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Yield Relationships and Population Dynamics of *Meloidogyne* spp. on Flue-cured Tobacco[1]

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Abstract: The complex nature of nematode-induced diseases of tobacco, the relationships between nematode levels and damage, the methods of determining these relationships, and the evolving nematode communities on tobacco in eastern North Carolina are described. Crop damage associated with these pathogens varies with nematode race and species, crop cultivar, microflora, and environmental conditions. Root-gall indices as well as initial and mid-season numbers of *Meloidogyne* spp. in soil are useful for estimating nematode-induced damage on tobacco. The increased occurrences of *M. arenaria, M. javanica,* and *M. incognita* races 2 and 4 on tobacco during the last 20 years in North Carolina are having an important economic impact on growers and pose new challenges to researchers.

Key words: chemical soil treatment, damage threshold, *Meloidogyne* spp., *Nicotiana tabacum,* population dynamics, root-knot nematode, tobacco.

Parasitic nematodes, especially *Meloidogyne* spp., have been known to cause direct and indirect damage on tobacco (*Nicotiana tabacum* L.) since the work of Tisdale in Florida in the 1920s (21,22). He, along with Tyler (23) in 1933, suggested that root-knot nematodes may predispose tobacco to attack by other disease-causing agents, including *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker (induces black shank). Thus, in considering the population dynamics of nematodes and their damage potential, we should be cognizant also of the roles that nematodes play in predisposing tobacco to other pathogens (15). This review focuses on characteristics of nematode damage on tobacco, principles involved in considering nematode-to-bacco relationships, how these relationships may be determined, and changes in nematode communities on this crop in North Carolina.

Characteristics of nematode damage on tobacco: Nematode damage on most crops is indicated by the presence of typically contagious or spotty patterns of stunted plant growth in fields (1,2). In addition to highly virulent *Meloidogyne* spp., other taxa such as *Pratylenchus* spp. may be important on this crop (21). A characteristic common to plant-parasitic nematodes is the polyspecific nature of infestations within fields (16). Still, *Meloidogyne* spp. that reproduce on tobacco are its most serious nematode pathogens (1,8,12,20). They often occur in nearly monospecific populations on this crop. Because they are now becoming more widespread (19), their role as pathogens on tobacco must be more completely characterized. A number of new *Meloidogyne*

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species have been described recently, but their role on tobacco is unknown (21).

Meloidogyne spp. may indirectly damage root systems of tobacco by predisposing them to attack by fungi and bacteria (15). The extensive root galling associated with these nematodes is obvious, but the associated necrosis often involves a complex of fungi and bacteria (15). Inside these roots are the typically enlarged *Meloidogyne* spp. females and the associated giant cells and hyperplasia in surrounding root tissue. These altered tissues predispose plants to attack by associated fungi and bacteria by modifying the rhizosphere as well as the metabolic activity of the roots themselves (15,21). In addition, these nematodes predispose normally resistant and susceptible tobacco roots to attack by fungi or bacteria (15). Root-knot resistant cultivars, such as Speight G-28, are also damaged severely by *Meloidogyne grahami* Golden & Slana (or race 4 of *M. incognita* (Kofoid & White) Chitwood). Limited evidence has been published that *M. hapla* Chitwood and *M. arenaria* (Neal) Chitwood may predispose *M. incognita*-resistant tobacco to *M. incognita* race 1 or race 3 (6).

Principles useful in investigating nematode–tobacco relationships: In relating root-knot and other nematodes to the performance of tobacco, the population dynamics of each nematode species and related environmental effects and management practices must be considered. The population declines of *M. incognita* after completion of tobacco harvests may be determined with bioassays and by extraction of second-stage juveniles (1,21). Management practices have striking effects on this decline (1). For example, the decrease in population levels of *M. incognita* from fall to spring may be relatively minor (20–30% decine) in tobacco plots or fields treated with a nematicide at planting, especially where roots remain intact (1). In contrast, nematode levels can drop ≥ 90% in plots with no at-plant nematicide and where roots are destroyed at final harvest. Thus, management practices affect the population dynamics of nematodes which in turn impact the damage potential for subsequent crops.

