Probable Source of Meloidogyne incognita Resistance in Tobacco as Indicated by Reactions to Five Meloidogyne Isolates

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ABSTRACT

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Nicotiana species, an interspecific hybrid, and tobaccos that were reportedly parents of root knot-resistant tobacco, related *Nicotiana* lines as well as resistant *N. tabacum* 'NC95' and breeding line Bel 4-30 were inoculated with five root knot nematode isolates of the genus *Meloidogyne*. Resulting root knot indices indicated that the source of resistance to two of these nematodes in NC95 and Bel 4-30 is probably not tobacco introduction (T. I.) 706, as previously reported, but either *N. tomentosa* or perhaps *N. tomentosiformis*. *Nicotiana tomentosa,* but not *N. tomentosiformis* or T. I. 706, responded to each nematode almost identically to NC95 and Bel 4-30. Populations of *N. otophora* were highly variable in response to most of the nematode isolates and the pattern of response to the five nematode isolates eliminated this species as a possible source of the resistance in NC95 and Bel 4-30. *Nicotiana otophora* showed some resistance to two nematode isolates for which resistance is not now available. The results are discussed in relation to previous reports, the method by which resistance was transferred to tobacco, and their implications for future improvement of disease resistance in tobacco.

Additional key words: Meloidogyne arenaria, M. incognita acrita, M. incognita incognita, M. javanica.

Root knot, which is caused by species of *Meloidogyne* and most commonly by *M. incognita* (Kofoid & White) Chitwood, has been a major disease of tobacco, *Nicotiana tabacum* L., in the southeastern United States for many years (12, 19). Efforts to find resistance to this disease and transfer it to commercially acceptable tobaccos were initiated by Clayton et al. (4) in the mid-1930's. After screening numerous tobacco cultivars and tobacco introductions (T. I.'s), four resistant T. I.'s were selected for crossing with susceptible cultivars. Resistant progeny were obtained only from crosses with T. I. 706. Efforts then were initiated to transfer resistance from T. I. 706 to commercial cultivars. The resistance proved to be unstable; susceptible plants still occurred among test populations even after resistant plants were selfed for many generations. In the F_1, F_2, and subsequent generations the ratio of resistant to susceptible plants was not predictable. Furthermore, occurrence of intermediate levels of resistance complicated attempts to identify resistant plants. Resistance increased with continued backcrossing and selection, but resistant lines had small leaves and low yields.

Cytogenetic studies of *Nicotiana* to determine the ancestry of *N. tabacum* (n = 24) had led, before 1950, to the conclusion that it had originated from a cross of *N. sylvestris* Speg. & Comes (n = 12) with a species in Section Tomentosae—either *N. otophora* Griseb., *N. tomentosa* R. & P., or *N. tomentosiformis* Goodsp. (all n = 12). The last species was favored as the male parent by some workers in 1950 (5, 10, 11), and by most workers since then (1, 9, 16). Twelve of the 24 chromosomes of *N. tabacum* pair with those of *N. sylvestris* and the other 12 pair with those of the other three species at a relatively high frequency (5, 11).

In 1950, Clayton et al. (4) crossed the root knot-resistant line, RK42, with Kostoff's allopolyploid, reportedly *N. sylvestris* × *N. tomentosiformis* (Kostoff's hybrid) in an attempt to break the apparent linkage between small leaf size and resistance. This allopolyploid and other hybrid material evidently were obtained by Clayton from Kostoff, who had made crosses of *N. sylvestris* with *N. tomentosa* and *N. tomentosiformis*. Kostoff also crossed these species with *N. tabacum* and crossed the hybrids back to the parent species and *N. tabacum* (11). In 1972, Sheen (16) showed that the Kostoff hybrid, which was used by Clayton et al. (4), was a product of introgression with *N. tabacum* and not a

simple allopolyploid of *N. sylvestris* × *N. tomentosiformis*.

