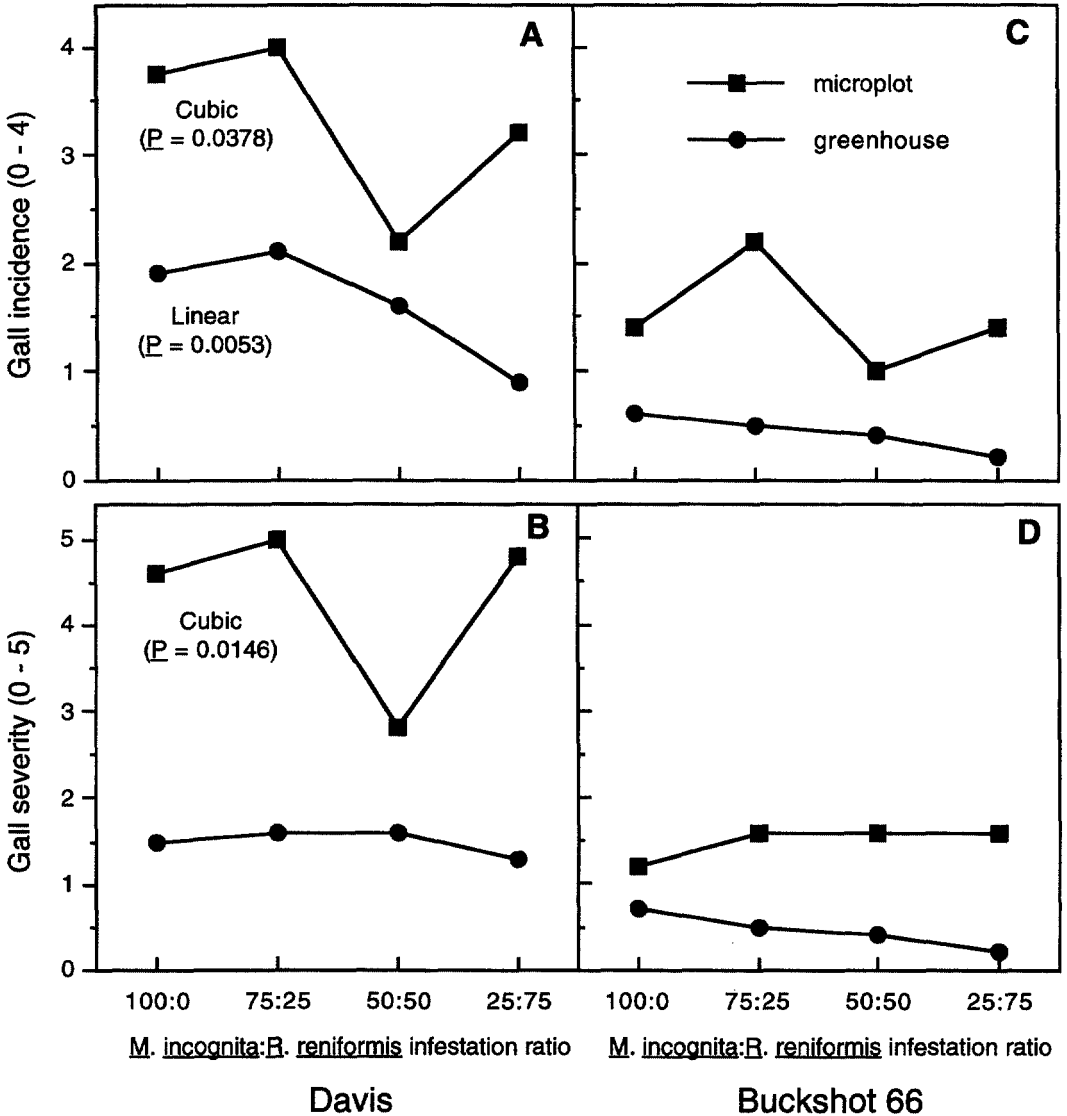


FIG. 2. Relationships between proportion of *Meloidogyne incognita* race 2 in the inoculum and severity and incidence of galling on the soybean cultivars Davis (susceptible to *M. incognita*) and Buckshot 66 (resistant to *M. incognita*) 91 to 93 days after infestation with nematodes. Incidence is rated on a 0-to-4 scale where 0 = no galls and 4 = galls appearing on 76% or more of the root system. Severity is rated on a 0-to-5 scale where 0 = no galls and 5 = galls >20 mm in diam. with major reduction in the number of feeder roots. The nature of the relationship and  $P > |t|$  are noted where significant. A) Incidence on 'Davis'. B) Severity on 'Davis'. C) Incidence on 'Buckshot 66'. D) Severity on 'Buckshot 66'.