A number of basic concepts have proven useful in elucidating nematode-damage functions (1,2,7,12,13,16,19,20). First, there is a general negative or inverse relationship between the initial population density and the growth and yield of annual crops (1,7,16,21). Theoretically, crop plants have a nematode tolerance limit below which they are not damaged by a given nematode species (19,20). There has been some debate as to whether the concept of tolerance should be viewed as a precise "tolerance limit" (19) or more as a range dependent on the effects of environmental parameters (1,2,16). In either case, the tolerance of plants to nematodes is affected by crop cultivar as well as by biotic and abiotic factors. Another concept (19) is that for most nematode-crop relationships there is a minimal yield, even with high numbers of nematodes. For highly aggressive root-knot nematodes, such as *M. arenaria,* this minimal yield can be zero. This situation is true particularly when they interact with soil-inhabiting fungi and bacteria (4,15). Unfortunately, little progress has been made in quantifying such disease complexes.

Additional parameters are useful in evaluating tobacco cultivars as hosts and for characterizing nematode damage. When a population is stable, an equilibrium density (20) is achieved (birth rate = death rate). A related parameter, reproduction rate (final population density/initial population density), also is useful in determining host status (16). The presence of poor hosts or nonhosts effects a decline in nematode numbers so that the population is not maintained. Where there is extensive reproduction of the nematodes, the populations may be much above the maintenance level or initial population density. Most susceptible tobacco cultivars are good or excellent hosts with high reproduction rates and equilibrium densities for *Meloidogyne* spp. Breeding programs are directed at identifying and exploiting the genetic potential

of poor hosts which are not damaged by nematodes.

At a more practical level, economic or action thresholds (nematode levels that warrant treatment) have promise for selecting management tactics and evaluating economic benefits (1,2,7). Economic thresholds can be calculated with an implied high degree of precision that is not achieved in reality with nematodes (1,7,16). This problem is due in part to the frequently unpredictable effects of environment on nematode–host interactions. Also, low numbers of some nematodes are reported to enhance plant growth (11). Some chemical soil treatments may suppress (14) or enhance plant growth (3) independent of their impact on the nematode population. Thus, use of nematicides in delineating these plant–nematode relationships must be considered carefully.

Approaches for determining nematode–tobacco relationships: A number of specific experimental methods are useful in determining nematode damage functions. Much of the research described in the literature has been conducted in greenhouses or phytotrons. This type of research is useful in describing host suitability for various nematodes, but it may not realistically represent damage potential in a field situation. A number of researchers (1,4,17) have utilized microplots in which soil was infested with the desired numbers and kinds of nematodes to determine their effects on plant growth and to characterize associated population dynamics of the pathogen. Still, field experiments are essential to validate estimates of the impact of nematodes on plant growth and yield (2). Experimental components that might be included in field tests are the types and levels of microbial components (whether under monoxenic or polygenic conditions), crop cultivars, pesticide treatments, and environmental factors (12).

The spatial patterns of nematodes in naturally infested fields can be utilized to determine damage functions. Very low numbers or no nematodes occur in some portions, whereas other portions have moderate to high levels. By mapping these patterns, plots can be located to achieve a range of inital nematode numbers for determining their impact on plant growth without chemical soil treatments (2,12,13). Cultivars of tobacco also may be evaluated with a split-plot experimental design. If desired, split plots with and without chemical soil treatments may be used.

Another approach in field experimentation is to use a wide range of chemical soil treatments with various degrees of efficacy to create a range of nematode population levels. The population densities of target nematodes in various chemical soil treatments can be monitored periodically and related to tobacco growth and yield (2,4,5). Baseline information on the general response of populations of nematodes on tobacco with and without chemical soil treatments is helpful in these studies. The ideal time for sampling to determine nematicide efficacy in North Carolina on tobacco transplanted about 30 April generally is in mid to late July, depending on the exact time the crop is established, as well as on soil temperatures. The impact of nematicides on plant growth in the absence of nematodes should be considered in these studies.

Data from these types of experiments can be evaluated with regression analyses. A simple model would relate numbers of eggs and juveniles (independent variables) to the yield (dependent variable) of tobacco (4). More complex models are also useful. For example, the Seinhorst model includes the concept of a tolerance limit, minimal yield, and a constant (< 1.0) damage-rate parameter which is a function of nematode virulence (7). This model, as well as linear and quadratic equations, is useful for characterizing tobacco yield responses vs. numbers of nematodes (4).

Although nematicide treatments are useful for developing nematode damage functions (4), they have inherent problems that must be considered (3,14). Materials such as aldicarb may enhance the growth

TABLE 1. Comparative reproduction and damage potential of *Meloidogyne* spp. on tobacco in microplots.

