

Interrelations between *Meloidogyne javanica*, *Rotylenchulus reniformis*, and *Rhizobium* Sp. on *Vigna sinensis*

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Abstract: The interactions of *Meloidogyne javanica*, *Rotylenchulus reniformis*, and *Rhizobium* sp. on cowpea seedlings were investigated. Upon simultaneous inoculation with the two nematode species, *M. javanica* invaded first but did not affect root invasion by *R. reniformis*. *M. javanica* populations increased less in competition with *R. reniformis* than when present alone. Preinvasion by *R. reniformis* significantly suppressed the number of *M. javanica* in the roots. Inoculation of *M. javanica* and/or *R. reniformis* with rhizobia did not affect nodulation. Nodule formation was hindered only when *R. reniformis* infection preceded rhizobial inoculation. Nitrogen nodules were formed on *M. javanica* galls. Nodules and *M. javanica* galls served as infection sites for both nematodes. Although *R. reniformis* on the roots reduced the space for *M. javanica* infection, *M. javanica* is more competitive than *R. reniformis* and ultimately predominates as a result of its higher reproductive potential and shorter time spent in the soil before infection. **Key Words:** root-knot nematode, reniform nematode, legume, cowpea, bacterial nodulation.

Cowpea (*Vigna sinensis* Endl.) is a good host for both the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood (7) and the reniform nematode *Rotylenchulus reniformis* Linford & Oliveira (14).

It is probable that those two nematodes and the root-nodule bacterium (*Rhizobium* sp.) are forced to compete in the rhizosphere region of cowpea, with unknown results. Kheir and Osman (11) found that root penetration by *Meloidogyne incognita* (Kofoid & White) Chitwood in tomato roots and its development and growth rate were hampered by *R. reniformis*.

Another study (20) on intergeneric

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competition between sedentary nematodes on soybean revealed that *M. incognita* was suppressed by *Heterodera glycines* Ichinohe. *H. glycines* was first suppressed, but eventually stimulated, by *M. incognita*. Jatala and Jensen (10) found that preinvasion by *Meloidogyne hapla* Chitwood significantly increased the rate of development of *Heterodera schachtii* Schmidt on sugar beet, whereas preinvasion by *H. schachtii* significantly reduced the size of *M. hapla* galls. They also reported that when both nematodes were added simultaneously, neither population was affected thereby.

Competition between *H. glycines* or *M. hapla* larvae and root-nodule bacteria was postulated as a cause of reduced nodulation on soybean (6, 9), and hairy vetch (15), respectively. Other workers (2, 13), however, found that races of *H. glycines* on soybean differ in their influence on nodulation. Race 1 significantly reduced nodulation, especially when added simultaneously with rhizobia, whereas races 2 and 4 did not. Taha and Raski (22) showed that nodule formation took place either before or after the addition of larvae of *M. javanica* or *Heterodera trifolii* Goffart to white clover. They concluded that reduced nodulation occurred only as a result of overall reduction of the root system. The sites of nodule initiation are present in the cortex of the root, and more lateral roots augment the infectable potential of the root (17). *M. hapla* increased nodulation on soybean (8). *Meloidogyne* galls on the roots may also reduce nodulation by occupying space on the root system, e.g., *Meloidogyne* spp. on beans and cowpea (16), *M. hapla* on hairy vetch (15), and *M. incognita* on pea (1). On the other hand, white clover and soybean nodules frequently developed on galls of *M. javanica* (22) and *M. incognita* (1), respectively. The role of *R. reniformis* on nodulation has not been investigated.

The present studies were done to explore the influence of intergeneric competition on population densities of *M. javanica* and *R. reniformis*, and on nodulation.

MATERIALS AND METHODS

General: Cultures of *Rhizobium* sp. (cowpea group), *M. javanica*, and *R. reniformis* were maintained outdoors on cow-

peas grown in sterilized sandy soil (87.7% sand, 6.2% silt, 7.3% clay) in 25-cm-diam pots. These were watered daily with tap water and fertilized weekly with nitrogen-free or full-nutrient solution (19) for rhizobia and nematodes, respectively.

Rhizobial inoculum was prepared by grinding nodules in a mortar with water to make a heavy suspension (22). Ten ml of suspension was added to each pot. Nematode inoculum for all experiments except the one studying invasion was added as egg masses placed in holes around the roots. With fine needles and a dissecting microscope the egg masses were detached from females parasitizing fine lateral roots. For the invasion experiment, larvae were obtained from egg masses in a Baermann funnel and added around roots at the rate of 100 per replicate.

All treatments were replicated three times. Ten-day-old cowpea (cv Fetriat) seedlings were used for each treatment. Unless otherwise noted, studies on intergeneric nematode competition were carried out in 200-ml plastic cups placed along laboratory window sills, where they received sufficient sunlight and air through the wire screen. They were maintained for 40 days following the first inoculation with nematodes. Nematode interactions with rhizobia were studied outdoors in 15-cm pots for a 60-day period from the time the nematodes were added.

