Effects of Concomitant Development on Reproduction of Meloidogyne incognita and Rotylenchulus reniformis on Sweet Potato[1]

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Abstract: The influence of various factors on reproduction of concomitant *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr) on sweet potato were studied in the greenhouse. Reproduction of Rr was reduced by Mi at all inoculum levels and experiment durations used, while Mi reproduction was not inhibited. Both species failed to affect each other when inoculated simultaneously onto root systems developed in separate pots from different nodes of the same plant. Reproduction of each species was not significantly greater when inoculation of the second species was delayed 1–2 weeks compared to simultaneous inoculation. After shoot excision, Rr increased in the soil but Mi decreased. Fibrous root weights of plants inoculated with Rr + Mi in some tests were higher than those inoculated with Mi alone, indicating an early suppression of Mi and/or root stimulation by Rr. Drought stress delayed Rr egg hatching and movement of larvae into the soil, but had little effect on Mi reproduction.
Key words: Ipomoea batatas, root-knot nematode, reniform nematode.

Journal of Nematology 15(2):215-221. 1983.

Meloidogyne incognita (Kofoid & White) Chitwood and *Rotylenchulus reniformis* Linford and Oliveira are frequently found in the same field attacking the same host (1,10,15,20). Several authors have observed that reniform nematode was the most abundant species in a field while root-knot nematodes remained at low population densities (4,6,16). Little information is available on their possible interactions on a particular host. Greenhouse studies of concomitant reproduction of both nematodes on tomato and cowpea were contradictory but indicated that each species might be capable of inhibiting the other (13,15,18). Root tissue reactions of cowpea remained unique even when the different genera were feeding in close proximity (17).

Recently, both *R. reniformis* and *M. incognita* were shown to be capable of inhibiting each other in the field on sweet potato (21). Species predominance was dependent mainly on the initial inoculum level of *M. incognita*. The objective of this research was to study various factors which might affect this interaction under controlled greenhouse conditions.

Received for publication 28 May 1982.

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MATERIALS AND METHODS

Sweet potato *Ipomoea batatas* cvs. Centennial and Porto Rico, both susceptible to *R. reniformis* (Rr) and *M. incognita* (Mi), were used throughout this study. In each experiment one terminal vine cutting, pruned of leaves, was planted per 15-cm clay pot containing approximately 1,250 cm^3 steam sterilized 1:1 sand-soil mixture.

A Louisiana population of Mi (Race 1) was increased on Rutgers tomato, *Lycopersicon esculentum*, and eggs were harvested 45 days after inoculation (11). Eggs were extracted similarly from a Louisiana population of Rr increased on Centennial sweet potato (7). Reniform nematode larvae and young adults were extracted by sieving and Baermann funnel. Inoculum was pipetted around the base of the cuttings in 10–20 ml of water. The experimental design for each test was a complete randomized block with 4–6 replications. Data were subjected to analysis of variance and significant mean differences determined using Fisher's LSD method ($P = 0.05$).

At harvest roots and soil were removed from each pot, and the soil was shaken gently from each root system and mixed thoroughly. Nematodes were extracted from 500 cm^3 of soil from each pot using a semi-automatic elutriator and centrifugal flotation (5,12). Adhering soil was washed from the roots, and fresh weights were determined for fibrous and fleshly roots and tops. Eggs were extracted from root systems with 0.525% sodium hypochlorite for 4

min and processed by sieving and centrifugal flotation. Numbers of nematodes per pot were determined by multiplying the number per 500 cm^3 by 2.5 and adding the result to the number of hatched larvae extracted from the root systems with the eggs. Eggs were not identified by species.

Effect of inoculum level and harvest date: Centennial sweet potato cuttings were planted and inoculated 9 June 1979 with 500 or 2,000 Mi and/or Rr eggs and harvested after 46 and 78 days. In a separate experiment, Centennial cuttings were planted and inoculated 2 August 1979 with 0, 500, 2,000, 4,000, and 10,000 Rr eggs and 2,000 Mi eggs. Plants were harvested after 44 days.