The cross of RK42 × Kostoff's hybrid produced valuable progeny (4). The leaf-size problem was eliminated and the F_1, F_2, and subsequent generations had a high level of root knot resistance that was controlled by a single dominant factor. The F_2 segregated into two distinct classes, resistant or susceptible; no plants had intermediate resistance. Progress in developing resistant cultivars was rapid, resulting in the release of NC95 by Moore et al. (13) in 1960. Many other flue-cured cultivars released since then carry this same resistance (12, 19). Clayton et al. (4) reported that *N. sylvestris* and Kostoff's hybrid were susceptible to root knot and that *N. tomentosiformis* was only moderately resistant. They theorized that the apparent changes in the genetics of resistance and in leaf size following the cross with Kostoff's hybrid were due to elimination of modifier genes that previously had reduced the effectiveness of the gene for root knot resistance and contributed to small leaf size.

Prior to 1949, when the work was with the unstable T. I. 706 resistance, all root knot nematodes were considered to be a single species, *Heterodera marioni* (Cornu) Goodey. In 1949, Chitwood (2) established *Meloidogyne* as the correct generic name, and identified five species and one subspecies in the genus. Among these species, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, *M. incognita incognita* Chitwood, and *M. incognita acrita* (Kofoid & White) Chitwood all are pathogens of tobacco in the southeastern United States; the latter two subspecies are most common, however.

The apparent change that occurred in the genetics of resistance in Clayton's material in 1950 could have been due to causes other than those theorized by Clayton et al. (4). The revision of the taxonomy of the pathogen occurred at almost the same time that the change was observed in the inheritance of resistance. This suggested to us that Clayton et al. (4) might have been using nematode populations containing two or more *Meloidogyne* spp. After Chitwood's publication (2), care would have been taken to be sure that the inoculum consisted of only a single species, *M. incognita*. If T. I. 706 had a different gene for resistance to each of two or more *Meloidogyne* spp., narrowing the inoculum to a single *Meloidogyne* sp. would have resulted in detection of monogenic resistance.

The purpose of our study was to determine the reaction of the germplasm used by Clayton et al. (4) and related germplasm to each of the *Meloidogyne* spp., subspecies, and races pathogenic on tobacco. If T. I. 706 plants were resistant to several different *Meloidogyne* isolates, this could explain the results of Clayton et al. (4). Some initial results were reported in an abstract (17).

MATERIALS AND METHODS

Nicotiana spp. (and their U. S. Department of Agriculture accession numbers) used in these experiments included *N. sylvestris* (56G), *N. tomentosiformis* (59G), *N. tomentosa* (58G), and *N. otophora* (38G). Interspecific hybrids included that of Kostoff, used by Clayton et al. (4), and Burk's *N. sylvestris* × *N. tomentosiformis* (1). Tobacco accessions included a root knot-susceptible cultivar, Hicks; NC95; Bel 4-30, a resistant Beltsville breeding line from the 1950's; T. I. 706 (that originated from Honduras), and T. I. 708. Root knot indices also were obtained for all 28 other Honduran accessions in the T. I. collection (T. I.'s 75, 180, 285, 286, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 539, 561, 562, 564, 565,

TABLE 1. Root knot indices of eleven *Nicotiana* spp., interspecific hybrids, and *N. tabacum* cultivars and lines 8 wk after inoculation with five *Meloidogyne* isolates[a]

Nicotiana spp. or accession	Disease indices produced by *Meloidogyne* isolates:				
	M. incognita acrita	*M. incognita acrita* (G)[b]	*M. arenaria*	*M. incognita incognita*	*M. javanica*
N. otophora	66.6 B[c]	36.4 C	63.5 B	91.0 AB	34.8 C
N. sylvestris	87.9 A	94.8 A	93.8 A	92.9 AB	85.4 A
N. tomentosa	0 C	90.5 AB	72.9 AB	0.8 C	51.6 BC
N. tomentosiformis	89.4 A	76.6 AB	91.3 AB	100.0 A	91.9 A
Kostoff's *N. sylvestris* × *N. tomentosiformis*[d]	81.7 A	76.4 AB	96.9 A	100.0 A	79.8 AB
Burk's *N. sylvestris* × *N. tomentosiformis*	77.3 AB	80.9 AB	96.9 A	84.4 B	78.9 AB
N. tabacum 'Hicks'	86.8 A	83.9 AB	97.5 A	97.2 A	78.3 AB
N. tabacum 'NC95'	1.4 C	90.6 AB	74.3 AB	2.2 C	52.4 BC
N. tabacum 'Bel 4-30'	3.4 C	89.4 AB	87.5 AB	1.8 C	62.1 ABC
N. tabacum T. I. 706[e]	86.4 A	66.8 B	97.9 A	99.0 A	49.8 BC
N. tabacum T. I. 708[e]	78.4 AB	79.1 AB	100.0 A	97.5 A	62.3 ABC