Preliminary experiments were carried out to determine: 1) the time required for root invasion by the infective nematode stage; 2) the time required for appearance of swollen females of each nematode species with their egg masses; and 3) the inoculum level (1, 25, or 50 egg-masses) of *R. reniformis* which is equivalent to one egg mass of *M. javanica*. The treatments were maintained for 30 days, during which nematode-inoculated seedlings were examined at 2- or 3-day intervals for nematode invasion and for appearance of mature females with egg masses.

Nematode competition: a) *The effect of prior invasion by M. javanica on R. reniformis infection:* Four inoculum levels of *M. javanica* (0, 1, 2, and 3 egg masses) and four levels of *R. reniformis* (0, 50, 100, and 150 egg masses) were used. These were added as eight single treatments and in five

selected intergeneric combinations of the levels (Table 1). Treatments were arranged in randomized blocks. Results were subjected to analysis of variance, and differences between means were discerned with Duncan's multiple-range test.

b) *The effect of prior invasion by R. reniformis on M. javanica infection:* One egg mass of *M. javanica* was added 25 days after inoculation with the three levels of *R. reniformis*. Design and analysis was as in a, above.

c) *The reciprocal effects of prior invasion by either nematode on infection:* One egg mass of *M. javanica* was added 10, 20, and 30 days after adding 50 egg masses of *R. reniformis*. All plants were maintained outdoors in 15-cm-diam pots for 30 days after *M. javanica* was added. Treatments were paired, and compared with the *t*-test.

Nodulation: To explore the influence of nematode infection on nodulation, cowpea seedlings were inoculated concomitantly with rhizobia and: 1) no nematodes; 2) one egg mass of *M. javanica*; 3) 50 egg masses of *R. reniformis*; and 4) one egg mass of *M. javanica* plus 50 egg masses of *R. reniformis*. Inoculation with rhizobia was also made 30 days after adding 50 egg masses of *R. reniformis*. At the end of the experiments, the roots were washed, stained with acid fuchsin in cold lacto-phenol for at least 24 h, rinsed in water, and cut into pieces to facilitate counting of nematodes and nodules with a dissecting microscope. Treatments were arranged in randomized blocks. Results were subjected to analysis of variance, and differences between means were discerned with Duncan's multiple-range test.

RESULTS AND DISCUSSION

M. javanica and *R. reniformis* respectively invaded cowpea roots in 2 and 19 days, and started to deposit eggs 26 days following addition of larvae. Full egg masses of both species were observed after 30 days. At that time the number of *R. reniformis* resulting from inoculation with 50 egg masses was 286 per seedling. This was about the same as the 279 *M. javanica* per seedling resulting from one egg mass of that species. One egg mass of *R. reniformis* produced 21 nematodes per seedling; 25 egg masses produced 167. Differences in inoculum potential of egg masses between *M. javanica* and *R. reniformis* probably arise from differences in the number of eggs and the sex ratios in egg masses.

Nematode competition: a) *The effect of prior invasion by M. javanica on R. reniformis infection:* The final population of *M. javanica* increased significantly with inoculum level, whereas that of *R. reniformis* did not (Table 1). Mass invasion by *R. reniformis* at the highest inoculum level drastically reduced the root surface area available for any later invasion.

Because *M. javanica* invades roots more rapidly than *R. reniformis*, simultaneous inoculation with the two species gave *M. javanica* priority. Nevertheless, this advantage did not affect the number of *R. reniformis* invading. Increasing *M. javanica* inoculum levels caused no significant difference in the numbers of *R. reniformis* in roots. Readily formed *M. javanica* galls, and rootlets arising from them, were subjected to *R. reniformis* infection. A range of one to seven females of *R. reniformis*

TABLE 1. Average numbers of *Meloidogyne javanica* (M_j) and *Rotylechulus reniformis* (Rr) in roots of cowpea grown 40 days after inoculation with one or both nematode species at the levels indicated.

Level of Rr inoculum in egg-masses	Numbers of nematodes							
	Level of M _j inoculum in egg-masses							
	0	1		2		3		
Rr	M _j	Rr	M _j	Rr	M _j	Rr		
0	0	372cd	0	656b	0	977a	0	
50	376	320cd	467	416c	459	629b	458	
100	487	—	—	380d	423	—	—	
150	581	—	—	—	—	516b	517	

Values in both vertical and horizontal columns not followed by the same letter differ significantly from one another (a-b & b-c/d, P = 0.05); (a-c/d, P = 0.01).

were observed partially embedded in gall tissue. At the lowest inoculum level of mixed infection, *M. javanica* increased at the same rate as in separate infection because of the availability of sufficient space on the root surface. At the higher inoculum levels, in contrast, its populations increased less than in separate infections since the second larval generation could not find more space on the root surface.

