Parasitic Variability of *Meloidogyne incognita* Populations on Susceptible and Resistant Cotton

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Abstract: Root gall induction and egg production by the four recognized host races and two cytological races of *Meloidogyne incognita* were compared on cotton *Gossypium hirsutum* cvs. Deltapine 16 (root-knot susceptible) and Auburn 634 (highly resistant). The 12 nematode populations included in the study were from various parts of the world. No population increases occurred on the highly resistant cultivar. After 45 days, populations of host races 1 and 2 induced slight root galling on both cultivars with only limited reproduction. Host race 4 populations induced moderate root galling with higher reproduction on Deltapine 16 than that of race 1 or race 2 populations. Host race 3 populations induced severe root galling with population density increases of 7–30-fold. In a complementary study, 24 cotton cultivars or breeding lines were compared for suitability as hosts for a typical population of *M. incognita* race 3. The poorest hosts, 'Auburn 623,' 'Auburn 634,' and 'McNair 220,' yielded fewer eggs after 45 days than were added initially. The best hosts—'M-8,' 'DES 24-8,' 'McNair 235,' and 'Coker 201'—yielded > 5 times as many eggs as were added initially. *Key words:* root-knot nematode, *Gossypium hirsutum*, host suitability, Nematoda, Meloidogynidae, races, N.C. differential host test.

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Parasitic variability among populations of *Meloidogyne incognita* (Kofoid & White) Chitwood on cotton, *Gossypium* sp., has been reported since Chitwood's re-establishment of the genus *Meloidogyne* in 1949 (2). These reported population differences have been both qualitative (ability or inability to reproduce on cotton) and quantitative (varying degrees of reproduction on the same host species) (12,15,21). Such variation in the ability of *M. incognita* to parasitize the same cotton cultivar has been a persistent problem in the development and use of nematode-resistant cottons.

Based on differential host preferences, morphology, and cytological examination of the 222 *M. incognita* populations now in the International *Meloidogyne* Project Live Nematode Culture Collection, four distinct host races and two cytological races have been recognized (4,5,7,22,27,28). Only the host races designated as 3 and 4 have been reported to reproduce on cotton, and 33 populations belonging to these races have been collected from widely scattered geographical regions (19). This observation suggests that, in terms of cotton parasitism, the host race to which a given *M. incognita* population belongs is much more important than its geographical origin. Therefore, two populations of the same host race from quite different regions might be expected to respond similarly to a given cotton cultivar. So far, no correlations are evident between chromosome number and host response.

Only 15% of the *M. incognita* populations collected from around the world by the International *Meloidogyne* Project are capable of appreciable reproduction on 'Deltapine 16' cotton (19). While cotton-parasitic populations are somewhat limited in occurrence, they may be involved in significant cotton yield suppression either directly or through interactions with other micro-organisms (20). The importance of root-knot involvement in cotton disease is demonstrated by the active research on resistant cultivars over the past 30 years (11, 14,24,25,26,29).

The objectives of this study were as follows: i) determine the responses of *M. incognita* host races to various cotton cultivars; ii) compare reproduction of cotton-parasitic populations from different geographical areas on susceptible and resistant cotton; and iii) study host suitability of certain cotton cultivars and breeding lines to a "typical" race 3 population.

MATERIALS AND METHODS

Reproduction of M. incognita races: Twelve populations of *M. incognita*, representing the full range of diversity known to exist with respect to host preference, geographical distribution, and cytology, were

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selected from the International *Meloidogyne* Project collection at North Carolina State University (Table 1). They were increased on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) for 60 days in the greenhouse. Eggs were then extracted from the tomato roots by the NaOCl method (10) and collected.

Seeds of *Gossypium hirsutum* L. cv. Deltapine 16, which is susceptible to *M. incognita,* and cv. Auburn 634 RNR, which is resistant to *M. incognita,* were planted singly in 4-cm-d clay pots containing a potting mixture of steam-sterilized Norfolk sandy loam soil and river sand (1:1 v/v). At full expansion of the cotyledons, each seedling was carefully removed from the pot with root mass intact and transferred to an 11-cm-d clay pot partially filled with the potting mixture. Each plant was inoculated by pipetting 10 ml of a water suspension containing 2,000 eggs of the appropriate population over the root system. The pots were then filled with the potting mixture. To avoid contamination among pots, each 11-cm pot was nested inside an empty 16-cm-d clay pot, and the combined pots were set on individual saucers approximately 2 cm above the surface of a greenhouse bench. Pots were arranged according to a completely random design with seven replications per population for each cultivar. Noninoculated Rutgers tomatoes in 16-cm pots were included randomly in the experiment for assessment of splash-contamination. Plants were allowed to grow for 45 days at an average soil temperature of 26 C. Water and fertilizer were supplied as needed.