Effect of species isolation in double rooted systems: Centennial cuttings were grown in 710 cm^3 square plastic freezer containers taped together in pairs. Root systems were developed in separate containers from different nodes of the same plant. Plants were planted 27 January 1980 and inoculated 30 January with 4,000 Mi and/or Rr eggs and 18 February with 2,000 additional Mi eggs. Plants were harvested 73 days after planting.

Effect of watering method: Watering treatments consisted of adding water daily to the approximate water holding capacity of the soil and allowing plants to reach the wilting point and then watering to capacity. The water-holding capacity of the sand-soil mixture was 15.6% by weight, and daily water loss was estimated gravimetrically. Porto Rico sweet potato cuttings were planted and inoculated 9 September 1980 with 10,000 Mi eggs and/or Rr larvae + young adults. Plants were harvested after 94 days.

Effect of sequential inoculation: Porto Rico sweet potato cuttings were inoculated 9, 17, or 24 days after planting with 2,000 or 5,000 Mi eggs and at 9 or 17 days with 5,000 Rr larvae + young adults. Plants were harvested 92 days after the last inoculation.

Effect of shoot excision: Porto Rico cuttings were planted 6 February 1981 and inoculated 6 days later with 9,500 Mi eggs and/or 2,600 Rr larvae + young adults. The first harvest was made 60 days after inoculation and tops were excised at the soil line from half the remaining plants to simulate field harvest effects. New shoots were removed as they emerged. Subsequent harvests of excised and nonexcised plants were made at 70 and 92 days. The 60-day harvest included only nonexcised plants.

RESULTS

Effect of inoculum level and harvest date: At both inoculum levels and both harvest dates, reproduction of Rr was significantly reduced in the presence of Mi compared to Rr alone (Table 1). However, the number of Mi per pot and eggs per root system were not affected significantly by the presence of Rr. Fibrous roots of plants inoculated with the lower level of both species weighed more than those inoc-

Table 1. Effect of inoculum level and harvest date on the reproduction of *Rotylenchulus reniformis* (Rr) and *Meloidogyne incognita* (Mi) inoculated alone and in combination on sweet potato *Ipomoea batatas* cv. Centennial, 46 and 78 days after inoculation, and on the fibrous root weights.

Inoculum level*	Figrous root weight (g)		Rr/pot		Mi/pot		Eggs/ root system	
	46	78	46	78	46	78	46	78
Control	4.4	8.8	50	69	4	128	603	765
Rr-500	4.6	3.7	11,504	102,165	0	90	4,132	17,580
Rr-2,000	4.3	2.7	27,335	89,773	0	103	11,810	15,780
Mi-500	2.5	2.3	0	428	29,800	77,463	175,200	141,080
Mi-2,000	1.8	1.2	0	0	48,745	91,425	171,840	148,675
Rr + Mi-500	3.9	2.7	4,400	22,530	23,250	49,370	165,600	137,190
Rr + Mi-2,000	2.1	0.9	2,225	20,760	34,425	32,420	86,200	98,380
LSD (0.05)	1.5	2.9	3,499	69,258	24,536	68,487	109,132	111,208

*Treatments inoculated with 500 or 2,000 Rr and/or Mi eggs per 15-cm pot. Treatment means are averages of four replicates. Minor Rr or Mi contamination occurred in some control pots.

ulated with Mi alone after 46 days, but not at 78 days nor at the higher inoculum level. The difference was significant at $P = 0.05$ using Duncan's MRT but not with Fisher's LSD. The root systems of the plants with Rr + Mi also appeared to be more extensive and less necrotic than root systems of plants inoculated with Mi alone. Varying the inoculum level of Rr while using a single level of Mi resulted in increased recovery of Mi larvae and eggs over Mi alone in several treatments (Table 2), but at the 10,000 Rr level, Mi larvae and eggs declined. The total number of Rr recovered increased with increasing inoculum level, but the ratio of Rr recovered per unit of inoculum declined.

Effect of species isolation in double rooted systems: Reproduction of Rr and Mi on separate root systems of the same host plant (20,364 Rr/pot and 37,360 Mi/pot) was not significantly different from their reproduction on separate plants when the second root system of the plant was not inoculated (21,580 Rr/pot and 38,010 Mi/pot).