[a]Each disease index is an average from four tests of 15-20 inoculated plants of each species or accession for each test. An index of 0 indicates no traces of knotting and 100 indicates maximum severity. In two of the tests, each plant was inoculated with nematodes by mixing minced appropriately infected tomato roots with the soil, giving 800-1,000 larvae/pot of cultivar Hicks at the time of indexing. In the other two tests, inoculation was by pouring approximately 750 of the appropriate larvae onto the soil around each plant.

[b]*Meloidogyne incognita acrita* (G) is a race of this nematode that was first reported in 1969 by T. W. Graham to be virulent on previously resistant tobacco cultivar NC95.

[c]Values for the 11 species and accessions inoculated with each of the nematodes that are followed by the same letter are not significantly different by Duncan's new multiple range test ($P = 0.05$). These comparisons apply only to the vertical columns of figures.

[d]Kostoff's hybrid was reported to be a cross between these species, but may have had a different origin.

[e]T. I. = Tobacco Introduction (a U. S. Department of Agriculture designation).

566, 567, 568, 675, 704, 705, 710, and 711). All accessions were seeded in Beltsville soil (17) in 16.5-cm diameter clay pots. Seedlings were transplanted to a mixture of equal parts Beltsville soil (18) and silica sand in 6.6-cm diameter clay pots. Prior to use, soils and pots were autoclaved. Twenty potted plants of each accession, located on each