Table 1. Identification number, country of origin, cytological race designation, and host race designation of *M. incognita* populations.

Population No.	Country of origin	Cytological race*	Host race
83-1	USA/North Carolina	A	1
E89	Ghana	A	1
E34	Panama	A	2
E246	Nigeria	A	2
161	Philippines	A	3
188	Belgium	B	3
E233	Egypt	A	3
E264	Mexico	A	3
398	Peru	A	3
63	USA/Tennessee	B	3
401	USA/North Carolina	A	4
282	USA/South Carolina	A	4

*Race A populations have 40–46 chromosomes; race B populations have 32–36 chromosomes.

After 45 days, each root system was carefully washed free of soil and given a root gall index: 0 = no galls, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls, 5 = > 100 galls (27). All root systems were then stained with phloxine B (0.15 g/liter water) (3), and egg masses were rated according to the same scale used for root galls. After root ratings were completed, eggs were extracted by cutting roots into 1-cm segments, comminuting them in a Waring blender with 200 ml water at high speed for 15 sec, then shaking the mixture vigorously in 0.05% NaOCl for 3 min. Eggs were rinsed through a 75-μm sieve, collected on a 26-μm sieve, and counted. Oostenbrink's R factor (final egg count/initial inoculum density) was determined for each plant (18). Gall indices, egg mass indices, and total eggs per root system were compared by the Waller-Duncan K-ratio T-test. The experiment was repeated at an average soil temperature of 28 C.

Host suitability studies: Twenty-four cotton cultivars or advanced breeding lines were selected to study host suitability for a typical population of race 3. IMP population 178-3 was used because it was relatively aggressive and had responded consistently to Deltapine 16 cotton in previous IMP studies (unpublished). Acid delinted seeds of the cultivar or breeding lines were planted as described in the previous experiment. At full expansion of the cotyledons, each seedling was transferred into 11-cm pots and inoculated with 3,500 *M. incognita* eggs in 10 ml water. Seven replications of each cultivar or line were completely randomized. Average soil temperature during the study was 26 C. Plants were fertilized at weekly intervals. After 45 days, each plant was removed from the pot, washed free of soil, and rated for galling, egg mass production, and total eggs per root system as described previously. A Waller-Duncan K-ratio T-test was conducted for each set of data.

RESULTS

Reproduction of M. incognita races: On Deltapine 16 cotton, galls were induced by all *M. incognita* populations, but gall indices were significantly lower for host races 1 and 2 than for host races 3 and 4 (Table 2). Eggs were extracted from roots inoculated with host races 1 and 2, but intact egg masses were found only on roots inoculated with E246 (host race 2), and R factors were always <1. Gall indices were greater than 4.4 for all race 3 populations, and egg mass indices ranged from 3.2 to 4.8. Except for population 188, egg counts and R factors were significantly higher for race 3 than for the other races. Host race 4 populations had gall indices, egg mass indices, and egg counts that were higher than those of host races 1 and 2, but lower than those of race 3.

Table 2. Gall and egg mass indices, egg counts, and R values for host races of *Meloidogyne incognita* on Deltapine 16 cotton.

Population No.	Host race	Gall† index	Egg mass† index	Eggs/plant (thousands)	R‡ value
83-1	1	1.6 d*	0 d*	0.11 d*	0.05
E89	1	2.0 d	0 d	0.11 d	0.05
E34	2	1.4 d	0 d	0.01 d	0.01
E246	2	1.8 d	0.2 d	0.31 d	0.16
161	3	4.8 ab	4.2 a	37.72 bc	18.86
188	3	4.4 ab	3.2 b	7.12 d	3.5
E233	3	5.0 a	4.6 a	61.76 a	30.87
E264	3	4.8 ab	4.6 a	51.92 ab	25.96
398	3	4.6 ab	4.2 a	24.64 c	12.32
63	3	5.0 a	4.8 a	41.40 b	20.70
401	4	4.2 b	2.4 c	4.54 d	2.27
282	4	3.0 c	2.4 c	0.44 d	0.22

†Gall or egg mass index based on 0–5 scale where 0 = none and 5 = more than 100 per total root system.