Effect of watering method: In both watering treatments Rr was significantly inhibited by Mi (Table 3). Reproduction of Mi was not significantly inhibited by Rr

Table 2. Effect of varying levels of *Rotylenchulus reniformis* (Rr) on *Meloidogyne incognita* (Mi) reproduction after 44 days on sweet potato *Ipomoea batatas* cv. Centennial.

Inoculum level*		Fibrous root			Eggs/
Rr	Mi	weight (g)	Rr/pot	Mi/pot	root system
0	0	4.3	213	31	0
0	2,000	4.8	56	11,871	36,400
500	2,000	7.0	4,663	11,746	47,867
2,000	2,000	5.6	17,138	17,625	48,800
4,000	2,000	7.2	25,850	17,363	56,500
10,000	2,000	5.4	27,788	5,938	42,200
LSD (0.05)		1.8	10,420	11,245	10,295

*Treatments inoculated with Rr and/or Mi eggs in 15-cm pots at levels shown. Treatments are averages of four replicates. Minor Rr or Mi contamination occurred in some control pots.

Table 3. Effect of water stress in the presence of *Rotylenchulus reniformis* (Rr) and *Meloidogyne incognita* (Mi) alone and in combination on sweet potato *Ipomoea batatas* cv. Porto Rico fibrous root weight and nematode reproduction.

Treatment*	Fibrous root weight (g)	Rr/pot	Mi/pot	Eggs/ root system
Nonstressed				
Control	14.8	81	76	267
Rr	18.4	79,995	225	8,180
Mi	11.9	58	11,810	17,430
Rr + Mi	20.6	26,303	5,775	10,740
Stressed				
Control	4.3	83	19	62
Rr	5.3	53,605	520	37,900
Mi	3.7	208	12,215	11,430
Rr + Mi	3.5	15,220	10,490	6,180
LSD (0.05)	8.8	19,054	9,204	20,533

*Nonstressed plants were watered daily to approximate water holding capacity of the soil. Stressed plants were watered to capacity when plants reached wilting point. Treatments were inoculated with 10,000 Mi eggs and/or Rr larvae + young adults and harvested after 94 days. Treatments are averages of four replicates. Minor Rr or Mi contamination occurred in some control pots.

in water stressed or nonstressed treatments. There was a tendency for fibrous root systems to have higher weights with concomitant inoculation than with Mi alone in the nonstressed treatment, but differences were obscured by a tendency of some plants to produce fleshy roots at the expense of fibrous roots and vice versa. Water stress tended to reduce root weights in all treatments. This resulted in a higher concentration of nematodes per weight of root system, but overall reproduction (eggs + vermiform nematodes) was not affected by water stress. Water stress did, however, result in a delay of reniform nematode hatching and inhibited movement of larvae out of the egg mass into the soil. In the Rr alone treatments, eggs accounted for 41% of the total population with water stress and 9% in nonstressed. Of the Rr larvae and young adults, 12% were found in the egg mass with water stress and 2% in nonstressed. Water stress apparently had no affect on Mi hatching and movement into the soil.

Effect of sequential inoculation: Numbers of Rr were lower when Mi was inoculated simultaneously, or 1–2 weeks following Rr, compared to Rr alone, but timing of Mi inoculation had no effect on Rr recovered (Table 4). Numbers of Mi and eggs per root system for Mi alone (48,975 Mi/pot and 45,840 eggs) were not significantly reduced when inoculated with 5,000 Mi +5,000 Rr simultaneously, or when Rr was inoculated 1 week following Mi. Numbers of Mi with simultaneous inoculation (20,905 Mi/pot and 28,720 eggs) and when Rr inoculation was delayed (31,225 Mi/pot and 34,850 eggs) were not significantly different. There were no differences in root weight between treatments.

