Correlation of Tobacco Yield, Value, and Root-Knot Index with Early-to-Midseason, and Postharvest Meloidogyne Population Densities¹

HOWARD FERRIS²

Abstract: Certain nematicidal treatments for the control of root knot of tobacco in six field experiments in North Carolina were used to determine early, midseason, and postharvest densities of eggs and larvae of *Meloidogyne* spp., postharvest root-knot indices, and crop yield and value. Various statistical correlations showed that population densities determined 6-8 wk after transplanting were more indicative of treatment effectiveness than postharvest densities. Logarithmic transformation of early and midseason population data stabilized the variance. Key Words: logarithmic transformation, root-knot nematode, Nicotiana tabacum.

Root knot caused by *Meloidogyne* spp. is a major disease of flue-cured tobacco (*Nicotiana tabacum* L.) in North Carolina (16). Most fields used for tobacco culture are infested with one or more species of this parasite. The southern root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood is prevalent in tobacco fields, whereas *M. hapla* Chitwood, *M. arenaria* (Neal) Chitwood, and *M. javanica* (Treub) Chitwood occur only occasionally. An effective control program is widely accepted and used by growers. Even so, the evaluation and improvement of control practices are still an important part of the tobacco disease control program (16).

Many control practices now in use were developed and evaluated empirically. Although benefits to the tobacco production industry have been great, they have been achieved by a long and costly investment in extensive field experimentation. Improvements in testing procedures depend largely upon knowledge of population dynamics of *Meloidogyne* spp. and the epidemiology of root-knot. The present study was undertaken to determine the feasibility of employing early and midseason population data to evaluate the effects of control treatments upon the course of root-knot epidemics and to interpret final results in terms of postharvest population densities, root-knot indices and crop

performance. Several field experiments, in which initial *Meloidogyne* population densities were differentiated by nematicidal treatment, provided an abundance of suitable material for study. Laboratory assays of populations, hitherto based upon the numbers of second-stage larvae extracted from soil samples, were augmented by a new procedure for extracting the eggs (3).

MATERIALS AND METHODS

Selected plots in six nematicide evaluation experiments were chosen for study (Table 1). The root-knot susceptible tobacco cultivar 'Coker 411' was used throughout. Standard cultural practices were employed (16). Species identification by bioassay on root-knot susceptible 'Rutgers' tomato and resistant 'N.C. tobacco and by perineal pattern 95' examination of 25 females of each population indicated that *M. incognita* was present at all locations, except the Johnston County location which was infested with *M. arenaria*. The plots selected for study were either nontreated checks or treated with volatile nematicides. Plots were four rows wide (0.0067 ha) except in Moore County where they were six rows wide (0.0101 ha). There were four replications of each treatment in a randomized complete block design.

Soil samples for nematode assay were taken from all plots two or three times between 24 and 68 days after transplanting, and after harvest (about 100 days after transplanting). Sampling procedure was standardized to improve comparability of samples. A 2.5-cm diam sampling tube was pushed into the root zone about 15 cm from, and directed to about 30 cm below, the base of the stalk. In plots with less vigorous plants, a more acute angle of penetration was used to sample an equivalent region of the root zone. Samples of 12 cores were taken from each plot. Population densities

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Location	No. plots	Initial	Yield (kg/ha)		Root-knot index ^c	
(county)	sampled	infestation ^a	Mean	Range ^b	Mean	Range ^b
Edgecombe	28	L-M	2531	962	14.8	75.0
Granville	36	L-M	2095	1126	15.1	87.5
Johnston	8	M-H	1382	1494	44.3	82.5
Moore	8	M-H	2686	803	39.3	87.5
Pitt ^d	16	Н	2019	1291	65.2	90.4
Robeson	48	M-H	2154	1955	42.5	92.0

TABLE 1. Location, yields and characteristics of experimental tobacco plots used for *Meloidogyne* population studies in North Carolina, 1971.

^aBased upon preseason assays of population densities; ranges shown indicate relative variations in initial densities. L = low, M = moderate, H = high.

