Interactions Between *Meloidogyne incognita, M. hapla,* and *Pratylenchus brachyurus* in Tobacco[1]

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Abstract: In a greenhouse pot experiment on the pathogenicity and interactions of *Meloidogyne incognita, M. hapla* and *Pratylenchus brachyurus* on four cultivars of tobacco the cultivars 'Hicks' and 'NC 2326' were susceptible to each nematode and 'NC 95' and 'NC 2512' resistant only to *M. incognita.*

Mean heights of susceptible plants were depressed but fresh weight of tops did not differ significantly. *Meloidogyne* spp. increased fresh weight of susceptible (but not the resistant) roots.

Reproduction of *M. incognita* was decreased in the presence of *P. brachyurus* in one case. *M. hapla* reproduction was less with either of the other nematodes in five out of eight cases. In 12 combinations involving *P. brachyurus,* reproduction of this species was depressed in seven, not affected in four and increased in one.

Mechanisms involved in associative interactions were not identified but appeared to be indirect and to involve individual host-nematode responses. *Key Words: Meloidogyne, Pratylenchus,* Root-knot nematode, Lesion nematode, *Nicotiana tabacum,* Tobacco, Interaction, Mixed populations.

Much information on the host/parasite relationships of plant-parasitic nematodes has been gained from greenhouse and laboratory observations of one species of nematode on one kind of plant, a situation rarely occurring in the field. Oostenbrink (13) observed that plant-parasitic nematodes generally occur in polyspecific communities and that most species have a wide host range with considerable overlap. Thus, many opportunities exist for competition, synergism, and other types of interaction. The relevance of such associations to studies of nematode population dynamics has been recognized (12).

In North Carolina three important nematode parasites of tobacco commonly occur in mixed communities. The southern root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, is the most prevalent and injurious in tobacco (3, 15). In the northeastern counties the northern root-knot nematode, *M. hapla* Chitwood, is more common where it is an important parasite of peanut but less injurious to tobacco than *M. incognita* (3). Both species coexist, particularly where tobacco is rotated with peanuts. The lesion nematode, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven is prevalent throughout the state. It is frequently associated with root-knot nematodes and is damaging to certain tobacco cultivars (7, 8, 9).

The present study was designed to characterize the interactions of these three nematodes on tobacco.

MATERIALS AND METHODS

TOBACCO VARIETIES: Criteria for selecting the four tobacco cultivars used in this experiment included: (i) susceptibility to the *Meloidogyne* spp., (ii) susceptibility to *Pratylenchus brachyurus,* (iii) genetic pedigree, and (iv) yield and market quality characteristics. 'Hicks' and 'NC 2326' are susceptible to *M. incognita* and *M. hapla* (7). The former is a suitable host for *P. brachyurus* (7, 8, 16), whereas the latter has not been characterized. Both cultivars produce moderate yields of high quality leaf. 'NC 2326' closely resembles 'Hicks', its backcross parent, both phenotypically and genetically, ex-

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cept that 'Hicks' is susceptible to blackshank, caused by *Phytophthora parasitica* (Dast.) var. *nicotianae* (Breda de Haan) Tucker and 'NC 2326' is resistant. 'NC 95' (4, 11) and 'NC 2512' (14) are resistant to *M. incognita* but are susceptible to *M. hapla*. 'NC 95' is a less suitable host for *P. brachyurus* than 'Hicks' (7) whereas the status of 'NC 2512' is not known. 'NC 95' and 'NC 2512' have the same source of root-knot resistance, but differ considerably in field type and other characteristics.

PRODUCTION AND CARE OF PLANTS: A seeding and potting medium was prepared 3:1 (v/v) methyl bromide-treated (454 g/1.4 m^3) sandy loam and builder's sand sieved through a 7-mm screen. Tobacco seed were sown in 20-cm plastic pots and covered with a thin layer of vermiculite. At the three- or four-leaf stage (approximately 3 weeks old), the seedlings were transplanted to 5-cm clay pots and grown 2 weeks before inoculation. Selected transplants of uniform size were transferred to 15-cm pots containing potting medium with little disturbance of the root system and surrounding soil. A complete nutrient solution (840 g of a commercial fertilizer, VHPF®[3] plus 148 g of $MgSO_4 \cdot 7H_2O$ in 100 liters of tap water) was added 100 ml per pot each week for 2 weeks then 100 ml per pot semiweekly until the experiment was terminated 50 days after inoculation. Tap water was supplied by means of an automatic watering system with a plastic tube supplying water to each pot.