Effect of shoot excision: Rr reproduction was inhibited by Mi in both excised and nonexcised treatments at 70 and 92 days (Fig. 1A). Rr increased in the soil following shoot excision, but in nonexcised plants Rr peaked and then declined. At 92 days, counts of Rr alone in excised plants were higher than in nonexcised plants. Counts of Mi larvae in nonexcised plants were not reduced by Rr (Fig. 1B). Excision reduced Mi larvae in the single species inoculation at 70 days and in the mixed inoculation at 92 days compared to nonexcised plants (Significant at $P = 0.05$ by Duncan's MRT but not Fisher's LSD). At 60 and 70 days egg counts found for Mi alone and for Mi + Rr in nonexcised plants were similar, but at 92 days the most eggs were found in the Mi + Rr treatment (Fig. 1C). Egg counts declined for nonexcised and excised Mi alone and for excised Mi + Rr. Egg counts for Rr alone were 18,053 the first harvest, declining to 4,040 and 2,177 at 70 and 92 days in the nonexcised plants and 1,380 and 568 in the excised plants. At 92 days significantly higher root weights were found for the nonexcised Mi + Rr infected plants (19.1 g) than for nonexcised Mi alone (6.3 g) and the Mi + Rr infected roots appeared to be more extensive and less necrotic. Shoot excision resulted in significant root weight losses in all treatments, and no difference was found between Mi and Mi + Rr treatments (both 3.1 g).

DISCUSSION

In the field experiments (21) we found that Rr and Mi were each capable of inhibiting the other and becoming the predominant species in a sweet potato field.

Table 4. Effect of simultaneous and delayed inoculation of *Meloidogyne incognita* (Mi) on reproduction of *Rotylenchulus reniformis* (Rr) on sweet potato *Ipomoea batatas* cv. Porto Rico.

		Time & level of Mi inoculation*				
	Rr Alone	Simultaneous		1 week following Rr		2 weeks following Rr
		2,000	5,000	2,000	5,000	5,000
Rr/pot	54,200	19,650	19,000	24,975	18,200	22,787
LSD (0.05) =	9,580					

*Treatments inoculated with 5,000 or 2,000 Mi eggs and/or 5,000 Rr larvae + young adults and harvested 107 days after first inoculation. Treatments are averages of four replicates.

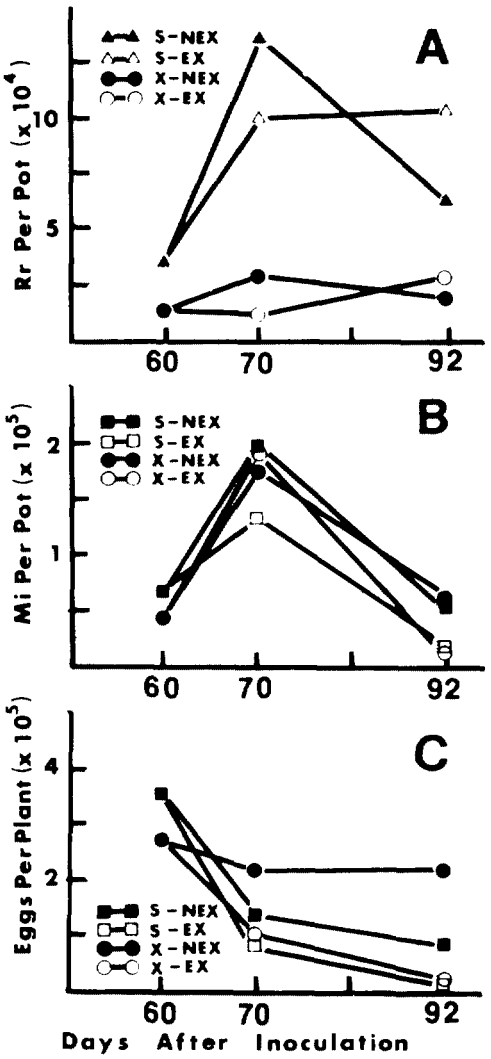

Fig. 1. Effect of shoot excision and harvest date on concomitant population dynamics of *Rotylenchulus reniformis* (Rr) and *Meloidogyne incognita* (Mi). A) Rr. B) Mi. C) Mi alone and Mi + Rr egg counts at 60, 70, and 92 days after inoculation. S = single species, X = with concomitant species, NEX = shoot not excised, EX = shoot excised at 60 days. Treatments are averages of six replicates. LSD (0.05) = A) 41,990, B) 57,934, C) 117,022.

