Variation in Pathogenicity of Seventeen Isolates of *Meloidogyne incognita*

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Abstract: Pathogenicity of 17 isolates of *Meloidogyne incognita* collected in Tennessee was studied in the greenhouse on: Rutgers tomato, N.C. 95 tobacco, McNair 1032 cotton, Dixie Queen watermelon, California Wonder pepper and line M57-13N cowpea. Root-knot indices of the isolates on the different hosts differentiated six physiological races. The host reactions of each race are discussed. *Key words:* host-parasite relations, physiological races.

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Physiologic variation within species of *Meloidogyne* Goeldi 1887 presents problems to taxonomists, plant breeders and other investigators, since certain populations possessing similar morphologic characteristics produce different reactions on the same host. Efforts to control these nematodes through

breeding resistant varieties and by crop rotation have been hampered by variation. Variation in pathogenicity among populations of *Meloidogyne* is not uncommon. Christie and Albin (2) demonstrated the existence of races within the former species *Heterodera marioni* and formed the basis on which Chitwood (1) reclassified the group into the genus *Meloidogyne*. Martin (10) found ranges from no parasitism to severe pathogenicity in cultures of *M. incognita* (Kofoid and White) Chitwood, and *M. incognita* var. *acrita* Chitwood, on different cultivars of cotton. Colbran (3) observed distinct physiological races in *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* and *M. javanica* (Treub) Chitwood. Giamalva et al. (6) and Davide and Struble (4) found similar situations with *M. incognita* on sweet potatoes. Goplen et al. (8), tested 20 collections of root-knot nematodes on five alfalfa cultivars and found three biotypes of *M. incognita* var. *acrita* and two each of *M. javanica* and *M. hapla*. Sasser (12) worked with world-wide collections of *Meloidogyne* spp. on nine host differentials and found physiological races in *M. incognita* and *M. arenaria*. Triantaphyllou and Sasser (15) described variation both in perineal patterns and host specificity of *M. incognita*. Riggs and Winstead (11) reported that new strains of *M. incognita* developed in the greenhouse which were capable of attacking resistant tomato plants. Graham (9) discovered a new race of *M. incognita* in field plots of flue-cured tobacco which attacked N.C. 95 tobacco, a cultivar resistant to *M. incognita*. More information is needed on the nature and extent of variation in root-knot nematodes if breeding programs and crop rotation practices are to succeed.

The purpose of this investigation was to determine the extent of physiologic variation exhibited by 17 Tennessee isolates of *Meloidogyne incognita* on six host differentials.

MATERIALS AND METHODS

Seventeen isolates of root-knot nematodes, subsequently identified as *M. incognita* on the basis of morphological characters, were obtained from root and soil samples collected in nine counties in Tennessee in 1969 (Table 1). Each field composite sample was placed in a 15-cm pot to which one tomato plant, *Lycopersicon esculentum* Mill. 'Rutgers', was transplanted; the pots then were kept in a greenhouse for 50 days. Nematode isolates were initiated from a single egg mass and established on Rutgers tomato in 15-cm pots containing a 1:1 mixture of fine sand and Etowah silt loam soil. Pots were placed on saucers and spaced approximately 25 cm apart. Plants were watered as needed and fertilized once a week with 200 ml of the following nutrient solution: 0.2 g VHPF® (Miller Chemical Co., Baltimore 15, Md.) + 0.2 g KNO_3/liter of water. Each isolate was subcultured by a transfer of egg masses to disease-free tomato seedlings every 50 days to provide inoculum for host-parasite studies.

TABLE 1. Collection sites and associated hosts of seventeen isolates of *Meloidogyne incognita* in Tennessee.

Isolate no.	County	Host
1	Johnson	Tobacco
2	Johnson	Tomato
3	Johnson	Tobacco
4	Rhea	Snapbean
5	Meigs	Snapbean
6	Knox	Tomato
7	Knox	Okra
8	Dyer	Lima bean
9	Dyer	Lima bean
10	Lake	Cotton
11	Lake	Cotton
12	Lake	Cotton
13	Greene	Tobacco
14	Monroe	Tobacco
15	Cocke	Lima bean
16	Dyer	Lima bean
17	Gibson	Sweet potato

Two studies (Test 1 and Test 2) were conducted in a greenhouse. Test 1 was initiated with seedlings approximately 3 weeks old of the following plants: tomato, *Lycopersicon esculentum* Mill. 'Rutgers'; watermelon, *Citrullus vulgaris* Schrad. 'Dixie Queen'; pepper, *Capsicum frutescens* L. 'California Wonder'; and tobacco, *Nicotiana tabacum* L. 'N.C. 95'. Four replications of each host and isolate combination were used. A plant was inoculated with eight egg masses of a given isolate, so that each isolate was tested on each cultivar. The temperature was maintained at approximately 26 C during the day and 24 C at night. The incubation period was 52 days. Test 2 was similar to Test 1, except that the test plants were: cotton, *Gossypium hirsutum* L. 'McNair 1032'; cowpea, *Vigna sinensis* (Torner) Savi breeding line M57-13N; and Rutgers tomato. The incubation period was 50 days.