INOCULATION TREATMENTS AND PROCEDURES: *M. incognita* and *M. hapla* inocula levels were approximately 1,000 nematodes or eggs per kg soil (considered a heavy infestation) supplied via well-developed egg masses hand picked from 50-day-old populations on heavily-galled tomato (*Lycopersicon esculentum* L., 'Rutgers') roots. The number of eggs per egg mass was estimated by the sodium hypochlorite method described by Loewenberg, et al. (10). Cultures of *P. brachyurus* were maintained via handpicked individual specimens transferred to snapbean (*Phaseolus vulgaris* L., 'Tendergreen') (16). Inoculum was extracted from infested roots by maceration and sieving according to Taylor and Loegering (17) and Seinhorst modified by Goodey (6). The resultant suspension of larvae and adults was washed, concentrated and calibrated as reported by Southards and Nusbaum (16).

Nematode species in the inoculum and their code designations follow: Control—no inoculum (CK); *M. incognita* (I); *M. hapla* (H); *P. brachyurus* (B); *M. incognita* + *M. hapla* (IH); *M. incognita* + *P. brachyurus* (IB); *M. hapla* + *P. brachyurus* (HB); and all three species combined (IHB). Each pot was filled with potting medium and an 8-cm deep, 5 × 5-cm depression formed in the surface. Inoculated pots received 50 ml of nematode suspension distributed at the periphery of that depression (CK's received comparable volume of water) then the transplants were set in the depression and the soil from the edge washed gently in around the roots. Three replicates for each treatment were arranged in a randomized block on a greenhouse bench where the ambient temperature ranged from 25 to 32 C.

COLLECTION AND ANALYSIS OF DATA: At the conclusion of the experiment, plant heights were measured and the tops were cut off at the surface of the soil and weighed. Roots were carefully freed from soil, washed, blotted, and weighed.

Reproduction of *M. incognita* and *M. hapla* was estimated from the total number of eggs produced per root system. Two 2-g samples of chopped root fragments (about

[3] Miller Chemical Company, Baltimore, Maryland. VHPF® contains 6% nitrogen, 25% available phosphoric acid, 15% potash and minor elements. Mention of trademark name or proprietary product does not constitute a guarantee or warranty of the product by North Carolina State University, or recommendation of it to the exclusion of other products that may be suitable.

1 cm long) were chemically treated with constant stirring in 40 ml of 10 percent NaOCl solution (10) in separate 150-ml beakers. After 5 min water was added to make 100 ml of suspension. Duplicate 1-ml aliquots of the suspension were placed in dishes and eggs were counted. Estimates of *P. brachyurus* were determined by macerating duplicate 5-g samples of chopped roots in 40 ml of tap water in a food blender at high speed for 20 sec (6, 17). The resulting slurry was washed through 40-, 200- and 325-mesh sieves and the residue from each sieve was placed in separate Baermann funnels. Lesion nematodes were recovered from the funnels and counted at 2-day intervals for 2 weeks. Nematode and egg counts and root weights were used to compute the total numbers of individuals per plant.

Results

Most nematode inoculation treatments depressed plant height of root-knot susceptible varieties, 'Hicks' and 'NC 2326', but not of resistant varieties, 'NC 95' and 'NC 2512' (Table 1). *P. brachyurus* alone (B) was the only species that failed to decrease plant height in any variety. *M. hapla* alone (H) caused a significant reduction only in 'NC 2326' plants. *M. incognita*, alone (I) or in combination with other species (IH, IB, and IHB) caused significant height reduction in susceptible varieties. Treatment means across all varieties showed that all inoculations, except *P. brachyurus* alone (B), reduced plant heights significantly. Variety means across all treatments showed that 'NC 2512' plants were tallest, 'NC 95' plants were shortest and plants of 'Hicks' and 'NC 2326' were intermediate. Fresh top weights of varieties were not affected significantly by the inoculations.

M. incognita alone (I) and *M. hapla* alone (H) significantly increased root weights of susceptible varieties, 'Hicks' and 'NC 2326' (Table 1). This was true also where 'Hicks' plants were inoculated with both *Meloidogyne* species (IH) and with *M. hapla* + *P. brachyurus* (HB). No treatment, except *M. hapla* alone (H) on 'NC 95', caused significant changes in root weights of resistant varieties. Treatment means across all varieties showed that root weights were significantly increased by inoculation with *M. incognita, M. hapla,* or a combination of both species. Variety means across all treatments showed that the root weights of 'NC 2512' plants were significantly lower than those of the other varieties.

