Populations of *Thielaviopsis basicola* and the Occurrence of Black Root Rot on Burley Tobacco in Western North Carolina

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ABSTRACT

Meyer, J., Shew, H. D., and Shoemaker, P. B. 1989. Populations of *Thielaviopsis basicola* and the occurrence of black root rot on burley tobacco in western North Carolina. Plant Disease 73:239-242.

Populations of the black root rot pathogen, *Thielaviopsis basicola*, and occurrence of black root rot on burley tobacco were determined in an extensive, random survey of 80 burley tobacco fields in the burley-growing region of the Appalachian Mountains in North Carolina. The pathogen was detected in 47 (59%) of the surveyed fields at a mean population of 40 cfu/g of soil and a range of 2–219 cfu/g of soil. Black root rot was observed in 32 (68%) of the infested fields. Disease severity was positively correlated with inoculum density of *T. basicola* and was lower on moderately resistant cultivars than on cultivars with low resistance to black root rot. No disease was detected on the very highly resistant cultivar, Tennessee 86, in any field. Disease severity on cultivars with low resistance was positively correlated with exchangeable soil Ca, Mg, K, cation exchange capacity, and base saturation. Disease severity on moderately resistant cultivars was not correlated with soil chemical factors. Pathogen populations were positively correlated with the number of years in tobacco. *T. basicola* was found less frequently and in lower inoculum densities in fields in which rotation had been practiced as compared with those in continuous tobacco.

Thielaviopsis basicola (Berk. & Br.) Ferr. is a common soil inhabitant and a pathogen of over 137 species of plants (16). *T. basicola* causes black root rot disease on tobacco, one of its major agricultural hosts. Black root rot is mainly a cortical rot disease, characterized by black-colored root lesions resulting from the dark chlamydospores and hyphae formed by the dematiaceous fungus. Stunting, wilting, and chlorosis caused by the impaired root system of infected plants are typical aboveground symptoms observed throughout the growing season (10).

Black root rot has been reported in all the major tobacco-growing areas of the world (10). In North Carolina it is probably the major field disease on burley tobacco (*Nicotiana tabacum* L.), which is produced in the mountainous western part of the state. Estimates based on agricultural extension agent reports indicate losses of 3% of yield or approximately $1 million in 1986 due to this disease (11).

Paper No. 11484 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh.

Accepted for publication 31 October 1988 (submitted for electronic processing).

A 1985 survey of 13 fields with a history of poor tobacco growth, located in three major burley-producing counties of North Carolina, confirmed the presence of the pathogen and of black root rot (Shew and Shoemaker, *unpublished*). The purpose of this study was to randomly survey the entire burley-growing region of North Carolina and determine the distribution of *T. basicola* and the extent of disease occurrence. Additional data on the cultivar grown, cropping history, soil fertility, and soil texture were collected from each field to begin identification of soil and cropping variables associated with the occurrence and severity of the disease.

MATERIALS AND METHODS

Geographical area sampled, soil sampling, and disease rating. The survey was conducted from May to July in 1987 and covered the 10 western North Carolina counties with the largest acreages in burley tobacco (Fig. 1). These counties together produce 94% of the burley crop in the state. The number of fields sampled per county ranged from three to 20, based on a weighting scheme of one field sampled per each 40.5 ha (100 acres) of burley tobacco grown in the county. This resulted in 80 sampled fields that were selected using a random number table and lists of growers' names supplied by the county agricultural extension agents.

Soil samples were collected during a 3-wk period in May before the fields were planted. Because of the hilly topography of the region, fields were divided into two to four quadrats of about 0.1 ha (0.25 acre) per field, based on slope or obvious changes in soil type. Each soil sample was a composite of 15 3-cm-diameter soil cores taken to a depth of 20 cm in a zigzag pattern across each quadrat. Samples were stored in polyethylene bags at 13 C