‡R value = total eggs extracted ÷ initial eggs inoculated.

*Means within columns followed by the same letter do not differ significantly at $P = .05$ by Waller-Duncan K-ratio T-test.

Table 3. Gall and egg mass indices, egg counts, and R values for host races of *Meloidogyne incognita* on Auburn 634 RNR cotton.

Population No.	Host race	Gall† index	Egg mass† index	Total eggs/plant	R‡ value
83-1	1	1.2 bcd*	0 a*	8 e*	0.01
E89	1	1.4 bc	0 a	44 de	0.02
E34	2	0.4 d	0.2 a	12 c	0.01
E246	2	1.6 bc	0 a	30 cde	0.04
161	3	3.0 a	0 a	216 ab	0.11
188	3	1.8 bc	0.2 a	14 c	0.01
E233	3	2.0 b	0.4 a	194 ab	0.10
E264	3	3.2 a	0.6 a	144 bc	0.08
398	3	1.8 bc	0.2 a	232 a	0.12
63	3	2.0 b	0.4 a	100 cd	0.05
401	4	1.6 bc	0.2 a	32 de	0.02
282	4	1.0 d	0.2 a	12 c	0.01

†Gall or egg mass index based on 0–5 scale where 0 = none and 5 = more than 100 per total root system.

‡R value = total eggs extracted ÷ initial eggs inoculated.

*Means within columns followed by the same letter do not differ significantly at $P = .05$ by Waller-Duncan K-ratio T-test.

Auburn 634 RNR was a poor host for all *M. incognita* populations regardless of host race (Table 3). Slight root galling was induced by all populations, and eggs were detected in all race 3 and race 4 populations, but R factors were always <1.

Since none of the noninoculated tomato plants were infected at the end of the host race experiment, splashing of inoculum among pots was negligible.

Host suitability: A wide range of root galling and nematode reproduction occurred on the 24 cotton cultivars and breeding lines (Table 4). R factors ranged from .01 to 6.9. 'DES 24-8' and 'M-8' had gall indices, egg mass indices, and egg counts significantly higher than all other cultivars. 'McNair 220' and the Auburn breeding lines had the lowest nematode reproductive rates ($R \leq 1$). Cultivars 'DES 56,' 'Delcot 277,' 'Coker 310,' and 'Tamcot 788' were distinguished by intermediate resistance ($1 < R < 2$). Both the gall index and egg mass index were significantly correlated with the final egg counts (R = .46 and .50, respectively, at $P = .001$).

DISCUSSION

Our studies confirm that host races 1 and 2 of *M. incognita* do not reproduce well on either susceptible or resistant cottons. However, some root galling is induced by populations of these host races, and a low level of reproduction does occur. Since a few nematodes do reproduce, the possibility exists for genetic selection within non-cotton-parasitic host races for increased reproductive ability on cotton. This result agrees with previous reports of selection for biotypes of *M. incognita* under field conditions (9, 17).

Race 3 is the most successful host race on cotton in terms of reproduction. While quantitative population differences in reproductive abilities on susceptible cotton

Table 4. Gall and egg mass indices, egg counts, and R values for *Meloidogyne incognita* host race 3† on cultivars and breeding lines of cotton.

Cultivar or line	Gall‡ index	Egg mass index	Eggs/plant (thousands)	R§ value
Acala SJ-1	3.4 efgh*	3.1 bc*	14.7 cde*	4.2
Acala B3080	3.8 cde	3.4 abc	9.2 efgh	2.6
DES 24-8	4.4 ab	4.0 a	24.3 a	6.9
DES 56	3.1 ghi	2.4 def	5.2 hijk	1.5
Delcot 277	3.5 defg	2.9 cde	4.3 ijk	1.4
Delcot 311	3.4 efgh	3.1 bc	10.3 efgh	2.9
Coker 201	3.7 cdef	3.3 bc	17.8 bc	5.0
Coker 304	3.7 cdef	3.0 bcd	6.9 fghij	2.0
Coker 310	3.0 hi	2.4 def	6.3 ghij	1.8
Coker 315	3.1 ghi	2.4 def	7.4 fghij	2.1
Deltapine 16	4.0 bcd	3.3 bc	16.6 bcd	4.7
Deltapine 41	3.8 cde	3.0 bcd	13.8 cde	3.9
Deltapine 61	4.1 abc	3.0 bcd	8.1 fghij	2.3
McNair 220	3.3 fghi	2.4 def	3.1 jk	0.9
McNair 235	3.7 cdef	3.0 bcd	21.3 ab	6.1
Stoneville 213	3.8 cde	3.0 bcd	11.4 defg	3.3
Stoneville 506	3.3 fghi	3.0 bcd	7.8 fghij	2.2
Stoneville 825	3.8 cde	3.0 bcd	10.8 efgh	3.0
Tamcot 788	3.5 defg	2.9 cde	5.8 ghijk	1.6
Auburn 56	2.9 i	1.9 f	3.4 jk	1.0
Auburn 623	0.6 j	0.3 g	0.4 k	0.1
Auburn 634	0.4 j	0.3 g	0.3 k	0.1
M-8	4.6 a	4.0 a	23.7 a	6.7