b) *The effect of prior invasion by R. reniformis on M. javanica infection:* Delaying the addition of *M. javanica* for 25 days gave *R. reniformis* priority in invading the roots and establishing its sedentary semi-endoparasitic life. Consequently, the number of invading *M. javanica* within a 15-day period decreased ($P = 0.01$) from 93 per seedling at the lowest inoculum level of *R. reniformis* to 31 and 30 per seedling at the higher inoculum levels. The population of *M. javanica* attained in separate infection (101 per seedling) was not statistically different from that attained in combination with the lowest inoculum level of *R. reniformis*. The concomitant populations of *R. reniformis* resulting from the three increasing inoculum levels were respectively 425, 455, and 562 per seedling, and were not statistically different from their single populations (Table 1).

c) *The reciprocal effects of prior invasion by either nematode on infection:* When *M. javanica* inoculation was delayed 10, 20, and 30 days, the respective resulting infections preceded, accompanied, and followed infection by *R. reniformis*. Therefore, the significant reduction in the number of *M. javanica* where the inoculum was delayed 30 days (Table 2) may be due to prior invasion and occupation of root surface by *R. reniformis*. Moreover, *R. reniformis* causes hypertrophy of the pericycle cells which ultimately prevents the production of lateral roots (18). Christie (4) reported that *Meloidogyne* larvae prefer to enter the apical meristem but could enter more mature tissue, depending upon the point of inoculation. *R. reniformis*, in contrast, shows preference of maturation zone of the roots, but never enters through the root tip (12, 18). Further work is needed to elucidate the interaction of *M. javanica* and *R. reniformis*. Addition of *M. javanica* inoculum 10 and 20 days after that of *R. reniformis*

TABLE 2. Average numbers of *Meloidogyne javanica* (M_j) and *Rotylenchulus reniformis* (Rr) in roots of cowpea when M_j inoculation was delayed for 10, 20, and 30 days after inoculation with Rr.

Delay (days)	Av. no. of nematodes			
	M_j		Rr	
	Alone	After Rr	Alone	Before M_j
10	394	368	277	238
20	288	237	265	250
30	212*	105	469	537

*Differs from the result with inoculation with both nematodes ($P = 0.05$).

caused no significant difference in numbers of *M. javanica* (Table 2). Populations of *R. reniformis* in separate and mixed infections at each period were not statistically different.

Although *R. reniformis* suppressed the population of *M. javanica* on cowpea roots, *M. javanica* has an advantage over *R. reniformis* because of its shorter time spent free in soil, and its higher reproductive potential. Furthermore, galls of *M. javanica* can be repeatedly invaded by its larvae because this tissue is as attractive as root tips to *M. javanica* (3). Therefore, in a mixed infestation, the population of *M. javanica* ultimately predominates (Table 3). These data differ from the observation that the development and growth rate of *M. incognita* in tomato roots were retarded by *R. reniformis* since a few *M. incognita* female larvae reached the adult stage, and each adult female laid few numbers of eggs (11). Reproduction of *Meloidogyne* is a maximum when thick-walled multinucleate giant cells with dense cytoplasm develop (5). Our histopathological studies (21) revealed such cells lying in contact with the feeding sites of *R. reniformis* in cowpea roots.

Nodulation: When added simultaneously with rhizobia, neither nematode, singly or in combination, significantly reduced the number of nodules (Table 3). Nodule formation took place at about the same time as root invasion by *M. javanica* and before invasion by *R. reniformis*. The number of nodules developing on *R. reniformis*-infected roots (43 nodules/seedling) was lower ($P = 0.05$) than on healthy roots (97

TABLE 3. Average numbers of *Rhizobium* nodules (Rh) and numbers of *Meloidogyne javanica* (M_j) and *Rotylechulus reniformis* (Rr) on roots of cowpea 60 days after inoculation with various combinations of these organisms.

Treatments	Rh	Rh + M _j	Rh + Rr	Rh + M _j + Rr
Av. no. of nodules	133	115	127	95
Av. no. of nematodes	-	2698*	576	1986 M _j 747 Rr

*Differs from Rh + M_j + Rr (P = 0.05).

nodules/seedling). Nutman (17) traced the course of rhizobial infection from a point outside the root, through the root hair, into the cortex where the sites of nodule initiation exist. He noted that rhizobial infection is not a haphazard process but is firmly controlled by influences within the root. *R. reniformis* infection may deplete root hairs through which rhizobial infection could take place. Also, suppression of lateral root formation by *R. reniformis* (18) may cause reduction in the number of sites for nodule initiation, since lateral roots bear their own sites (17).

Data obtained on nodulation provided no support for the hypothesis that *Meloidogyne* or *Heterodera* larvae reduce nodulation by competing with root-nodule bacteria for root invasion sites (6, 9, 15). Previous studies on white clover (22) showed that *M. javanica* or *H. trifolii* did not hinder nodule formation when added either before or after rhizobial inoculation. The formation of white clover nodules on *M. javanica* galls (22) and of soybean nodules on *M. incognita* galls (1) does not support Masefield's (16) conclusion that nodulation is prevented at sites occupied by nematode galls.

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