TABLE 2. Root-knot ratings of 17 isolates of *Meloidogyne incognita* on four hosts 52 days after inoculation.[a]

Isolate	Root-knot index[b]			
	Tomato	Watermelon	Pepper	Tobacco
1	8.6 ab	6.6 abc	5.3 bcd	1.0 a
2	8.8 ab	7.5 a	5.1 bcd	1.0 a
3	8.4 ab	7.6 a	4.9 bcd	1.0 a
4	8.5 ab	6.3 abcd	5.6 bc	1.0 a
5	8.6 ab	5.8 abcd	4.1 cd	1.0 a
6	8.0 bc	3.8 de	4.5 bcd	1.0 a
7	8.3 abc	6.7 abc	4.5 bcd	1.0 a
8	8.1 bc	4.9 bcde	5.8 bc	1.0 a
9	9.0 ab	7.3 ab	1.0 e	1.0 a
10	9.9 a	7.9 a	5.6 bc	1.0 a
11	8.5 ab	6.1 abcd	4.4 bcd	1.0 a
12	6.6 c	4.2 cde	4.9 bcd	1.0 a
13	8.5 ab	7.5 ab	6.0 abc	1.0 a
14	8.1 abc	7.9 a	7.7 a	1.0 a
15	8.8 ab	1.0 f	6.4 ab	1.0 a
16	8.1 abc	6.7 abc	5.0 bcd	1.0 a
17	8.8 ab	4.5 bcde	6.0 abc	1.0 a

[a]Numbers are means of four replications.
[b]Based on the degree of infection and reproduction: 1 = no galls or egg masses present; 10 = severe infection, galls, mature females and egg masses abundant on almost 100% of the root system. Corresponding gradations of infection and reproduction between these two limits are numbered accordingly. Means which have a small letter in common within a given category do not differ significantly from each other at the 1% probability level (Duncan's multiple range test).

After incubation, the soil was washed gently from the roots and each root system was rated on a relative scale of 1-10 where 1 = no infection, or if larvae entered the roots they did not develop into mature egg-laying females; 2 = 1-10% of root system galled, a few egg masses present; 3 = 11-20% of root system galled, egg masses present; . . . 10 = 80-100% of root system galled, mature females and egg masses numerous. When little or no infection occurred (ratings of 2.0 or under), tests were repeated for verification.

RESULTS AND DISCUSSION

All isolates used in Test 1 produced severe galling and numerous egg masses on Rutgers tomato (Table 2). No isolate produced galls or egg masses on N.C. 95 tobacco, which is resistant to *M. incognita*. A wide range of variation in host reaction was evident among the isolates. For example, isolate 15 reproduced moderately on pepper but did not infect watermelon (Table 2). Isolate 9 did not infect pepper but was moderately severe on watermelon. All other isolates had a moderate to high index on watermelon and pepper. Isolates 2, 3, 10 and 14 had significantly higher indices on watermelon than did isolates 6, 8, 12, 15 and 17. Isolate 10 had a significantly higher index on tomato and watermelon than did isolates 6, 8 and 12. Isolates 9, 10 and 14 reacted similarly on watermelon but significantly different from each other on pepper. Isolate 9 did not infect pepper, and isolate 14 had the highest index rating on pepper of all isolates.

Ratings in Test 2 were generally lower than in Test 1 (Table 3). The environmental conditions or the inoculum potential could have varied sufficiently between the two tests to account for the overall lower ratings in Test 2. Otherwise, the response of tomato to the 17 nematode isolates in Test 2 was very similar to

TABLE 3. Root-knot ratings of 17 isolates of *Meloidogyne incognita* on three hosts 50 days after inoculation.[a]

Isolate	Root-knot index[b]		
	Tomato	Cowpea	Cotton
1	4.6 ab	1.3 bc	1.0 c
2	4.9 ab	1.5 bc	1.0 c
3	5.9 ab	1.5 bc	1.0 c
4	5.8 ab	2.3 ab	1.0 c
5	5.4 ab	1.0 c	1.0 c
6	5.3 ab	1.1 c	1.0 c
7	6.7 ab	1.4 bc	1.0 c
8	6.5 ab	2.3 ab	3.9 a
9	5.6 ab	1.0 c	2.1 ab
10	7.0 a	2.0 abc	3.4 ab
11	5.1 ab	1.8 abc	2.8 ab
12	4.3 b	1.4 bc	4.2 a
13	5.7 ab	1.5 bc	1.0 c
14	6.0 ab	1.6 abc	1.1 c
15	6.0 ab	2.5 a	1.3 bc
16	6.4 ab	2.3 ab	2.6 ab
17	6.0 ab	1.4 bc	1.3 bc

[a]Numbers are means of four replications.
[b]Based on the degree of infection and reproduction: 1 = no galls or egg masses present; 10 = severe infection, galls, mature females, and egg masses abundant on almost 100% of root system. Corresponding gradations of infection and reproduction between these two limits are numbered accordingly. Egg masses present on cowpea and cotton only where the letter "a" appears after the number. Means which have a small letter in common within a given category do not differ significantly from each other at the 1% probability level (Duncan's multiple range test).