†IMP Live Nematode Culture Collection No. 178-3.

‡Gall or egg mass index based on 0–5 scale where 0 = none and 5 = more than 100 per total root system.

§Reproductive value = total eggs extracted ÷ initial eggs inoculated.

*Means within columns followed by the same letter do not differ significantly at $P = .05$ by Waller-Duncan K-ratio T-test.

exist, all races are capable of significant population increases. Race 4 is much less successful than race 3; however, reproduction is significant. Race 4, encountered much less frequently than any of the other races, is characterized by the ability to reproduce on both Deltapine 16 cotton and *M. incognita*-resistant 'NC 95' tobacco. Population 282, originally reported as a resistance-breaking biotype of *M. incognita* (9), has been described as *M. grahami* (8).

Cytological race B populations (188 and 63) of host race 3 differed significantly in the number of eggs produced on susceptible cotton. Race B populations are very rarely encountered, and no correlation has been apparent between cytological race and host race (28). Because only two race B populations with very different responses on cotton were included in this study, no conclusions as to the relationship between chromosome number and response on cotton can be drawn.

Resistance in cotton to *M. incognita* has been actively sought for many years. Resistance in cotton is not simply inherited, and development of highly resistant cultivars with acceptable agronomic qualities has been slow. Auburn 634 RNR, a transgressive segregate of a cross between the moderately resistant cultivar 'Clevewilt' and a moderately resistant wild race of *G. hirsutum,* has exhibited an exceptional degree of resistance to a limited number of *M. incognita* populations (23). In our studies, this level of resistance was retained regardless of host race or geographical origin of the population.

A plant's suitability as a nematode host, as measured by the R factor, is usually indicative of its degree of resistance (6). However, root galling is many times not an adequate measure of the level of nematode reproduction. Galls on cotton roots are not necessarily correlated with the number of egg masses (7) or eggs (23). This general trend was also evident in our studies where gall indices were usually slightly higher than egg mass indices. Although both gall indices and egg mass indices were significantly correlated with final egg counts ($P = .001$), correlation coefficients were not high enough to permit direct prediction of absolute egg numbers with confidence.

In the field, both augmentation and suppression of root-knot nematode populations have been reported to result from the growth of resistant cotton cultivars (1,13, 16,23,25). In our greenhouse study, the low nematode reproduction on Auburn 623 and Auburn 634 indicates that these lines should suppress high root-knot nematode populations in the field during the first growing season.

LITERATURE CITED

1. Brodie, B. B., L. A. Brinkerhoff, and F. B. Struble. 1960. Resistance to the root-knot nematode, Meloidogyne incognita acrita, in Upland cotton seedlings. Phytopathology 50:673-677.

2. Chitwood, B. G. 1949. Root-knot nematodes. I. A. revision of the genus Meloidogyne Goeldi. 1887. Proc. Helminthol. Soc. Wash. 16:90–104.

3. Dickson, D. W., and F. B. Struble. 1965. A sieving-staining technique for extraction of egg masses of Meloidogyne incognita from soil. Phytopathology 55:497.

4. Eisenback, J. D., H. Hirschmann, J. N. Sasser, and A. C. Triantaphyllou. 1981. A guide to the four most common species of root-knot nematodes (Meloidogyne spp.), with a pictorial key. A cooperative publication of the Department of Plant Pathology, North Carolina State University, and the United States Agency for International Development. North Carolina State Graphics.

5. Eisenback, J. D., and H. Hirschmann. 1981. Identification of Meloidogyne species on the basis of head shape and stylet morphology of the male. J. Nematol. 13:513-521.