Rr predominated when field plots were inoculated with low levels of Mi (100/250 cm³) but not with high levels (4,000/250 cm³). Negative correlations between initial and final populations in a field naturally infested with both species also supported this observation. Our greenhouse experiments have consistently shown that Rr reproduction was inhibited in concomitant infections with Mi on sweet potato. In contrast, Mi reproduction was not signficantly inhibited by Rr.

Reproduction of Rr was significantly reduced by Mi with all inoculum levels and experiment durations used. Water stress, inoculation with Rr 2 weeks prior to Mi, and shoot excision had little effect on inhibition of Rr by Mi. Reproduction of Rr was not inhibited in a double root system, suggesting that the mechanism of inhibition does not involve a translocatable substance, as postulated on the basis of tomato split root experiments in which *Pratylenchus penetrans* was inhibited by *M. incognita* (9). The increase in Rr numbers in the soil following shoot excision (which presumably speeds root decay) may be similar to what occurs in the field following harvest. *Rotylenchulus reniformis* is able to survive between crops in high numbers in the soil (3,8). In greenhouse tests the majority of the population was found in larval or young adult stages in the soil rather than as eggs on roots.

In most tests Mi larval and egg counts were not significantly different between Mi inoculated and concomitantly inoculated treatments, regardless of inoculum level or length of time. The majority of the Mi population was generally found in the egg stage and in much higher numbers than Rr eggs. While Mi and Rr eggs can not be distinguished, the assumption can often be made that the majority of the eggs in concomitant treatments are Mi eggs, particularly when egg counts for Mi alone are very high relative to Rr alone. In most tests, equal numbers of Mi and Rr were used for inoculum, which probably gave Mi the advantage because of its higher reproductive capacity. Reports of eggs per egg mass range from 30 to 200 for Rr (2,14) and more than 1,000 for Mi (19). Also, Rr populations usually have about 50% males which do not feed. A small amount of contamination probably due to splashing late in the test period was detected in some control pots but significant reproduction did not occur in control pots relative to inoculated pots. When the Mi inoculum level was kept constant and Rr was increased, the highest Rr level resulted in the lowest final Mi counts. Tests using larger differentials

between Mi and Rr inoculum levels need to be conducted to confirm this apparent inhibition of Mi by Rr. In the field Mi counts decrease in the soil over winter and by spring planting may fall to undetectable levels (Thomas, unpublished data). Shoot excision resulted in a decline in Mi larvae and eggs recovered. The low survival rate of Mi and the high survival rate of Rr create a large differential between initial inoculum levels at planting. This is probably the main factor that allows Rr to predominate in the field, but we also found that Mi juvenile concentration in soil was significantly inhibited by Rr (21).

Interactions of Mi and Rr can not be interpreted fully without considering their effects on the plant. As Mi increases in a greenhouse pot on sweet potato, it will eventually become selflimiting as roots begin to die and numbers of feeding sites decline. This destructive effect on the root system may also be an important part of the mechanism by which Mi inhibits Rr. *Rotylenchulus reniformis* is usually not as damaging and probably takes longer to become self limiting. In several tests (Table 1, 2, and shoot excision test) fibrous root weights were higher with concomitant infection than with Mi alone. This can be interpreted in several ways: 1) Rr caused root growth stimulation in the presence of Mi. 2) There was an inhibition of early generations of Mi by Rr that slowed the rate of root-destruction and was not significantly manifested in later Mi population counts. 3) There may be a combination of both factors.

Whatever the mechanism, the effect of Rr may be to delay the time when Mi becomes selflimiting. In the shoot excision test, higher root weights in concomitant infections than in Mi infections apparently resulted in higher egg counts in nonexcised plants. The root growth effect did not occur in all tests and can apparently be overcome by higher Mi inoculum levels or with time (Table 1). No stimulation of Mi population or sweet potato yield was observed in the field (21).

In summary, greenhouse studies showed that Rr was inhibited and Mi became predominant in concomitant infections of sweet potato. The higher reproductive capacity of Mi and its destructive and self-limiting effect on the root system are probably of major importance in inhibiting Rr. These studies failed to confirm field observations that Rr can inhibit Mi (21), probably because Mi levels used were too high. Higher root weights in concomitant treatments than in Mi treatments may indicate an early suppression of Mi and/or root stimulation by Rr.

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