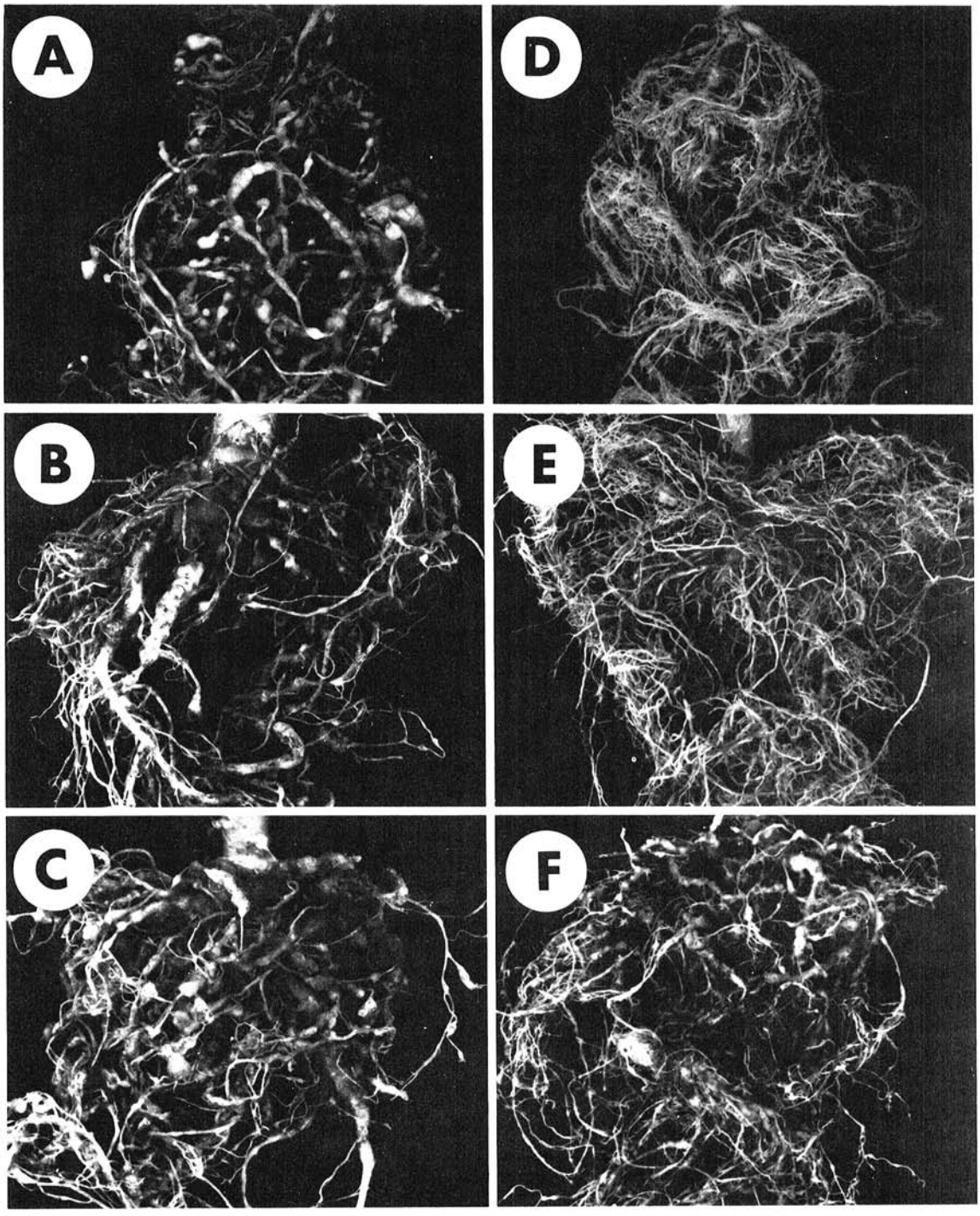

Fig. 1-(A to F). Root knot development 8 wk after inoculation with root knot nematodes. Roots A, B, and C were inoculated with *Meloidogyne arenaria* and D, E, and F with *M. incognita acrita*. Roots A and D are *Nicotiana tomentosa,* B and E are *N. tabacum* 'NC95', and C and F are *N. tabacum* T. I. 706. Note the similarity in the resistance of *N. tomentosa* and NC95 to knotting from *M. incognita acrita* and the susceptibility of T. I. 706 to this nematode. All three accessions are susceptible to *M. arenaria*.

of five isolated greenhouse benches, were inoculated. The inoculations of the Honduran T. I.'s were repeated on two replicate sets of plants. Those of all other accessions were repeated on four replicate sets of plants.

The nematode species, subspecies, and races that were tested included *Meloidogyne incognita incognita, M. incognita acrita, M. arenaria, M. javanica,* and Graham's (7, 8) new race of *M. incognita acrita;* the latter is highly pathogenic on NC95 and other formerly resistant cultivars. Each of the *Meloidogyne* isolates was derived from a single egg mass. The nematode populations were maintained and increased on tomato, *Lycopersicon esculentum* Mill. 'Rutgers'. Each of the five *Meloidogyne* isolates was maintained on an isolated greenhouse bench. The *Nicotiana* species, interspecific hybrids, tobacco cultivars, Bel 4-30, T. I. 706, and T. I. 708 were inoculated with each of the five nematode isolates, but the Honduran T. I.'s were not inoculated with *M. javanica* or Graham's race of *M. incognita acrita*. All plants were inoculated and maintained on the same greenhouse benches where the inoculum was produced. These benches were filled with sand to a depth of 15 cm and contained electric heating cables that maintained a temperature of 30-34 C for *M. javanica* and 25-29 C for all of the other nematodes. Air temperatures in the greenhouses were kept close to that of the sand. The pots containing soil and plants were buried in sand to the rim to stabilize the soil temperatures.

Two methods of inoculation were used, and half of the replicated tests were inoculated by each method. In one method, the plants were transplanted after about 3 wk from the 6.6-cm to 8.5-cm diameter pots containing soil infested with the desired nematode population. This soil was infested by mixing minced, infected tomato roots with the soil in which they were grown and diluting this with the usual soil-sand mixture. 'Hicks' tobacco plants inoculated by this method had root knot indices equivalent to those resulting from inoculation with 800-1,000 second-stage *Meloidogyne* larvae/pot. In the other inoculation method, water was added to the soil and tomato roots and the mixture was poured through 250-μm and 38-μm screens. Larvae were collected on the 38-μm screen. The nematodes then were cleansed and concentrated by the funnel technique of Christie and Perry (3). The collected larvae were rinsed in sterile water and surface-sterilized by the method of Peacock (14). After sterilization, 750 larvae in 2 ml of water were pipetted onto the soil containing each plant of the *Nicotiana* spp., interspecific hybrids, and *N. tabacum* accessions. A lower inoculum level, 500 larvae/plant, was used for the Honduran T. I.'s in an attempt to detect moderate levels of resistance. Plants inoculated by the second method had been in the 6.6-cm diameter pots for 3 wk and were kept in these same pots after inoculation.