and severity ( $t = -2.85, P > |t| = 0.0146$ ) in the microplot test (Fig. 2A,B). For both indices, minimum values were associated with the 50:50 ratio. In the greenhouse test, gall incidence decreased linearly in proportion to lower levels of *M. incognita* in the inoculum ( $t = 3.03, P > |t| = 0.0053$ ) (Fig. 2A). No relationship between nematode ratio and severity of galling was detected on 'Davis' in the greenhouse (Fig. 2B). On 'Buckshot 66', no relationships between nematode ratio and either incidence or severity of galling

were detected in greenhouse or microplot tests (Fig. 2C,D).

On 'Davis', relative *M. incognita* yields in the greenhouse test were significantly higher than predicted ( $F = 4.26, P = 0.0099$ ), notably at the 50:50 ratio. Relative nematode yields of *R. reniformis* were not influenced by infection of the same host by *M. incognita* ( $F = 0.60, P = 0.6171$ ) (Fig. 3A). In the microplot test, both *M. incognita* and *R. reniformis* relative nematode yields were lower than predicted (for *M. incognita*,  $F =$

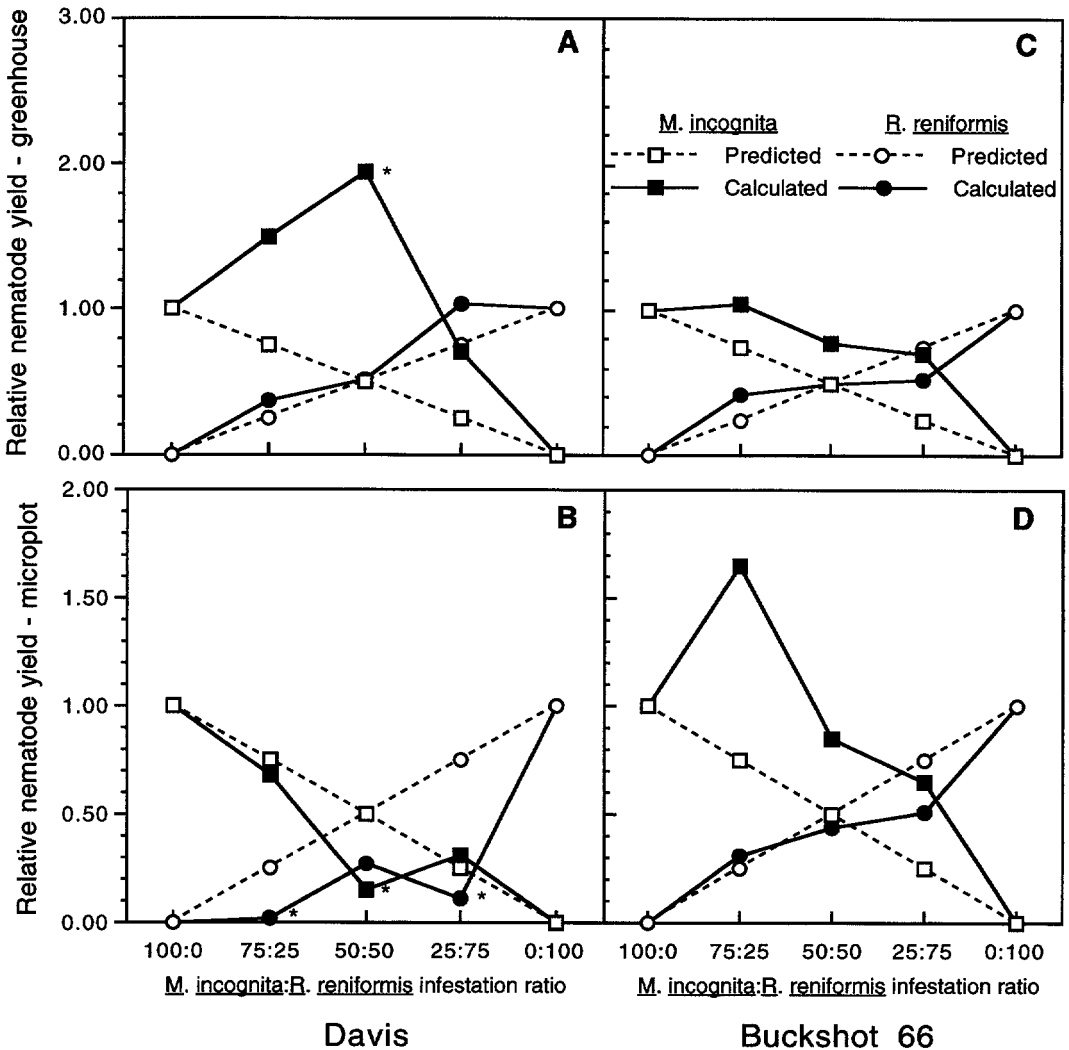


FIG. 3. Relative nematode yield of *Meloidogyne incognita* race 2 and *Rotylenchulus reniformis* 91 to 93 days after infestation of the soybean cultivars Davis and Buckshot 66 in greenhouse (10 replications) and microplot (5 replications) tests. Calculated values that differ significantly ( $P \leq 0.05$ ) from predicted relative nematode yields are indicated with an asterisk to the right of the calculated mean. A) 'Davis', greenhouse. B) 'Davis', microplot. C) 'Buckshot 66', greenhouse. D) 'Buckshot 66', microplot.

3.28,  $P = 0.0422$ ; for *R. reniformis*,  $F = 12.80$ ,  $P \leq 0.0001$ ) (Fig. 3B). Significant reductions in relative nematode yields occurred at the 50:50 ratio for *M. incognita* and at the 75:25 and 25:75 ratios for *R. reniformis* (Fig. 3B). On 'Buckshot 66', no significant differences were detected between calculated and predicted relative nematode yields for either species in greenhouse or microplot tests (Fig. 3C,D).