Table 1. Effect of *Meloidogyne incognita* (I), *M. hapla* (H), and *Pratylenchus brachyurus* (B), singly and combined upon plant height and fresh root weight of four tobacco varieties.

Inoculation Treatment	Variety: Hicks	NC 2326	NC 95	NC 2512	Treatment means
	Mean plant height (cm)				
CK	116	132	83	126	114
I	87**[a]	88**	79	118	93
H	105	114*	79	113	103
B	119	118	89	112	110
IH	86**	95**	75	120	94
IB	90**	92**	87	116	96
HB	98*	108**	87	116	102
IHB	94**	87**	82	116	95
Variety means	99	104	83	117	

LSD .05: Variety means = 8; treatment means = 7; varieties across treatments = 15.

	Mean root weights (g)				
CK	128	146	144	139	140
I	165**[b]	181**	145	133	156
H	172**	197**	167*	150	171
B	123	138	140	130	133
IH	174**	150	157	133	153
IB	145	145	126	130	137
HB	159**	156	148	134	149
IHB	148	131	140	119	135
Variety means	155	154	147	133	

LSD .05: Variety and treatment means = 11; varieties across treatments = 23.

[a] * and ** = lower than the CK treatment at the .05 and .01 levels of significance, respectively.

[b] * and ** = higher than the CK treatment at the .05 and .01 levels of significance, respectively.

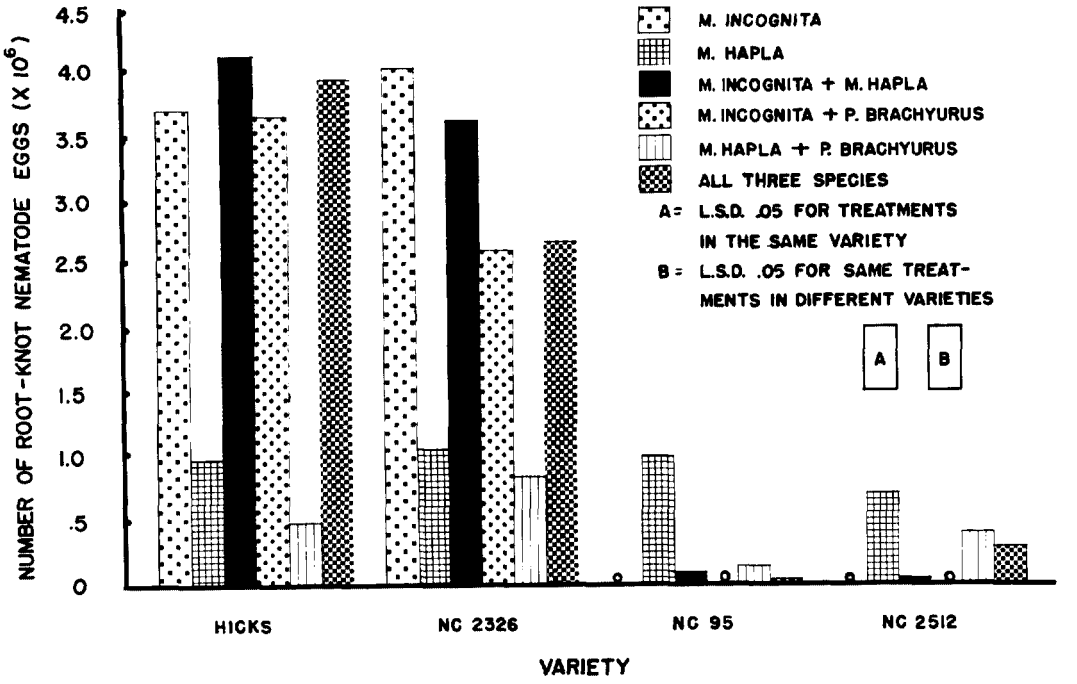

FIG. 1. Number of root-knot nematode eggs recovered from roots of four varieties of tobacco 50 days after inoculation with *M. incognita, M. hapla* and *P. brachyurus,* singly and combined.

