Development of Black Root Rot on Burley Tobacco as Influenced by Inoculum Density of *Thielaviopsis basicola*, Host Resistance, and Soil Chemistry

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ABSTRACT

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Twelve fields infested with *Thielaviopsis basicola*, selected from 80 surveyed the previous year, were planted with cultivars of burley tobacco with either low (B21 × Ky 10) or moderate (Ky 14) resistance to black root rot. One week after transplantation, soil samples were taken around 20 individual plants in each field to determine the inoculum density of the pathogen and to conduct soil chemical analyses. Severity of black root rot, evaluated on each of the same plants 6 wk later, depended on inoculum density, level of host resistance, and soil chemistry. Inoculum densities of < 50 cfu/g of soil resulted in severe root rot on 52% of the plants with low resistance but on only 3% of the plants with moderate resistance. At high inoculum densities, however, levels of disease on the two cultivars were similar. Black root rot did not develop on plants of either cultivar in three of the six fields with an average pH of < 5.2. These soils appeared to suppress disease development, since the pathogen was present at densities sufficient to cause disease (> 50 cfu/g of soil). Black root rot developed, however, in soils with a pH of < 5.2 if the base saturation was > 70%. The suppressive effect was confirmed under controlled experimental conditions with fumigated field soils reinfested with *T. basicola* and was reversed by altering soil chemistry with calcium hydroxide.

Black root rot has been reported in all the major tobacco-growing areas of the world (13). In North Carolina, it is the major field disease on burley tobacco (*Nicotiana tabacum* L.) (14), which is grown in the mountainous western part of the state. The disease is caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris (synanamorph: *Chalara elegans* Nag Raj & Kendrick), a dematiaceous fungus for which no teleomorph is known. The fungus is a common soil inhabitant in both cultivated and noncultivated soils (22), causing characteristic black, necrotic lesions on the main and lateral roots of over 137 plant species (19). Roots and shoots of infected plants are stunted, and yield loss is often sustained because of stunting, root dysfunction, and delayed maturation of the crop (13).

Inoculum density of *T. basicola* in soil has been positively correlated with the severity of black root rot (3,18,21). However, this relationship can be modified by cultural and environmental factors, including soil temperature (6,9-11,15), genetic resistance of the host (3,11), and soil pH (1,6,7). Soil temperatures affect development of black root rot when temperatures slow the growth of the host relative to that of the fungus (9,11,15). Black root rot rarely occurs when pH is below 5.4 and is most severe above pH 5.6 (1,7). The mechanism for the interaction between black root rot development and soil pH is not yet understood.

In a large, random survey of 80 burley tobacco fields in western North Carolina, severity of the black root rot disease was associated with populations of *T. basicola*, the level of host resistance, and factors generally associated with soil pH, including percent base saturation and amount of base cations in the soil (18). The objectives of the present study were: 1) to resample selected fields from the previous survey more intensively, 2) to determine more precisely how host resistance and soil pH modify the relationship between inoculum density of *T. basicola* and the development of black root rot, and 3) to demonstrate under controlled conditions how soil chemistry modifies the susceptibility of tobacco to *T. basicola*. This information will be useful in establishing pathogen threshold levels and in predicting disease losses under various field conditions.

MATERIALS AND METHODS

Field study. Twelve commercial fields were selected for study from 80 fields surveyed the previous season (18). Fields that satisfied the following criteria were chosen: 1) *T. basicola* was present; 2) the cultivar to be grown in the current season was either B21 × Ky 10 or B37 (low resistance) or Ky 14 (moderate resistance), which were the same cultivars grown in the previous season; and 3) the average soil pH was either < 5.2 (low) or > 5.6 (high). These pH values were chosen because the severity of black root rot in 80 randomly surveyed fields was significantly lower when the average soil pH in the field was < 5.2 ($n = 21$) than when the soil pH was > 5.6 ($n = 14$) (17; J. R. Meyer, *unpublished*).

The fields were grouped into four categories based on the level of host resistance of the cultivar to be grown and the soil pH: low pH and low host resistance (fields 1-3), low pH and moderate host resistance (fields 4-7), high pH and low host resistance (fields 8 and 9), and high pH and moderate host resistance (fields 10-12) (Table 1). The crop was maintained in each field by the growers according to their standard practices; no treatments were imposed.

Soil sampling and disease rating. Soil samples were collected during a 3-day period in early June within 1 wk after the seedlings were transplanted into the field. Each soil sample was a composite of four 3-cm-diameter soil cores taken to a depth of 20 cm around each plant. Twenty plants per field were sampled systematically (every fifth plant of every other row in eight rows) and tagged with flagging tape. Soil samples were stored in polyethylene bags at 13 C for 1-3 wk prior to pathogen assays and chemical analyses. Six weeks after transplantation, the roots of the same 20 tagged plants in each study field were inspected for symptoms of black root rot. Disease was rated by estimating the percentage of the root system that had characteristic black lesions caused by *T. basicola*.