Nematode	Reproduction factor (R)	Root-gall indices/Pi†			Yield losses/10-fold increase in Pi (%)
		750	1,500	3,000+	
M. hapla	27	18	24	29	3.4–4.6
M. incognita	527	34	46	61	8.9–10.0
M. arenaria	610	64	73	86	15.6–16.5
M. javanica	1,083	45	56	73	13.0–19.0

After Barker et al. (4). R = midseason population density/initial population density (at plant).
† Pi = eggs + juveniles/500 cm^3 soil at plant. Gall indices: 0 = healthy roots; 100 = maximum root galling (4).

of tobacco in the absence of nematodes (3). Also, rainfall patterns may affect the growth responses of tobacco to this pesticide in the absence of nematodes. With low level moisture, 1 μg aldicarb/cm^3 soil may give a significant increase in yield of tobacco, whereas 3 μg may have only a slight impact. Regardless of the level of moisture provided, there was some benefit in the growth of tobacco, particularly at 1 or 2 μg of aldicarb (3). On the other hand, soil fumigants may actually suppress tobacco growth with low nematode levels. Fumigation of fields with slight to moderate root-knot nematode infestations gave a decreased return in about 50% of treated plots, relative to untreated plots; in contrast, 71 of 87 fields with a severe problem gave an increase in yield when fumigated (14).

Microplots have been used extensively in North Carolina (1,4) and Florida (17) to determine damage functions for various nematodes on tobacco. Linear regressions usually were adequate for depicting the impact of increasing numbers of eggs and juveniles of *Meloidogyne* spp. on the yield of tobacco. For every 10-fold increase in initial population densities of *M. arenaria* or *M. javanica* (Treub) Chitwood, a yield loss in the range of 15–19% occurred (Table 1). Hence, relatively low numbers of these nematodes can result in significant yield losses of tobacco. In comparison, the more common root-knot nematode in North Carolina, *M. incognita,* causes an 8–10% loss for every 10-fold increase in initial numbers, and *M. hapla* causes only 3% loss (4). Similar studies with *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven, *P. scribneri* Steiner, and *Tylenchorhynchus claytoni* Steiner showed these nematodes to have only slight or no impact on tobacco yield under the test conditions used.

Effects of *M. incognita* on resistant cultivars also were investigated (4). For each 10-fold increase in the initial level of *M. incognita* race 3 on the resistant Speight G-28, there was a 3% loss in yield. Although this is a relatively small loss, corrective action such as nematicide treatment still would be needed with high numbers of nematodes.

Data comparing the four common species of root-knot nematodes on tobacco provide some clues about the wide range in virulence or aggressiveness and damage induced by them. First, the degree of root-gall induction was inversely related to yield. *Meloidogyne hapla* caused only slight root galls and had the smallest impact on yield. In contrast, *M. incognita* resulted in about 2–2.5 times greater root galling than did *M. hapla,* and this trend corresponds to the increased yield loss. The two most damaging nematodes on tobacco, *M. arenaria* and *M. javanica,* cause near maximum root galling, especially at high initial population levels. A quadratic equation has been found to adequately describe the relationship between root galling and tobacco yields across a range of population levels of these four nematodes (4). This model accommodates the observed tolerance of tobacco for low to moderate numbers of *M. hapla.*

Associated with these differences in magnitude of gall induction by the four *Meloidogyne* spp. is the relative increase in

their reproductive factors (4) (Table 1). By midseason, a 750 Pi of *M. hapla* increased 27-fold. The increase for *M. incognita* was about 527-fold, again suggesting that fecundity may be important in the damage brought about by these nematodes. For *M. arenaria,* the increase was even greater (610-fold), whereas *M. javanica,* the most aggressive of all these nematodes, increased 1,083-fold from the time the experiment was established to midseason. All nematodes that reproduce rapidly on tobacco do not necessarily inflict yield losses. For example, the so-called tobacco-stunt nematode, *T. claytoni,* increases rapidly on this crop, but causes little or no damage under microplot or field conditions (Barker and Nusbaum, unpubl.)

Another reliable approach for evaluating damage is to rate roots for necrosis, which reflects the combined impact of nematodes and associated fungi (4). Little root necrosis was associated with *M. hapla.* The relative root necrosis associated with *M. incognita, M. arenaria,* and *M. javanica* followed a pattern similar to that for root galls. The effects of this necrosis on yield can be depicted with linear regressions (4).

Damage functions that have an implied high degree of reliability can be developed for specific field conditions. Nevertheless, in considering crop responses in the same field from year to year or from one field to the next, researchers who have worked with nematodes know that there is much variability in plant responses to these pests. Because of this variation, experiments are being conducted to determine the effects of a number of factors that might impact these nematode level–tobacco yield relationships. In addition to cultivar and nematode level, soil fertility, moisture, soil texture, water holding capacity, soil strength, temperature, and associated micro-organisms may play major roles in modifying damage functions. These effects have been determined in tests over a range of soil textures in microplots in North Carolina (unpubl.). *Meloidogyne incognita* can be devastating in some soils, as in a Cecil sandy clay or in Fuquay loamy sand, whereas in a fertile Norfolk loamy sand or a Cecil clay loam, the damage from similar infestation levels may be minimal. Soil moisture levels may have similar but less striking effects (Wheeler, unpubl.).