When ratios of fresh top weight to fresh root weight were calculated, the values for noninoculated checks of all varieties were similar, ranging from 2.18:1 to 2.36:1. In root-knot susceptible varieties, certain inoculation treatments caused marked shifts in the ratios reflecting the influence of nematodes on weights of tops and roots, particularly the latter. With 'Hicks', for example, the ratio for check plants was 2.36:1. Ratios for *M. incognita, M. hapla,* and the combination of these species were 1.86:1, 1.87:1, and 1.72:1, respectively. The ratio for *P. brachyurus* was 2.46:1.

Where plants were inoculated with single nematode species, final population densities provided an estimate of relative host suitability of each variety for each species. 'Hicks' and 'NC 2326' plants were highly suitable for *M. incognita* (Fig. 1). Over 3.5×10^6 eggs were produced per plant. This species failed to reproduce in resistant varieties. Host suitability of the four varieties for *M. hapla* was characterized as fair. Egg production ranged from about 0.7 to 1.0×10^6 per plant. Host suitability of the varieties for *P. brachyurus* showed marked similarities between 'Hicks' and 'NC 2326' and between 'NC 95' and 'NC 2512', as well as differences between the two pairs (Fig. 2). Final population densities were nearly 15,000 per plant in the former, more than twice that of the latter.

Single nematode species used in combination with either one or both of the other species, had final population densities, in some instances, different from those obtained with corresponding single species inoculations (Fig. 1, 2).

On susceptible varieties inoculated with

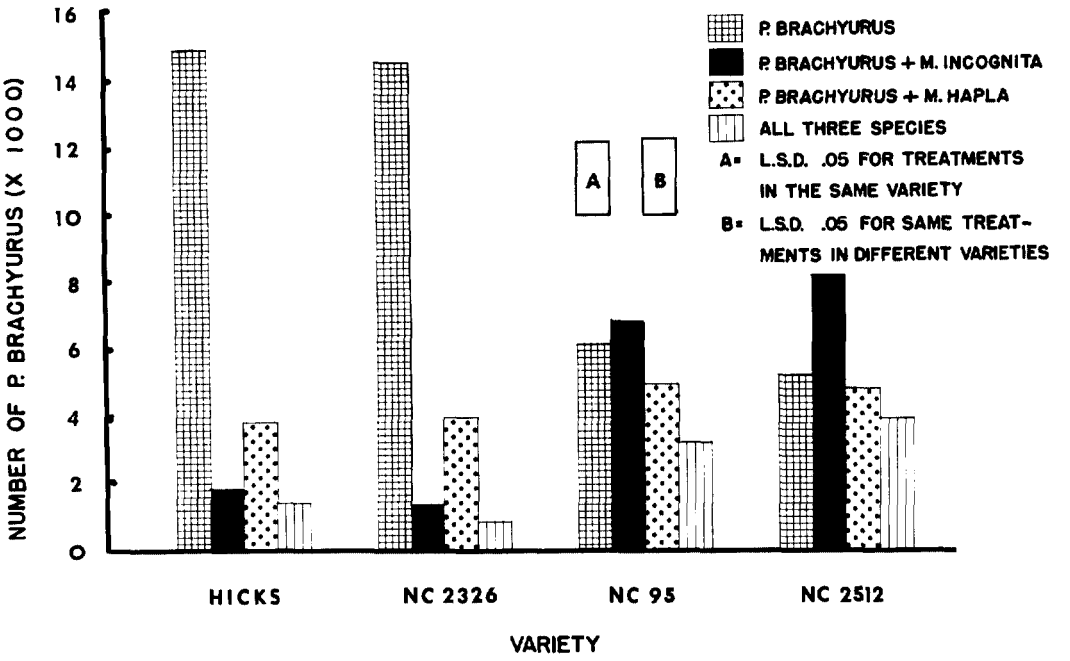

FIG. 2. Number of lesion nematodes recovered from roots of four varieties of tobacco 50 days after inoculation with *P. brachyurus, M. incognita* and *M. hapla,* singly and combined.

M. incognita + *M. hapla*, number of eggs was not significantly different from those obtained with *M. incognita* alone (Fig. 1). Reciprocal effects were not determined because of the problem of distinguishing between the eggs of *M. incognita* and those of *M. hapla*. It is apparent, however, that there was little, if any, associative effect between these species in 'Hicks' and 'NC 2326' plants. Reproduction of *M. hapla* was depressed by association with *M. incognita* in 'NC 95' and 'NC 2512' plants.