#### DISCUSSION

The genotype of the soybean host influenced the relationship between *M. incognita* and *R. reniformis*. In the greenhouse test, the susceptibility of 'Davis' to *M. incognita* increased when *R. reniformis* infected the same host, as evidenced by higher relative nematode yields of *M. incognita*. In the microplot test, enhanced *M. incognita* reproduction probably began early in the season, resulting in severe galling, destruction of feeder roots, and dramatic population declines for both species, reflected in the relative nematode yields observed 91 to 93 days after infestation. The reduction in shoot weight seen in the microplot test also may be attributable to the significant damage caused by the large *M. incognita* population. The microplot infestation method may have concentrated high numbers of nematodes in a small area, thereby increasing the potential of individual nematodes to locate and infect roots at the beginning of the experiment. In addition, the microplot environment was less subject to temperature and moisture fluctuations, which can impact nematode population development, than was the greenhouse environment. A combination of these factors may have contributed to the apparent increase in susceptibility to *M. incognita* on 'Davis' in the microplot test. In spite of these factors, enhanced susceptibility to *M. incognita* was not observed on 'Buckshot 66', which remained resistant to this species even when colonized by *R. reniformis*. Host resistance, therefore, is a key factor in determining the nature of the relationship between *M. incognita* and *R. reniformis*.

The relationship between *M. incognita* and *R. reniformis* was defined based on vermiform and swollen individuals because these life stages could be readily classified as one species or the other. Eggs, however, were not identifiable to species; therefore, egg counts were not included in population totals. Preliminary hatch, morphology, and differential staining studies were conducted in an attempt to identify eggs to species, but we were not able to identify a reliable method by which the entire egg cohort could be classified. In our experience, root-associated populations of *M. incognita* are generally larger than root-associated populations of *R. reniformis* (Stetina et al., 1997a). This inequality would be exaggerated on a cultivar susceptible to *M. incognita*, when *R. reniformis* and *M. incognita* occur in the same community.