The soil was washed from the roots of all plants and root knot indices were determined for each plant 8 wk after inoculation. The indexing method resembled the one used by Clayton et al. (4). Each plant was rated in one of six classes as follows: (i) (class 0) no visible symptoms; (ii) (trace, 0.5) traces of knotting present; (iii) (class 1) 3-15% of the maximum possible amount of knotting; (iv) (class 2) 15-30%; (v) (class 3) 30-65%; and (vi) (class 4) 65-100%. These indices were converted to a 0-100 disease index for each accession, with zero indicating no knotting. The identity of each nematode population was checked by microscopic examination of the stylet lengths, excretory pore locations, and perineal patterns of at least 16 random adult females, as well as the total and tail lengths of at least 16 larvae. This was done at the times of inoculation and indexing to determine whether accidental mixtures had occurred; none was detected.

The root knot index from each plant in each of the replicate tests was used in a statistical analysis of the data. Duncan's new multiple range test was used to detect significant differences ($P = 0.05$) among the average indices for the various *Nicotiana* species and accessions or T.I.'s when inoculated with each of the individual *Meloidogyne* isolates. Due to the design and objectives of these experiments, the differences among the indices from various *Meloidogyne* isolates on individual *Nicotiana* entries were not analyzed.

RESULTS

In each test, 20 plants were inoculated, but as many as five plants per accession died in some tests from

TABLE 2. Percent of *Nicotiana otophora* plants in each of six root knot severity classes 8 wk after inoculation with five *Meloidogyne* isolates[a]

Meloidogyne sp. isolate	Percent of plants in disease severity classes: 0	Tr	1	2	3	4	Disease index[c]
M. incognita acrita	0	7.4	22.2	14.8	22.2	33.3	66.6
M. incognita acrita (G)[d]	12.0	28.0	20.0	16.0	12.0	12.0	36.4
M. arenaria	0	7.1	0	10.7	21.4	60.7	63.5
M. incognita incognita	0	0	2.9	8.8	20.6	67.6	91.0
M. javanica	25.0	16.7	8.3	41.7	0	8.3	34.8

[a]The results are from four tests of 15-20 plants/test. The plants in two tests were inoculated by mixing minced, appropriately infected tomato roots with the soil. In the other two tests inoculation was by pouring a suspension of approximately 750 of the appropriate larvae onto the soil around each plant.

[b]Each figure is the percentage of all of the tested plants that were rated in each class. Disease severity classes include: 0, no symptoms; Tr, a trace of root knot detectable; 1, 3-15%; 2, 15-30%; 3, 30-65%; and 4, 65-100% of the maximum possible knotting on the root system.

[c]The disease index was obtained by converting the readings for each plant to an overall index with a minimum possible value of 0 for no symptoms and 100 for maximum knotting of the roots.

[d]*M. incognita acrita* (G) is a race of this nematode that was first reported in 1969 by T. W. Graham to be virulent on previously resistant tobacco cultivar NC95.

transplanting shock or other causes apparently unrelated to *Meloidogyne* infection. These plants were not included in the results.

There was no significant difference between the indices of NC95 and Bel 4-30 with any of the nematodes (Table 1). Symptoms caused by *M. arenaria* infection on NC95 are shown in Fig. 1-B. A few plants of NC95 and Bel 4-30 had a trace of knotting from *M. incognita acrita* and *M. incognita incognita,* but roots of the remainder were free of knotting (Fig. 1-E). Both NC95 and Bel 4-30 had average indices below 65 with *M. javanica,* but neither accession had any individual plants with lower indices than the 11.8 and 5.6% of the plants, respectively, that were in class 1. In 1964, Graham (6) reported that NC95 and a breeding line with resistance derived from the same source as in NC95 had some resistance to *M. javanica.* The cultivar Hicks had relatively high root knot indices with all five *Meloidogyne* isolates, but a slightly lower index with *M. javanica* than with the others. Root knot indices of NC95 and Bel 4-30 both were high when they were inoculated with Graham's race of *M. incognita acrita.*