The influences of *P. brachyurus* on *M. incognita* and on *M. hapla* reproduction differed with host variety (Fig. 1). *P. brachyurus* affected *M. incognita* adversely in 'NC 2326' but not in 'Hicks', whereas it depressed *M. hapla* reproduction in 'Hicks' and 'NC 95' but not in 'NC 2326' or 'NC 2512.' Where plants were inoculated with all three species egg yields with 'Hicks' and 'NC 2326' were similar to those obtained from *M. incognita* + *P. brachyurus,* whereas with 'NC 95' and 'NC 2512' they were similar to those obtained from *M. hapla* + *P. brachyurus.*

In 'Hicks' and 'NC 2326' plants, *P. brachyurus* was depressed markedly by *M. hapla* and even more by *M. incognita* (Fig. 2). In all species combinations that included *M. incognita,* final populations densities were below the maintenance level of 2,500 per plant. In plants of root-knot resistant varieties, however, the only significant depression of *P. brachyurus,* occurred in the three-species mixture on 'NC 95'. In 'NC 2512' plants a significantly higher population of *P. brachyurus* was found in plants inoculated with *P. brachyurus* + *M. incognita* than in those inoculated with the lesion nematode

TABLE 2. Summary of reciprocal effects of nematode species on each other when used in certain mixtures on four varieties of tobacco.[a]

Species combinations	Rating by variety[b]			
	Hicks	NC 2326	NC 95	NC 2512
Paired				
Meloidogyne incognita +	?	?		
M. hapla	?	?	××	××
M. incognita +	O	××		
Pratylenchus brachyurus	×××	×××	O	+
M. hapla +	×	O	××	O
P. brachyurus	××	××	O	O
3-way				
M. incognita +	?	?		
M. hapla +	?	?	××	O
P. brachyurus	×××	×××	×	O

[a] Based upon final population densities of species used singly versus those of corresponding species used in combination with either one or both of the other species.

[b] The following rating symbols were used: O = no significant change in population; ×, ××, and ××× = significant, marked, and drastic population reduction, respectively; + = significant population increase; ? = rating doubtful because of the problem of distinguishing between eggs of *M. incognita* and *M. hapla*. No data are shown for *M. incognita* on NC 95 and NC 2512 because no reproduction occurred on these varieties.

alone. This was the only case of apparent stimulation noted.

A summary of associative effects between nematode species observed in the 36 combinations of nematode species mixtures and tobacco varieties (Table 2) provides a simplified key to the interpretation of the nematode population data presented in Figs. 1 and 2. Each nematode species was represented in 12 combinations. The effects of other nematode species on *M. incognita* reproduction was ascertained in only two cases and both involved *P. brachyurus*. *M. hapla* reproduction was adversely affected by other species in five of the eight comparisons. In the 12 comparisons involving *P. brachyurus*, populations of this species were depressed in seven, were not affected in four and were increased in one. Greatest depression appeared to be caused by *M. incognita* in root-knot susceptible varieties.

DISCUSSION

The experimental variables employed in this study were limited to nematode species, used alone and in various mixtures, and host genotypes. Single inoculum levels of each nematode species were employed and all inocula were applied simultaneously. Environmental conditions were variable but comparable for all treatments throughout the 50-day period of the test. It is likely that different results would have been obtained if other variables and environmental regimes had been employed. For example, Chapman (2), studying coincident infections by *M. incognita* and *M. hapla* in tomato, showed that both temperature and inoculum level differentially affected infection and reproduction of each species. In our study, ambient greenhouse temperatures (25–32 C) were considered more favorable for *M. incognita* and *P. brachyurus* than *M. hapla*. This may account for the relatively low reproduction of *M. hapla* in all varieties.

Although the mechanisms responsible for nematode species interactions observed were not studied, knowledge of host parasite relationships of individual species may provide clues to them in certain instances. Chapman (1) studied an interaction between *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa and *Tylenchorhynchus martini* Fielding in alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) and reported that *P. penetrans* had an adverse effect on *T. martini* populations whereas *T. martini* had little, if any, effect upon *P. penetrans*. He postulated that root damage caused by *P. penetrans* reduced the food supply available to *T. martini*. Ferris, Ferris, and Bernard (5) studied *Pratylenchus alleni* Ferris alone and in combination, in soybean (*Glycine max* Merr.). Regardless of the initial population densities, final populations of *P. penetrans* were higher than those of *P. alleni*. It was suggested that the higher proportion of fe-

males in *P. penetrans* populations than in those of *P. alleni* might have accounted for these differences.