The ability of one nematode population to influence the reproduction of a second nematode population is a key factor affecting interspecific competition (Eisenback, 1993). The results of the current study support those of Erwin et al. (1995) and Stetina et al. (1997b), who first reported that infection of Davis soybean by *R. reniformis* consistently increased *M. incognita* reproduction. The stimulatory effect of *R. reniformis* on *M. incognita* is not an isolated example of enhanced reproduction by nematodes in coexistence. Increased reproduction of *Belonolaimus longicaudatus* in the presence of *Hoplolaimus galeatus* on cotton (Yang et al., 1976), *Hoplolaimus columbus* in the presence of *M. incognita* or *Scutellonema brachyurum* on cotton (Kraus-Schmidt and Lewis, 1981), *Criconemella xenoplax* in the presence of *Meloidogyne hapla* on grape (Santo and Bolander, 1977), *Pratylenchus brachyurus* in the presence of *M. incognita* on tobacco cv. NC 2512 (Johnson and Nusbaum, 1970), and *Paratrichodorus minor* in the presence of *P. brachyurus* on soybean (Johnson and Nusbaum, 1968) have been documented. Mutual stimulation of *P. minor* and *Pratylenchus zeae* on corn (Johnson and Nusbaum, 1968) and *H. columbus* and *S. brachyurum* on cotton (Kraus-Schmidt and Lewis, 1981) also have been reported. Further studies are required

to elucidate the mechanism behind the stimulatory effect of *R. reniformis* on *M. incognita* on 'Davis' soybean in this system.

It is widely believed that *Rotylenchulus* is replacing *Meloidogyne* throughout the soybean production region in the southern United States. Our current findings that show an increase in the *M. incognita* population in the presence of *R. reniformis* on susceptible 'Davis' seem to contradict this. However, an explanation may be found in examining results from the resistant cultivar Buckshot 66, which did not support enhanced reproduction by *M. incognita* even when coinfecting by *R. reniformis*. Soybean cultivars resistant to *Meloidogyne* spp. are employed commonly in nematode management programs. Because *R. reniformis* has a longer infective period (Robbins et al., 1994; Sivakumar and Seshadri, 1976) and a shorter life cycle than *M. incognita*, it has greater potential to reach a damaging population level on cultivars resistant to *M. incognita*. In addition, commercial soybean cultivars resistant to *R. reniformis* are lacking. Consequently, planting *M. incognita*-resistant soybean likely favors *R. reniformis* over time.

The influence of the host is evident in other nematode-host-nematode systems as well. Inoculation with *M. incognita* inhibited subsequent penetration of tomato roots by *P. brachyurus* but stimulated penetration of cotton roots by the latter species (Gay and Bird, 1973). Johnson and Nusbaum (1970) documented inhibitory, neutral, and stimulatory associations among *M. incognita*, *P. brachyurus*, and *M. hapla* on tobacco, which differed in both nature and magnitude depending on the host cultivar. The associations were species-specific, as *M. incognita* did not have the same impact on *P. brachyurus* as did another root-knot nematode species, *M. hapla*. In split-root experiments, Eisenback (1983) reported that inoculation of root-knot nematode-resistant tobacco with *Meloidogyne arenaria* or *M. hapla* masked the resistance of that cultivar to *M. incognita* race 1 when this species was subsequently introduced. Griffin (1980) found that infection by *Ditylenchus dipsaci* reduced the resistance

of the alfalfa cultivar Vernal 298 to *M. hapla*. McGawley and Winchell (1987) reported that galling of soybean induced by a combination of *M. incognita* and *M. javanica* was significantly greater than when either species was tested independently.

Relationships defined on one host may be quite different on other cultivars or host species. However, host suitability is not the only factor capable of influencing the ecological association among nematode species. Edaphic factors such as soil texture, soil moisture, and temperature, nematode population densities, timing and method of nematode inoculation, pesticide application, and the influence of other biological entities within the system may alter nematode relationships. To fully document the interrelationships between two nematode species, the species should be evaluated under a range of biotic and abiotic conditions.

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