^bThe difference between the largest and smallest of the sample observations.

^CIndex of Todd and Nusbaum (16). Six categories assessing disease development on a 0-100 scale.

^dMidseason development of blackshank caused by *Phytophthora parasitica* var. *nicotianae* was a source of variation in the data obtained at this location.

of both eggs (3) and larve (4) were determined. Root-knot indices were determined after harvest. The index used is based upon the degree of galling and root damage, recognizing

TABLE 2. Means and relationship of standard deviation and variance to mean, of *Meloidogyne* eggs and larvae in experimental tobacco plots in North Carolina, arranged in ascending order of means by location.

Location (county)	Sampling day ^a	Mean (× 10 ³)	s/x	$s^2/\bar{x} (\times 10^3)$
Edgecombe	44	1.4	2.9	11.9
0	58	2.0	2.3	11.1
	Final	11.6	1.3	20.1
Granville	49	0.1	3.5	1.7
	63	3.1	3.0	28.2
	Final	6.6	2.2	31.0
Johnston	40	1.0	1.8	3.3
	68	8.0	1.0	8.4
	54	9.0	1.4	17.5
	Final	26.9	1.0	27.5
Moore	34	0.2	1.6	0.5
	48	8.3	1.4	15.7
	Final	8.3	1.4	15.3
	62	19.4	2.3	101.6
Pitt	33	0.2	1.4	0.3
	47	14.2	1.5	31.3
	Final	77.3	0.6	24.0
	61	101.5	1.0	96.7
Robeson	24	0.02	1.4	0.04
	38	2.1	1.6	5.1
	Final	3.0	1.2	4.3
	52	5.2	1.8	16.9

^aDays after transplanting.

six categories (16). Data on tobacco yields and value were based upon the weight of cured leaf and the average selling price by grade on North Carolina markets.

The relation of the variance and standard deviation to the mean of population densities, measured by the sampling and assay methods employed, were determined. Such relationships indicate any need for variance stabilization in the data before using statistical methods (15). Similar relationships were examined for root-knot indices and the yield, value/0.4 ha and value/45 kg of tobacco.

Correlations were drawn between root-knot indices and original and transformed data from early, midseason, and final (postharvest) samplings. The total density of eggs plus larvae in each sample was considered. The final nematode population density was correlated with early and midseason estimates of population density. To determine which estimate of the population density was most useful in testing treatment effectiveness in nematicide trials, yield and value of tobacco were correlated with early-to-midseason and final nematode densities.

RESULTS AND DISCUSSION

At locations in Edgecombe and Granville Counties where initial infestations were low (Table 1), mean root-knot indices were low and mean yields were high with a fairly narrow range, indicating little nematode damage. The infestation level was somewhat higher at the other four locations; mean root-knot indices were high and yield data had a wide range in most cases, indicating nematode damage in some plots.

Transformation of data: Means of nematode population densities at each location, with the relationships of variance and standard deviation to the mean, were arranged in ascending order of means (Table 2). The value s/\bar{x} was approximately constant whereas the variance was not directly proportional to the mean. In skewed distributions, the variance tends to be a function of the mean, and (based on their relationship) a transformation can be chosen to place the data on a scale in which the variance is more stable (5). When the standard deviation is proportional to the mean, as in this case, a log transformation will stabilize the variance (15).

Log transformations of nematode population data are commonly used, particularly in linear regressions between plant yield and nematode density (8, 9, 11). Seinhorst (14) and Oostenbrink (12) explained that log transformations of nematode density have no biological meaning and are empirical, being used because the regression of plant growth upon them is often linear. Increases in numbers within biological populations are often proportional to numbers already present, so that variations in sample means are themselves proportional to the mean, which necessitates log transformation (1). With root-zone sampling there is a biologically based statistical necessity for data transformation, dependent upon the distribution of the nematodes in the soil. If the population density is low and variable, the sample mean will be low and the range wide, particularly in the case of a population aggregated in egg masses. As the season progresses and the roots grow, the nematode population becomes more uniformly distributed throughout the root zone. Early season sampling may thus involve errors which are not normally and independently distributed but tend to increase as the mean increases. This skewness decreases as the population distribution becomes more uniform.