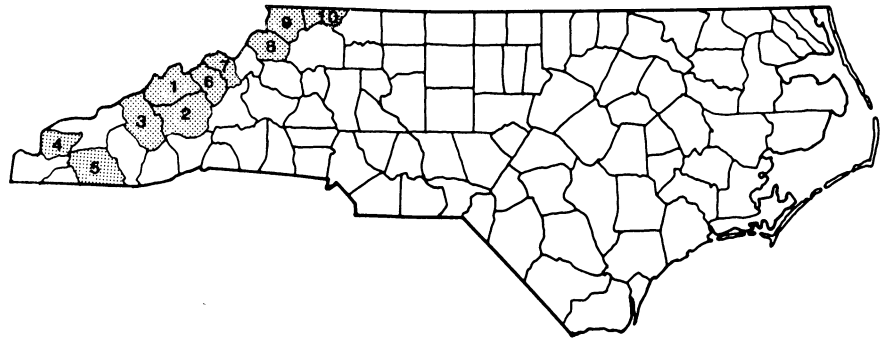

Fig. 1. Location of the counties included in the 1987 survey of *Thielaviopsis basicola* and black root rot on burley tobacco in western North Carolina. One field was sampled for each 40.5 ha (100 acres) of burley tobacco grown in each of the 10 major burley-producing counties, resulting in the following sampling scheme: Madison, 19 fields (1); Buncombe, 13 fields (2); Haywood, 10 fields (3); Graham, five fields (4); Macon, three fields (5); Yancey, 10 fields (6); Mitchell, five fields (7); Watauga, four fields (8); Ashe, seven fields (9); and Alleghany, four fields (10).

for the duration of the sampling period.

The fields in which *T. basicola* was detected were revisited in mid-July, approximately 6 wk after transplanting, and inspected for incidence and severity of black root rot. Six plants per quadrat (12–24 per field) were selected arbitrarily by pacing off a zigzag pattern similar to that used during the soil sampling. The plants were rated for disease severity by estimating the percent of the root system with the characteristic black lesions caused by *T. basicola* according to the following rating scale: 1 = no symptoms, 2 = trace (few, small, discrete lesions), 3 = <5% of the root system with symptoms, 4 = 5–25% of the root system with symptoms, and 5 = >25% of the root system with symptoms. The cultivar grown and the cropping history were also recorded for each survey field.

Soil analysis and pathogen assays. Populations of *T. basicola* were determined by using a modification of a soil plating technique developed by Specht and Griffin (18). Ten milliliters of a 1:100 (w/v) soil suspension in deionized water was pipetted into 300 ml of molten medium. The medium contained 50 ml of canned carrot juice, 18 g of agar, 950 ml of deionized water, and the antibiotic solution described by Specht and Griffin (18). The agar:soil suspension was mixed thoroughly on a magnetic stir plate, divided equally into 10 plastic petri plates, and incubated for 14 days at room temperature (22–25 C) in the dark. Thorough mixing of the soil suspension into the agar and pouring thick plates were important to obtain distinct colonies of *T. basicola*. Populations of *T. basicola* were calculated as the mean value of three replicate 1-g subsamples per soil sample. This was the optimum number of subsamples as determined in preliminary work by using the equation $n = (\hat{\sigma}/\hat{\sigma}_s)^{1/2}$, where n = optimum number of subsamples, $\hat{\sigma}$ = variance of the subsample, and $\hat{\sigma}_s$ = variance of the sample (4).

Populations of *Phytophthora parasitica* var. *nicotianae*, which causes black shank of tobacco, were determined by plating 1 ml of a 1:100 (w/v) soil suspension on PARP selective medium (9) modified by adding 50 mg/L of hymexazol to a 5% V-8 juice basal medium. Populations of plant parasitic nematodes were determined by the North Carolina Department of Agriculture (NCDA) nematode testing lab.

All soil samples were analyzed by volume for P, K, Ca, Mg, Na, Zn, Cu, and Mn by the NCDA soil testing lab by means of the Mehlich-3 extractant and atomic absorption spectrophotometry. The NCDA lab also determined the cation exchange capacity, calculated by summing the basic cations (including Na) and buffer acidity, and soil pH in a 1:1 soil:water suspension. Percent sand, silt, and clay were determined by the hydrometer method for each sample (5).

Data from the quadrats within a field were averaged to obtain one date set per field. Relationships between inoculum density of *T. basicola*, rating for black root rot, and soil and cropping variables were tested by means of t tests, one-way analysis of variance (17), and factor analyses (15) using principal components analysis and oblique rotation. Oblique rotation was chosen because, unlike orthogonal rotation, it allows for correlation between factors, which was an appropriate assumption for soil variables. Those variables not associated with disease in the initial analyses were excluded from the final factor solution. Variables with factor loadings greater than 0.40 were considered interrelated.

RESULTS

T. basicola was detected in 47 of the 80 fields (59%) at an inoculum density ranging from 2 to 219 cfu/g of dry soil and a mean inoculum density of 40 cfu/g of dry soil. Black root rot disease occurred in 32 (68%) of the infested fields. Disease severity was positively correlated with inoculum density of *T. basicola* (Tables 1 and 2). In most fields, an inoculum density greater than about 60 cfu/g of soil was necessary to cause more than trace symptoms of black root rot (Table 1).

Disease severity was significantly different among cultivars with low, moderate, or very high resistance to black root rot (Table 3). The very high resistance class comprised a single cultivar, Tennessee 86. No black root rot was observed on this cultivar in any field. Fields planted in cultivars with low resistance to black root rot had the highest disease rating (Table 3).