In our studies, the depressive effects of *M. incognita* upon *M. hapla* in plants of 'NC 95' and 'NC 2512' may be related to rapid necrosis of root tips caused by invasion of *M. incognita* larvae (4). This hypersensitive reaction could reduce the number of infection sites available for successful colonization by larvae of *M. hapla*. In these varieties, however, *P. brachyurus* populations were not adversely affected by *M. incognita* indicating that root tip necrosis did not interfere with the activities of lesion nematodes. Although these migratory endoparasites sometimes enter root tips and the region of root elongation, they are characteristically found in the cortex of roots.

The possible mechanisms by which the root-knot nematodes suppressed reproduction of *P. brachyurus* in 'Hicks' and 'NC 2326' plants or which caused reciprocal interference between *M. incognita* and *P. brachyurus* in 'NC 2326' and between *M. hapla* and *P. brachyurus* in 'Hicks' are not known. It may be an indirect expression of host response not only to individual species but also to a combination of species. This study, although exploratory and descriptive in nature, may stimulate further studies of host-parasite-parasite interactions designed to elucidate the physiological processes involved.

Literature Cited

1. Chapman, R. A. 1959. Development of *Pratylenchus penetrans* and *Tylenchorhynchus martini* on red clover and alfalfa. Phytopathology 49:357–359.
2. Chapman, R. A. 1965. Infection of single root systems by larvae of two coincident species of root-knot nematodes. Nematologica 12:89. (Abstr.)
3. Clayton, E. E., T. W. Graham, F. A. Todd, J. G. Gaines, and F. A. Clark. 1958. Resistance to the root-knot disease of tobacco. Tob. Sci. 2:53–63.
4. Drolsom, P. N., and E. L. Moore. 1958. Reproduction of *Meloidogyne* spp. in flue-cured tobacco lines of root-knot resistant parentage. Plant Dis. Rep. 42:596–598.
5. Ferris, Virginia R., J. M. Ferris, and R. L. Bernard. 1966. Relative competitiveness of two species of *Pratylenchus* in soybeans. Nematologica 13:143. (Abstr.)
6. Goodey, J. B. 1963. Laboratory methods for work with plant and soil nematodes. Ministry of Agr., Fisheries and Food, Tech. Bull. 2, pp. 2–3. Her Majesty's Stationery Office, London.
7. Graham, T. W. 1965. Tobacco varieties used to show differential response to root-lesion and root-knot nematode. Plant Dis. Rep. 49:822–826.
8. Graham, T. W., and H. E. Heggestad. 1959. Growth response and root decay development in certain tobacco varieties and breeding lines infected with root lesion nematodes. Helminthol. Abstr. 31:454. (Abstr.)
9. Graham, T. W., Z. T. Ford, and R. E. Currin. 1964. Response of root-knot resistant tobaccos to the nematode root disease complex caused by *Pratylenchus* spp. and *Meloidogyne incognita acrita*. Phytopathology 54:205–210.
10. Loewenberg, J. R., T. Sullivan, and M. L. Schuster. 1960. The effect of pH and minerals on the hatching and survival of *Meloidogyne incognita incognita* larvae. Phytopathology 50:215–217.
11. Moore, E. L., N. T. Powell, G. L. Jones, and G. R. Gwynn. 1962. Flue-cured tobacco—variety NC 95—resistant to root-knot, black shank and wilt diseases. N. C. Agr. Exp. Sta. Bull. 419. 18 pp.
12. Mountain, W. B. 1965. Pathogenesis of soil nematodes, pp 285–301. *In* K. F. Baker and W. C. Snyder (eds.), Ecology of soil borne plant pathogens. Univ. of Calif. Press, Berkeley.
13. Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Medel. Landbouwhogesch. Wageningen 66-4. 46 pp.
14. Powell, N. T., and G. R. Gwynn. 1965. A new leaf variety. Res. Farming (N. C. Agr. Exp. Sta.) 23:15.
15. Sasser, J. N., and C. J. Nusbaum. 1955. Seasonal fluctuations and host specificity of root-knot nematodes in two-year tobacco rotation plots. Phytopathology 45:540–545.
16. Southards, C. J., and C. J. Nusbaum. 1967. Genetic variability of tobacco response to *Pratylenchus brachyurus*. Phytopathology 57:18–21.
17. Taylor, A. L., and W. Q. Loegering. 1953. Nematodes associated with root lesions in abaca. Turrialba 3:8–13.