6. Fassuliotis, G. 1979. Plant breeding for root-knot nematode resistance, Pp. 425-453 *in* F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (Meloidogyne species) systematics, biology and control. London & New York: Academic Press.

7. Fox, J. A., and L. I Miller. 1973. Comparison of gall and egg-mass indices of two races of Meloidogyne incognita on differential hosts. Phytopathology 63:801.

8. Golden, A. M., and L. J. Slana. 1978. Meloidogyne grahami n. sp. (Meloidogynidae), a root-knot nematode on resistant tobacco in South Carolina. J. Nematol. 10:355-361.

9. Graham, T. W. 1969. A new pathogenic race of Meloidogyne incognita on flue-cured tobacco. Tob. Sci. 13:43-44.

10. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne spp. including a new technique. Plant Dis. Rept. 57:1025-1028.

11. Jones, R. W., and W. Birchfield. 1967. Resistance of the experimental cotton variety, Bayon, and related strains to root-knot nematode and Fusarium wilt. Phytopathology 57:1327-1331.

12. Kirby, M. F., D. W. Dickson, and G. C. Smart, Jr. 1975. Physiological variation within species of Meloidogyne occuring in Florida. Plant Dis. Rept. 59:353-356.

13. Kirkpatrick, T. L., and J. N. Sasser. 1981. Evaluation of selected nematicides and a resistant cultivar for control of Meloidogyne incognita on cotton. P. 22 *in* 1981 Beltwide Cotton Production Research Conferences, National Cotton Council of America, Memphis, TN.

14. Kappelman, A. J., Jr., and L. S. Bird. 1981. Indirect selection for resistance to Fusarium wilt-root-knot nematode complex in cotton. Crop Sci. 21:66-68.

15. Martin, W. J. 1954. Parasitic races of Meloidogyne incognita and M. incognita var. acrita. Plant Dis. Rept. Supplement 227:86-88.

16. Minton, N. A., E. J. Cairns, and A. L. Smith. 1960. Effect on root-knot nematode populations of resistant and susceptible cotton. Phytopathology 50:784-787.

17. Netscher, C., and C. E. Taylor. 1979. Physiologic variation with the genus Meloidogyne and its implications on integrated control. Pp. 269-294 *in* F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (Meloidogyne species) systematics, biology and control. London & New York: Academic Press.

18. Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Meded. Landbouwhogesch. Wageningen 66-4.

19. Sasser. J. N. 1979. Pathogenicity, host ranges, and variability in Meloidogyne species. Pp. 257-268 *in* F. Lamberti and C. E. Taylor, eds. Root-knot nematodes (Meloidogyne species) systematics, biology and control. London & New York: Academic Press.

20. Sasser, J. N. 1972. Nematode diseases of cotton. Pp. 187-214 *in* J. M. Webster, ed. Economic nematology. London & New York: Academic Press.

21. Sasser, J. N. 1966. Behavior of Meloidogyne spp. from various geographical locations on ten host differentials. Nematologica 12:97-98.

22. Sasser, J. N., and A. C. Triantaphyllou. 1977. Identification of Meloidogyne species and races. J. Nematol. 9:283.

23. Shepherd, R. L. 1979. A quantitative technique for evaluating cotton for root-knot nematode resistance. Phytopathology 69:427-430.

24. Shepherd, R. L. 1974. Breeding root-knot resistant Gossypium hirsutumL L. using a resistant wild G. barbadense L. Crop Sci. 14:687-691.

25. Shepherd, R. L. 1974. Transgressive segregation for root-knot nematode resistance in cotton. Crop Sci. 14:872-875.

26. Smith, A. L. 1954. Problems on breeding cotton for resistance to nematodes. Plant Dis. Rept. Supplement 227:90-91.

27. Taylor, A. L., and J. N. Sasser. 1978. Biology, identification and control of root-knot nematodes (Meloidogyne species). A cooperative publication of the Department of Plant Pathology, North Carolina State University, and the United States Agency for International Development. North Carolina State Graphics.

28. Triantaphyllou, A. C. 1981. Oogenesis and the chromosomes of the parthenogenetic root-knot nematode Meloidogyne incognita. J. Nematol. 13: 95-104.

29. Viglierchio, D. R. 1978. Resistant host responses to ten California populations of Meloidogyne incognita. PJ. Nematol. 10:224-227.