T. basicola was found more frequently and at higher inoculum densities in fields planted in continuous tobacco. *T. basicola* was found in 70% of the fields planted in continuous tobacco (59 fields), but in only 23% of the fields in which an alternate crop (usually field corn, *Zea mays* L.) had been planted in the previous season (13 fields). Mean inoculum density following tobacco was 46 ± 9 cfu/g of soil (n = 41); mean inoculum density in rotated fields was 21 ± 12 cfu/g of soil (n = 3).

The variables measured during the survey were analyzed with the factor analysis technique to identify groups of related variables. The results of the final factor analysis solution are presented in Table 4. In factor 1, black root rot disease severity and seven soil chemical variables were highly related. Disease severity was not associated with variables in factors 2

Table 1. Inoculum density of *Thielaviopsis basicola* and severity of black root rot of burley tobacco in 10 western North Carolina counties

Disease severity	Number of fields[x]	*T. basicola*[y] (cfu/g of soil)
No symptoms	7	9 (± 5)
Trace[z]	20	15 (± 3)
Symptoms in <5% of root system	6	64 (± 19)
Symptoms in 5–25% of root system	6	109 (± 31)

[x] Does not include fields planted in the very highly resistant cultivar Tennessee 86 (eight fields).

[y] Mean (± standard error); linear correlation of disease severity and inoculum density = 0.62 (P = 0.001).

[z] Few, small, discrete lesions.

Table 2. Linear correlations of soil variables and inoculum density of *Thielaviopsis basicola* with disease severity of black root rot in infested burley tobacco fields planted to cultivars with low or moderate black root rot resistance

Variable	Linear correlation with black root rot rating[x]: Low resistance (eight fields)	Moderate resistance (26 fields)
Inoculum density[y]	0.57[z]	0.63**
Ca	0.93**	0.24
Mg	0.63	0.20
K	0.71*	0.11
Sum of cations	0.89**	0.26
CEC	0.89**	0.24
pH	0.65	0.02
% Base saturation	0.74*	0.16
% Ca saturation	0.86**	0.10

[x] Fields in which cultivars with very high (eight fields) or unknown (five fields) resistance were planted were not included in the analysis.

[y] Colony-forming units of *T. basicola* per gram of dry soil.

[z] Correlation coefficient for disease rating and a given variable are significant at P = 0.05 (*) and P = 0.01 (**).

Table 3. Severity of black root rot in fields planted in burley tobacco cultivars differing in resistance to *Thielaviopsis basicola* and estimated production area planted in each resistance class in western North Carolina

Cultivar resistance class	Mean disease severity index[y]	Estimated acreage planted (%)
Low resistance	2.2 a[z]	15
Moderate resistance	1.7 b	68
Very high resistance	1.0 c	9

[y] Disease severity was an estimate of the percentage of the root system with the characteristic black lesions caused by *T. basicola* using the following rating scale: 1 = no symptoms, 2 = few, small, discrete lesions, 3 = <5% of the root system with symptoms, 4 = 5–25% of the root system with symptoms, and 5 = >25% of the root system with symptoms.

[z] Means followed by the same letter are not significantly different at P = 0.05 using the Student-Newman-Keuls multiple range test.

or 3, but was highly correlated with the cultivar resistance class in factor 4. As indicated by the communality rating, the five-factor solution accounts for 83% of the variance in disease severity. In factor 5, inoculum density was associated with the number of years in continuous tobacco.

Linear correlation analyses of soil variables and disease severity were performed separately with each cultivar resistance class. The results are shown in Table 2. Disease severity on cultivars with low resistance was highly correlated with the soil chemical variables Ca, Mg, K, and CEC, and with Ca saturation and base saturation. Disease severity on moderately resistant cultivars was not correlated with soil chemical factors (Table 2). Inoculum density was the only significant correlation observed with disease severity on moderately resistant cultivars.

Most soils were classified as loam, clay loam, or silty loam and contained less than 1% organic matter. Inoculum density and disease severity were not correlated with soil texture. Over 76% of the fields had a soil pH below 6.0, which is the recommended value for burley tobacco in North Carolina.

P. parasitica var. *nicotianae* was not detected (detection limit of assay = 1 cfu/10 g of soil) in any of the fields surveyed. Symptoms of black shank, however, were observed in one of the survey fields in July. Root-knot nematodes (*Meloidogyne* spp.) were detected in 14% of the fields at a range of 10–270 nematodes per 500 cm^3 of soil and a mean population level of 82 nematodes per 500 cm^3 of soil.