This same type of response to soil texture also has been observed in field experiments where nematicides were used to establish a wide range of initial or midseason numbers of nematodes (4). Approximately one-half of the crop was lost at the highest population levels in a sandy soil. In contrast, fields located in the finer textured soils supported near normal growth and yield of tobacco, even though many plants had moderately high gall indices (4).

Multiple regressions were used to weigh factors that might have the greatest impact on tobacco yield. A large list of variables including various soil factors, numbers of nematodes, root gall indices, root necroses, and various combinations were analyzed. The combination of sand percentage, acidity, calcium level, and root necrosis gave the highest coefficient of determination (R^2 = 0.62) (unpubl.). Thus, one or more potentially important factors, such as soil moisture, were not considered in these analyses. Further studies in which additional factors are being monitored are in progress.

The evolving nematode problems on tobacco in North Carolina: A major problem we have encountered in North Carolina and much of the general region is that the nematode species present are shifting over time (Tables 2, 3). With the extensive use of resistant cultivars and changes in use patterns of nematicide treatments, a striking increase of *M. arenaria* and *M. javanica* has occurred during the last two decades (18). These nematodes often severely damage *M. incognita*- resistant as well as susceptible tobacco cultivars. An exception is *M. arenaria* race 1; it causes only slight damage on resistant tobacco. Similar observations for some populations of *M. arenaria* and *M. hapla* have been made by researchers in Florida (10). In a 1988 nematode survey in North Carolina, most populations of both of these nematodes from peanut fields in-

TABLE 2. Responses of *Meloidogyne incognita*-resistant tobacco cultivar Speight G-28 to selected populations of *Meloidogyne arenaria*.

Population†	Root-gall indices (0–100)	Root-necrosis indices (0–100)
Greenhouse cultures		
54-VA	6	1
56-NC	86	68
CJN	30	1
BD-H	23	6
Recently isolated cultures from resistant tobacco		
Denton	90	83
Apple 1	90	70
Summerville	43	10
Clemmons	95	95
Windborn	93	88

† Populations of *M. arenaria* were 54-VA from Virginia, 56-NC from North Carolina, CJN from a >25-year-old greenhouse culture from tobacco, BD-H from holly (*Ilex crenata* Thunb. var. 'rotundifolia'). Names for last five cultures were growers (names) reporting problems on the *M. incognita*-resistant tobacco from which they were isolated.

TABLE 3. Frequency (%) of occurrence of *Meloidogyne* species on tobacco in North Carolina based on three separate surveys.†

Nematode	1968	1976	1983
M. incognita (races 1 and 3)	67.3	42.9	29
M. incognita (races 2 and 4)	0.9	18.1	37
M. arenaria	2.7	19.5	15
M. javanica	2.1	5.0	14
M. hapla	43.0‡	7.8	6

† Total numbers of samples per year were 692 in 1968 (survey by C. J. Nusbaum), 433 in 1976, and 442 in 1983.
‡ Primarily from peanut (samples came via nematode assay service).

duced little or no root galls on 'NC-95' tobacco (Schmitt, Barker, and Bailey, unpubl.). These observations indicate that the differential host test for *Meloidogyne* spp. (9) should be used with care. In contrast, populations of *M. arenaria* (usually race 2) isolated from *M. incognita*-resistant tobacco fields inflict severe damage on these cultivars (Table 2).

The situation with the most common root-knot nematode, *M. incognita*, is complex. Populations of races 1 and 3, for which we have effective resistance, have been declining in recent years (Table 3). In contrast, populations of race 2 and especially race 4, which attack our resistant cultivars, have increased significantly. Thus, the changing nature of *M. incognita* species is complex, and the increasing incidence of *M. arenaria* and *M. javanica* in North Carolina add to the difficulties in managing root knot on tobacco.

In conclusion, a number of approaches may be utilized in characterizing tobacco responses to nematodes. The objective of all of these studies is to develop management strategies and tactics that can bring about a near optimum crop whether using resistant cultivars, nematicides, rotation, or a combination thereof. Understanding how nematodes damage crops under various environmental and cultural conditions should contribute toward reaching this goal.

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