In these studies, correlations were conducted with both original nematode population densities and log (X + 1) of these densities. The value X + 1 was used because zero values of X occurred. Ranges of root-knot indices and those of tobacco yield appeared independent of their means (Table 1), indicating independence of the respective variances. Similar results were obtained with tobacco value data. Correlations with these data

		Correlation coefficients between root-knot indices and population densities			
Location (county)	Sampling day ^a	Density	Log density		
Edgecombe	44	0.78** ^b	0.82**		
	58	0.91**	0.80**		
	Final	0.93**	0.58**		
Granville	49	0.62**	0.62**		
	63	0.67**	0.58**		
	Final	0.81**	0.70**		
Johnston	40	0.38	0.78*		
	54	0.60	0.84 **		
	68	0.80*	0.85**		
	Final	0.66	0.38		
Moore	34	0.16	0.26		
	48	0.50	0.65		
	62	0.70	0.80*		
	Final	0.84**	0.89**		
Pitt	33	0.31	0.51*		
	47	0.55*	0.74**		
	61	0.54*	0.63**		
	Final	0.21	0.29		
Robeson	24	0.18	0.27		
	38	0.60**	0.76**		
	52	0.58**	0.76**		
	Final	0.51**	0.53**		

TABLE 3. Correlation coefficients between root-knot indices and population densities in experimental tobacco plots in North Carolina.

^aDays after transplanting.

b* and ** indicate that the probability of a greater |r|under H₀: rho = 0 is <0.05 and <0.01, respectively.

were conducted with the original values only. Root-knot indices themselves represent transformations of nematode densities, although not logarithmic, and may be rectilinearly related to yields (12).

Correlations of root-knot indices with nematode densities: Root-knot indices were highly correlated with nematode population densities when the initial infestation was low and variable as at Edgecombe and Granville Counties (Table 3). Since the nematode populations were non-detectable in many plots, the variance was low and stable and correlations were not improved by transformation. Root-knot indices were highly correlated with final density data, suggesting little early season damage to the tobacco.

In the locations with moderate to high initial infestations, root-knot indices were

<u> </u>		Correlation coefficients between						
Location (county)	Sampling day ^a	Final density and early ^b density	Final density and log early ^b density	Log final density and early ^b density	Log final density and log early ^b density			
Edgecombe	44	0.70**c	0.71**	0.33	0.57**			
-	58	0.83**	0.74**	0.41*	0.44*			
Granville	49	0.34*	0.45**	0.35*	0.45**			
	63	0.84**	0.52**	0.40*	0.45**			
Johnston	40	-0.31	0.17	-0.64	-0.11			
	54	-0.02	0.45	-0.45	0.16			
	68	0.28	0.49	-0.15	0.20			
Moore	34	0.44	0.53	0.47	0.50			
	48	0.72*	0.57	0.68	0.76*			
	62	0.92**	0.68	0.65	0.87**			
Pitt	33	-0.11	-0.22	0.00	-0.13			
	47	0.21	0.03	0.26	0.02			
	61	-0.18	-0.26	-0.04	-0.22			
Robeson	24	0.03	0.16	0.13	0.24			
	38	0.17	0.42**	0.33*	0.58**			
	52	0.35*	0.49**	0.33*	0.59**			

TABLE 4. Correlation coefficients between final and early-to-midseason densities of *Meloidogyne* eggs and larvae in experimental tobacco plots in North Carolina.

^aDays after transplanting.

^bDensity determined on sampling day specified in second column.