Both T. I. 706, the reported source of root knot resistance in NC95 and Bel 4-30 (4, 13), and related T. I. 708 had relatively high root knot indices with *M. incognita acrita* and *M. incognita incognita,* the nematodes to which NC95 and Bel 4-30 are resistant (Table 1). The knotting that occurred on T. I. 706 inoculated with *M. incognita acrita* is shown in Fig. 1-F and the freedom from knotting of NC95 when inoculated with the same nematode is shown in Fig. 1-E. None of the T. I. 706 plants had lower indices than the 3.0% that were in class 1 with *M. incognita acrita.* Only 3.2% of the T. I. 706 plants inoculated with *M. incognita incognita* were in class 2; the remainder had higher indices. The reported source of resistance, T. I. 706, was less susceptible to Graham's race of *M. incognita acrita* and to *M. javanica* than it was to the nematodes to which NC95 and Bel 4-30 are resistant. This T. I. had a numerically but not significantly lower index to Graham's race than did NC95 and Bel 4-30. Ten percent of the T. I. 706 plants had a trace of infection with Graham's race and 26.7% were in class 1. Neither NC95 nor Bel 4-30 had any plants in the trace or class 1 categories with this nematode. Twenty percent of the T. I. 706 plants inoculated with *M. javanica* were in class 1 and the remainder were in higher classes. When inoculated with *M. arenaria,* root knot indices of all T. I. 706 plants were in classes 3 or 4 (Figure 1-C).

The two interspecific hybrids had relatively high root knot indices with each of the nematodes (Table 1). Kostoff's hybrid had the lowest index with Graham's race of *M. incognita acrita;* 4.8% of the plants had a trace of knotting, and none was entirely free. Burk's hybrid had a significantly lower index with *M. incognita incognita* than did Kostoff's hybrid. Otherwise, the disease indices of the two hybrids were not significantly different.

Nicotiana tomentosa had very similar disease indices to those of NC95 and Bel 4-30 with each of the nematodes (Table 1, Fig. 1-A, D). It was highly resistant to both *M. incognita acrita* and *M. incognita incognita* and, like NC95, it was significantly less susceptible to *M. javanica* than were *N. sylvestris* and *N. tomentosiformis.* The breeding line Bel 4-30 also had a relatively low index with *M. javanica.* The similarity of the reaction of *N. tomentosa* to that of NC95 and Bel 4-30 when inoculated with Graham's race of *M. incognita acrita* was particularly striking and suggests that all three have the same type of resistance. The root knot index of *N. tomentosa* inoculated with *M. arenaria* was also close to that of NC95 and Bel 4-30.

Nicotiana tomentosiformis had very high root knot indices with all of the nematodes except Graham's race of *M. incognita acrita.* When plants of *N. tomentosiformis* were inoculated with that nematode, 5.9% of the plants were in class 1, 26.5% in class 2, and the remainder were more severely knotted. Inoculation with each of the *Meloidogyne* isolates produced high indices on *N. sylvestris.*

Indices of *N. otophora* were usually lower than those of *N. sylvestris* or *N. tomentosiformis.* There was some (perhaps useful) resistance in *N. otophora* to *M. incognita acrita,* Graham's race of *M. incognita acrita, M. arenaria,* and *M. javanica.* However, the response of *N. otophora* to each of the *Meloidogyne* isolates was more variable (Table 2) than that of any of the other *Nicotiana* species, hybrids, *N. tabacum* accessions, or T. I.'s. Twelve and 25% of the *N. otophora* plants had no root knot symptoms when inoculated with Graham's race of *M. incognita acrita* and *M. javanica,* respectively, but 12.0 and 8.3% (respectively) of the plants were in class 4 with these respective nematodes.