TABLE 4. The reaction of 17 isolates of *Meloidogyne incognita* on six hosts (+ = infection and reproduction; – = no infection and reproduction).

Isolate	Host					
	Tobacco	Cotton	Cowpea	Watermelon	Pepper	Tomato
1	–	–	–	+	+	+
2	–	–	–	+	+	+
3	–	–	–	+	+	+
5	–	–	–	+	+	+
6	–	–	–	+	+	+
7	–	–	–	+	+	+
13	–	–	–	+	+	+
17	–	–	–	+	+	+
4	–	–	+	+	+	+
14	–	–	+	+	+	+
15	–	–	+	–	+	+
9	–	+	–	+	–	+
12	–	+	–	+	+	+
8	–	+	+	+	+	+
10	–	+	+	+	+	+
11	–	+	+	+	+	+
16	–	+	+	+	+	+

that of Test 1. In both tests, isolate 10 had the highest rating on tomato, and isolate 12 had the lowest.

None of the isolates reproduced well on the root-knot nematode-resistant cowpea line M57-13N. Isolates 4, 8, 10, 15 and 16 produced galls and small egg masses on 10-20% of the roots, mainly associated with or on *Rhizobium* nodules. The remainder of the isolates either produced no or few galls on cowpea with no visible egg masses.

Approximately 50% of the *M. incognita* isolates did not infect McNair 1032 cotton (Table 3). Isolates 8, 9, 10, 11, 12 and 16 produced a moderate number of galls and egg masses; isolates 14, 15 and 17 produced some galls, but no egg masses were found. The other isolates did not infect cotton.

The variation exhibited by these 17 Tennessee isolates of *M. incognita* was greater than that reported by Sasser (12, 13) among 18 populations of this species from various geographic regions of the world. Sasser (12) distinguished three "biotypes" of *M. incognita* with nine differential hosts. We distinguished six physiological races on six hosts.

A schematic representation of the six race groupings is shown in Table 4. Isolates 1, 2, 3, 5, 6, 7, 13 and 17 comprise one race, since they colonized and reproduced on Dixie Queen watermelon and California Wonder pepper but not on N.C. 95 tobacco, McNair 1032 cotton or line M57-13N cowpea. Isolates 4 and 14 are of a different race, since they colonized and reproduced on cowpea, although rather poorly, in addition to watermelon and pepper. Isolates 9, 12 and 15 were each distinct physiologic races. Isolate 9 reproduced on cotton and watermelon but not on pepper and cowpea. Isolate 12 colonized and reproduced on cotton, watermelon and pepper but not on cowpea or tobacco. Isolate 15 reproduced on cowpea and pepper but not on cotton, watermelon or tobacco. Isolates 8, 10, 11 and 16 were members of another race, since they colonized and reproduced on watermelon, pepper, cowpea and cotton but not on tobacco.

Nematode populations that differ in their pathogenicity on a given host or hosts, especially when there are qualitative or large quantitative differences, have been referred to in the literature as biotypes, strains, isolates, pathotypes and races. Golden et al. (7) proposed the term "race" to apply to infraspecific forms of *Heterodera glycines* and suggested guidelines for identifying and designating races of this nematode. Dropkin (5) devised a bioassay system for separating races of root-knot nematodes. The physiological variants that were distinguished by these tests were based on the ability of the nematodes to reproduce on certain plants and should meet the criteria for designating them as races. The only possible exception might be those distinguished by their reaction on cowpea, which required closer scrutiny and more

replications for rating than did other host-parasite combinations.

Sturhan (14) stated that variation in pathogenicity was the principal distinguishing characteristic of physiological races, primarily due to physiological or biochemical differences within the species. More specifically, he attributed the variation largely to an enzymatic process which determines the ability of nematodes to invade plants, feed and reproduce on them.

We conclude that populations of *M. incognita* in Tennessee differ considerably in pathogenicity. There is evidence that six physiologic races exist among 17 isolates collected, and it is reasonable to assume that more will be distinguished with additional collections in other localities or with additional differential hosts. Thus, it is evident that the control of root-knot nematodes through plant breeding and crop rotation is more complicated than we thought prior to this investigation. More information is needed concerning the nature and extent of variation in root-knot nematodes and the processes by which variants arise for plant breeding programs to succeed. Factors that may exert selection pressures and influence variability of populations of root-knot nematodes should be investigated. Additional information on the host ranges and responses must be obtained in order for crop rotations to be used effectively. These data should provide a base for further studies in characterizing populations of *Meloidogyne* spp.

Eventually, it may be necessary to devise a nomenclature for designating races within certain species of *Meloidogyne*. However, since so many races are already known, and there probably are numerous others still unknown, a formal designation for each race would be rather complex. Perhaps it would be more feasible to consider first a race designation for certain special races that are encountered often over a regional or larger area.

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