^{c*} and ** indicate that the probability of a greater |r| under H₀: rho = 0 is ≤ 0.05 and ≤ 0.01 , respectively.

usually better correlated with early-to-midseason densities, than with final densities. The correlation coefficients of these early-to-midseason data were increased somewhat by transformation. Tobacco at the Pitt County location was damaged by blackshank, caused by *Phytophthora parasitica* var. *nicotianae*, and nematode increase was restricted to the noninfected plants. Hence, root-knot indices were not directly correlated with final densities; the latter were a function of plant vigor.

Nontransformed final densities usually gave higher correlation coefficients than the transformed data; presumably increased stability of variance by this stage, resulting from more uniform nematode and root distribution, reduced the need for transformation.

Root-knot indices are widely used as indicators of treatment effectiveness (6, 7, 16). Much of the galled root system examined was invaded early in the season and is indicative of the nematode density at that time. Extracting *Meloidogyne* eggs from soil samples (3) improves the accuracy of population measurement early in the season so that root-knot index assessments could be compared with early-to-midseason and final densities. The indices were generally better-correlated with early and midseason densities than with final densities, provided the samples were taken at least 40 days after transplanting, to ensure that the initial population had reproduced, and that log transformation was used to stabilize and reduce inflation of the variance of these early samples.

Correlations of final nematode densities with early and midseason densities: Final nematode densities were highly correlated with early-to-midseason densities when the initial infestation was low as in Edgecombe and Granville Counties (Table 4). Correlation coefficients were lower at the other locations where the higher early season densities affected plant vigor and hence the final densities. The low correlation coefficients at Pitt County reflect both nematode and blackshank damage to the plants. Highest correlation coefficients were obtained between the log-transformed

	- Sampling day ^a	Yield		Value/45 kg		Value/0.4 ha	
Location (county)		Density	Log density	Density	Log density	Density	Log density
Edgecombe	44	-0.17	-0.28	-0.06	-0.06	-0.17	-0.28
U	58	-0.26	-0.26	-0.27	-0.20	-0.27	-0.27
	Final	-0.30	-0.23	-0.15	0.07	-0.30	-0.21
Granville	49	-0.24	-0.30	-0.45**	-0.18	-0.29	-0.31
	63	-0.33**b	-0.14	-0.10	-0.33*	-0.33*	-0.18
	Final	-0.50**	-0.57**	-0.26	-0.33*	-0.51**	-0.59**
Johnston	40	-0.54	-0.42	-0.27	0.11	-0.54	-0.42
	54	-0.54	-0.19	-0.14	0.12	-0.53	-0.18
	68	-0.49	-0.21	0.12	0.16	-0.48	-0.20
	Final	0.28	0.35	0.78*	0.76*	0.30	0.37
Moore	34	-0.80*	-0.83*	0.28	-0.10	-0.71*	-0.83*
	48	-0.82*	-0.35	0.34	0.62	-0.72*	-0.19
	62	-0.81*	-0.59	0.12	-0.17	-0.76	-0.76
	Final	-0.72*	-0.53	0.19	0.09	-0.66	-0.50
Pitt	33	0.19	-0.10	0.20	-0.05	0.21	-0.11
	47	0.10	0.00	-0.54*	-0.40	0.04	-0.04
	61	-0.01	-0.25	-0.46	-0.43	-0.06	-0.29
	Final	0.60*	0.65**	0.13	0.01	0.60*	0.63
Robeson	24	-0.32*	-0.21	-0.25	-0.24	-0.33*	-0.22
	38	-0.39**	-0.42**	-0.19	-0.43**	-0.40**	-0.45**
	52	-0.33*	-0.34*	-0.40**	-0.49**	-0.36*	-0.38**
	Final	-0.03	-0.07	-0.26	-0.24	0.00	-0.10

 TABLE 5. Correlation coefficients between tobacco yield, value/45 kg and value/0.4 ha and Meloidogyne spp. densities throughout the growing season in experimental plots in North Carolina.

^aDays after transplanting.

b* and ** indicate that the probability of a greater |r| under H_0 : rho = 0 is ≤ 0.05 and ≤ 0.01 , respectively.

early-to-midseason densities and either original or transformed final densities, again indicating less need for the transformation of postharvest density data.