All of the Honduran T. I.'s had high disease indices and none had indices significantly lower than those of T. I. 706 with any of the three nematodes tested (Table 3). A few of the plants of some T.I.'s were rated in class 1; the remainder were in higher classes. Only T. I.'s 486, 488, 704, and 708 had indices as low as T. I. 706 with *M.*

TABLE 3. Root knot indices of the least susceptible Honduran tobacco introductions 8 wk after inoculation with three *Meloidogyne* isolates

Tobacco introduction	Disease indices produced by *Meloidogyne* isolates:[a]		
	M. incognita acrita	*M. arenaria*	*M. incognita incognita*
486	79.6 BC[b]	92.5 A	87.5 ABC
488	82.1 ABC	89.0 A	94.6 AB
562	88.3 AB	91.0 A	75.8 C
568	84.2 AB	83.5 A	83.3 ABC
675	83.3 AB	94.5 A	81.2 BC
704	80.0 BC	94.5 A	91.7 AB
705	85.0 AB	84.8 A	95.8 AB
706	70.8 C	88.5 A	81.3 BC
711	93.3 A	96.3 A	97.9 A

[a]Each figure is the average disease index from two tests of 17-20 plants/test. The higher the index the more knotting was present on the root systems. The plants in one test were inoculated by mixing minced, appropriately infected tomato roots with the soil. In the other test inoculation was by pouring a suspension containing approximately 500 of the appropriate larvae onto the soil around each plant.

[b]Values for the nine tobacco introductions inoculated with each of the nematodes that are followed by the same letter are not significantly different by Duncan's new multiple range test ($P = 0.05$). These comparisons apply only to the vertical columns of figures.

incognita acrita (Tables 1 and 3). Eight of the T. I.'s, including 486, 488, 562, 568, 675, 704, 705, and 708 did not differ significantly from T. I. 706 with *M. incognita incognita*.

DISCUSSION

In our tests, none of the parental lines used by Clayton et al. (4) in developing the breeding lines from which the root knot-resistant tobacco cultivars were obtained had resistance like that of NC95 or Bel 4-30. However, we found the resistance of *N. tomentosa* to be virtually identical with that of NC95 and Bel 4-30. In another series of inoculations (L. J. Slana and J. R. Stavely, *unpublished*), none of the other *Nicotiana* spp. had a pattern of root knot responses to the several *Meloidogyne* isolates similar to those of NC95, Bel 4-30, and *N. tomentosa*. These results strongly suggest that the source of root knot resistance in flue-cured tobacco cultivars was *N. tomentosa* and not T. I. 706.

Kostoff (11) reported the production of hybrids between *N. sylvestris* and both *N. tomentosa* and *N. tomentosiformis*. These hybrids were self-sterile, but he also studied several crosses involving these species and *N. tabacum* in various combinations, some of which produced abundant seed. Sheen (16) showed that the Kostoff hybrid used by us and Clayton et al. (4) is a product of introgression with *N. tabacum*.

Nicotiana tomentosa is highly polymorphic (5, 10) and there are at least two strains of *N. tomentosiformis* (5). We were able to test only one uniform selection of each of these species. Reactions of other selections of these species may differ from those of the selections tested by us. Clayton et al. (4) reported that neither *N. tomentosa* nor *N. tomentosiformis* had a particularly low disease index. Thus, they apparently had a strain of *N. tomentosa* different from the one used by us or their inoculum was not pure *M. incognita*. When *N. tomentosa* was inoculated with *M. incognita acrita* in the field at Beltsville in 1955 (J. J. Grosso and H. E. Heggestad, *unpublished*), only one plant survived and it had a trace of infection.

Clayton et al. (4) reported that the F_1 progeny from RK42 × Kostoff's hybrid and the BC_1 plants from the cross of resistant F_1 plants with tobacco segregated for resistance to *M. incognita*. They obtained 12 homozygous resistant and three segregating F_3 populations from 25 resistant F_2 plants. Populations of F_2 plants from crosses between homozygous resistant selections and susceptible tobacco cultivars segregated into distinct resistant and susceptible classes and no plants had intermediate levels of resistance. Clayton et al. (4) concluded from their results that the resistance obtained following the cross with Kostoff's hybrid was controlled by a monogenic dominant factor derived from T. I. 706.