Table 4. Factor analysis (principal components-oblique rotation) of black root rot, inoculum density of *Thielaviopsis basicola*, and soil and cropping variables in 80 randomly chosen burley tobacco fields in western North Carolina

Variable	Factors 1	2	3	4	5	Communality
Disease severity[x]	0.42	...	...	−0.78	...	0.83
Inoculum density[y]	...	...	...	...	0.59	0.45
Cultivar resistance class[z]	...	...	...	0.92	...	0.90
Years in tobacco	...	0.52	...	...	0.54	0.70
P	...	0.80	...	...	...	0.67
K	...	0.84	...	...	...	0.77
Ca	0.92	...	...	...	...	0.90
Mg	0.84	...	...	...	...	0.74
pH	0.82	...	...	...	...	0.77
CEC	0.68	0.53	0.46	...	...	0.91
Buffer acidity	−0.72	...	0.61	...	...	0.92
Sum of cations	0.95	...	...	...	...	0.98
Base saturation	0.91	...	...	...	...	0.94
Mn	...	...	−0.68	...	...	0.57
Organic matter	...	...	0.79	...	...	0.68
Variance (%)	35	18	12	8	7	
Cumulative variance	35	53	65	72	79	

[x] Disease severity was an estimate of the percentage of the root system with characteristic black lesions caused by *T. basicola* using the following rating scale: 1 = no symptoms, 2 = few, small, discrete lesions, 3 = <5% of the root system with symptoms, 4 = 5–25% of the root system with symptoms, and 5 = >25% of the root system with symptoms.

[y] Colony-forming units of *T. basicola* per gram of dry soil.

[z] Burley tobacco cultivars planted in each field were grouped into a low, moderate, or very high resistance class based on the reported resistance to *T. basicola*.

DISCUSSION

T. basicola was widely distributed in the burley tobacco growing region of western North Carolina. The inoculum densities detected in this region were similar to those detected by Specht et al (20) in a recent survey of flue-cured and burley tobacco fields in Virginia.

Black root rot developed in 68% of infested fields, which indicates that disease is likely to occur when the pathogen is present. Disease severity was dependent on the inoculum density of *T. basicola*. For example, significant root rot developed in most fields only when populations of *T. basicola* were greater than 40–60 cfu/g of soil. This observation is in agreement with results from recent greenhouse and field experiments (19) in which a positive linear correlation between inoculum density of *T. basicola* and black root rot ratings on burley and flue-cured tobacco cultivars was demonstrated. Yield loss of burley tobacco has also been correlated with inoculum density of *T. basicola* in field experiments (Shew and Shoemaker, *unpublished*).

Control of black root rot is achieved mainly by the use of resistant cultivars (10,14). In 1986, about 68% of the burley fields in western North Carolina were planted in cultivars reported to have moderate black root rot resistance. Disease severity on these cultivars was significantly lower than on cultivars with low resistance to black root rot, which confirms the effectiveness of current cultivar resistance in controlling the development of black root rot in the field. Tennessee 86 has very high resistance conditioned by a single gene (13) and did not develop black root rot even in the presence of high inoculum densities. This cultivar offers an effective control in fields with high populations of *T. basicola*.

Crop rotation is a recommended cropping practice for aid in black root rot control (10). The positive correlations found between inoculum density of *T. basicola* and years in continuous tobacco, and the lower populations found in fields in which rotation had been practiced, suggest that rotation reduces inoculum density and subsequent disease severity. This observation is supported by experimental work of Bateman (3), in which populations of *T. basicola* increased only in the presence of a host plant and decreased in soil planted with nonhosts or in fallow soil. It is probable that pathogenic root infection is necessary for population buildup of this fungus. The most common rotation crop in western North Carolina is field corn, which is not a host of *T. basicola* (7).

The results of the factor analysis indicate that soil chemical factors strongly affect black root rot disease severity. The variables correlated positively with black root rot severity in this study (exchangeable soil Ca, Mg, cation exchange capacity, base saturation) are all related to soil pH. Severe black root rot associated with high soil pH is well-documented for tobacco and other host species (1,2,6,8,12), although the nature of this interaction is not yet understood. Previous studies have primarily used susceptible host cultivars. However, survey data from this study indicate that, although disease development on susceptible cultivars is influenced by soil pH or related chemical factors, disease development on moderately resistant cultivars is not. This observation warrants further investigation.

The results of this survey suggest that both inoculum density of *T. basicola* and soil pH-related chemical variables are good predictors of favorable conditions for black root rot development. We are currently investigating the interactions between these predictive variables in controlled experiments. Quantification of these variables and an understanding of their interactions should be useful in the prevention and management of black root rot in the field.

ACKNOWLEDGMENTS

We wish to thank the following agricultural extension agents for their valuable help in this survey: Bob Breland, Keith Clay, Wiley DuVall, Tom Harvey, Gary Hyatt, Raymond Norton, Robert Rollins, Jim Ray, and Jerry Washington. Their assistance is greatly appreciated.

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