Correlations of yield and value of tobacco with nematode population densities and root-knot indices: Justesen and Tammes (10) illustrated by hypothetical models that the effect of individual pathogens on crop growth diminishes as their numbers increase. This self-limiting effect results in a non-linear relationship between crop yield and initial pathogen population level. It has been used to explain the high correlations often obtained between yield and log transformations of initial nematode density; e.g. by Jones (9) and Lownsbery and Peters (11). To avoid the problem of determining the infectivity of nematodes from treated plots, initial population densities were not used in this study. Even in nontreated plots, laboratory

assay of the overwintered nematode population does not provide an accurate measure of its infectivity.

TABLE 6. Correlation coefficients between yield, value/45 kg and value/0.4 ha of tobacco and root-knot index in experimental plots in North Carolina.

	Correlation with root-knot index					
Location (county)	Yield	Value/45 kg	Value/0.4 ha			
Edgecombe	-0.33	-0.21	-0.34			
Granville	-0.55** ^a	-0.47**	-0.58**			
Johnston	-0.41	0.44	-0.39			
Moore	-0.38	0.14	-0.34			
Pitt	0.30	-0.54*	0.23			
Robeson	-0.27	-0.41**	-0.30*			

^{a*} and ^{**} indicate that the probability of a greater |r| under H_0 : rho = 0 is ≤ 0.05 and ≤ 0.01 , respectively. Correlation coefficients between tobacco yield, value per 0.4 ha and per 45 kg, and nematode densities were low when the initial infestation was low and variable as at Edgecombe and Granville Counties (Table 5). Yield and value were significantly negatively correlated with postharvest densities at the Granville County site, however, indicating a late season increase of the nematodes in this relatively heavy soil.

Yield and value at Pitt County were more related to the blackshank infection than to the early-to-midseason nematode densities. High positive correlations between tobacco yield and final densities indicated that nematode multiplication was greatest on the vigorous plants which had escaped early nematode damage and blackshank injury.

At the other locations, yield and value/0.4 were negatively correlated with ha early-to-midseason nematode densities, but the coefficients were low or positive with the final density data. Value/45 kg, an indicator of tobacco quality, was not consistently correlated with nematode density. Nematode population appeared unreliable for yield density prediction. Tobacco yield and value were sometimes hetter correlated with nontransformed than with transformed early-to-midseason densities, apparently contrary to the rationale of Justesen and Tammes (10). Yield was invariably negatively correlated with early-to-midseason nematode densities, but often positively correlated with final densities, again demonstrating that the former indicate nematicide effectiveness while the latter reflect crop vigor. Sasser et al. (13) found that peanut yield and value were negatively correlated with midseason larval densities of M. hapla.

Yield and value of tobacco were generally negatively correlated with root-knot indices, although the coefficients were low in most cases (Table 6). Value/45 kg of tobacco was not consistently correlated with root-knot index, the coefficients being positive at some locations and negative at others. The relationship of yield to root-knot index was not as striking as that in Daulton's (6) data as presented by Oostenbrink (12), indicating the influence of other factors upon yield in many of these cases. Brodie and Dukes (2) showed that tobacco yield was related to infection occurring early in the season as measured by root-knot indices at that time and that indices at the end of the season

might show little difference although yields differed significantly.

This study assesses the usefulness of early and midseason nematode sampling in measuring nematicide effectiveness, as compared with final densities which often reflect crop vigor rather than the original treatment. Final nematode population densities are customarily presented in reports of the nematicide evaluation program of the North Carolina Agricultural Extension Service (16). Differences in these data are often nonsignificant and inconsistent with the root-knot index assessments and crop yields. Presentation of early-to-midseason densities would provide a better assessment of nematicde effectiveness to supplement root-knot indices and crop yield and value data. Samples taken 6-8 wk after transplanting could indicate the effectiveness of each treatment while the crop was growing, without having to wait for root-knot index data. This would be especially useful in demonstration plots.

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