Our results, and the background information discussed above, support the theory that RK42 was crossed with a heterozygous resistant plant of Kostoff's hybrid. If the cross were made with a homozygous resistant plant, all F_1 plants would have been resistant. It seems likely that this Kostoff hybrid could have had a *N. tomentosa* parent similar to the strain that was resistant in our tests. However, we do not have sufficient information to eliminate the possibility that the parent was a resistant strain of *N. tomentosiformis,* not currently available. In hybrids between these two species, all 12 chromosomes form pairs, the seed have good viability, and the F_2 plants are fertile (5, 11). The susceptible population of Kostoff's hybrid that we tested probably was descended from susceptible plants in the segregating population that contained the heterozygous resistant plant crossed with RK42. Clayton et al. (4) also may have tested such an advanced population, but their high disease index for Kostoff's hybrid could also have resulted from the presence of a large proportion of susceptible plants in the population that contained the heterozygous resistant plant crossed with RK42.

The heterozygosity of the *M. incognita* resistance in the Kostoff hybrid that was crossed with RK42 could originate in the *N. tomentosa* or *N. tomentosiformis* parent or could result from pairing of the chromosome carrying the resistance factor with a *N. sylvestris* chromosome. However, it probably resulted from pairing with a tobacco chromosome carrying the recessive allele. The latter possibility is supported by the results of Sheen (16) and the studies on chromosome homologies by Kostoff and others (5, 11).

The occurrence of T. I. 706 plants with considerable resistance to Graham's race of *M. incognita acrita* and the lack of resistance to this race in NC95, Bel 4-30, and *N. tomentosa* is further evidence against T. I. 706 being the source of root knot resistance in NC95 and Bel 4-30. The root knot index of T. I. 706 was lower with Graham's race than with the *M. incognita* collections to which NC95 and Bel 4-30 are resistant. Furthermore, none of the other Honduran T. I.'s had low indices when inoculated with these nematodes. Our population of T. I. 706 seems to be less resistant to *M. incognita acrita* and *M. incognita incognita* than the population tested by Clayton et al. (4). Over the last 30 yr, seed increase for T. I. 706 has been done in the absence of nematode inoculum. Seed apparently has been harvested from the less resistant plants in a heterogeneous population.

Our results with *N. otophora* indicated that this species is highly variable in response to several of the *Meloidogyne* isolates. Schweppenhauser et al. (15) also found that *N. otophora* varied in response to *M. javanica,* the only nematode that they used. In our tests, *N. otophora* was most resistant to Graham's race of *M. incognita acrita* and to *M. javanica*. Seed is being saved from the *N. otophora* plants that were most resistant to these nematodes in an effort to obtain a uniformly resistant population to cross with *N. tabacum*. Although *N. otophora* is closely related to *N. tomentosa* and *N. tomentosiformis,* the lack of *N. otophora* plants highly resistant to the original race of *M. incognita acrita* and to *M. incognita incognita* as well as the presence of plants resistant to Graham's race of *M. incognita acrita* seem to eliminate this species as a possible source of the resistance in NC95 and Bel 4-30.

Our results strongly suggest that the resistance to two root knot nematodes in NC95 and Bel 4-30 originated from *N. tomentosa* or perhaps *N. tomentosiformis*. This resistance is now present in cultivars grown on over 50% of the flue-cured tobacco acreage in the southeastern United States. It has saved growers millions of dollars in disease losses since the first resistant cultivar, NC95, was

released 16 yr ago (13). In types of tobacco other than flue-cured, resistance to at least four diseases has been transferred from wild *Nicotiana* species to commercial cultivars (12). Prior to this report, all of the widely-used disease resistance in American flue-cured tobacco was thought to have come from other cultivated tobaccos or T. I.'s (12) and none from other wild *Nicotiana* species. As a result, a theory that resistance factors from wild *Nicotiana* species are very difficult to use in the flue-cured type has gained considerable support. We now have evidence that one of the most widely used sources of disease resistance in flue-cured tobacco came from a wild *Nicotiana* species, albeit one closely related to tobacco. In the future, resistance to other nematodes from such species as *N. otophora* and resistance from *Nicotiana* spp. to other pathogens must be considered potentially useful for improvement of disease resistance in the flue-cured type, bearing in mind the potential for pathogen variability and dispersal in relation to the genetics of resistance.

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