Soil analyses and pathogen assays. Populations of *T. basicola* were calculated as the mean value of three replicate 1-g subsamples per soil sample by using a modification of a soil plating technique (18,20) in which 10 ml of a soil suspension (1:100, w/v) in deionized water was pipetted into 300 ml of a molten medium. The medium contained 50 ml of canned carrot juice, 18 g of agar, 950 ml of deionized water, and the antibiotics described by Specht and Griffin (20). The agar/soil suspension was mixed thoroughly on a magnetic stir plate, divided equally into 10 plastic petri dishes, and incubated for 14 days at room temperature (22-25 C) in the dark.

The remaining soil was air-dried and analyzed by volume for P, K, Ca, Mg, Na, Zn, Cu, and Mn by the North Carolina Department of Agriculture Soil Testing Laboratory by means of the Mehlich-3 extractant (16) and atomic

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absorption spectrophotometry (Ca, Mg, Zn, Cu, Mn) or flame emission (K, Na). The cation exchange capacity was calculated as the sum of basic cations (including Na) and buffer acidity, and the base saturation was calculated as the percentage of the cation exchange capacity occupied by Ca, Mg, and K. Soil pH was measured with a glass electrode in a 1:1 soil/distilled water suspension.

Phytotron study. Soils from three survey fields with a soil pH of 4.5, 6.0, and 6.7 were collected in late fall, fumigated with methyl bromide, and stored in 20-L plastic bins. The methyl bromide was allowed to dissipate for several days before the soil was used or stored. No *T. basicola* was detected in any soil after fumigation as determined by assay on selective agar medium.

Chlamydospore suspensions of *T. basicola* were prepared from 6- to 8-wk-old colonies on 5% carrot juice agar by flooding the medium surface with water and scraping the spores off the surface. The resulting spore suspension was washed through nested 400-mesh (0.038-mm) and 500-mesh (0.022-mm) sieves. Most endoconidia passed through the sieves, the mycelium stayed on the top sieve, and the chlamydospores were harvested from the 500-mesh sieve. The chlamydospore suspension was homogenized in a Waring blender for 1 min at high speed, and the sieving process was repeated. The concentration of spores in the suspension was determined using a hemacytometer. Each chlamydospore chain was counted as a single propagule, although this may underestimate the inoculum density because spores in a chain may separate before or at germination (5,8).

The test soils were infested with 100 chlamydospores per gram of dry soil by adding an appropriate volume of a spore suspension to soil (225 cm^3) contained in a plastic bag. The soil and spore suspension were mixed thoroughly by hand agitation before each 7.5-cm-diameter pot was filled. One 6-wk-old seedling of burley tobacco was transplanted into each pot of infested soil. Three cultivars of burley tobacco, three field soils, and seven isolates of *T. basicola* were used in a factorial design with five replicate pots per treatment. The tobacco cultivars had low (B21 × Ky 10), moderate (Ky 14), or moderate-high (Ky 14 × L8) resistance to black root rot, and the isolates of *T. basicola* were isolated from a range of soil types. The plants were grown in a completely randomized design in a walk-in growth chamber with a 12-hr day length and 28/20 C day/night temperatures, which are similar to those in western North Carolina in the summer. Three weeks after transplantation, the plants were removed from the pots and rinsed, and the root systems were inspected for symptoms of black root rot. Disease severity was expressed as the percentage of the root system that had characteristic black lesions caused by *T. basicola*. Populations of *T. basicola* were determined after harvest in three 1-g soil samples per pot with the selective agar medium previously described. The experiment was conducted twice.

A second experiment was conducted in which the soil with a pH of 4.5 was amended with 2.2 mg of dry calcium hydroxide [$Ca(OH)_2$] per gram of soil, brought to 25% moisture, and allowed to incubate for 4–6 wk before being infested with 100 chlamydospores of *T. basicola* per gram of soil. Eight replicate pots per treatment (amendment or no amendment) were prepared as described above. Soil chemical analyses were performed before amendment and after incubation. The experiment was conducted twice.

RESULTS

Field study. *T. basicola* was detected in 11 of the 12 study fields. Propagule numbers varied greatly around individual plants, with a range of 0 to 577 cfu/g of soil in one field (Table 1).

Symptoms of black root rot were observed in all fields where the mean soil pH was 5.6 or higher (fields 8–12). Symptoms were significantly more severe on B21 × Ky 10 (low resistance) than on Ky 14 (moderate resistance). For example, when inoculum density was < 50 cfu/g of soil, severe root rot (10–25% necrosis) occurred on 52 and 3% of the plants of B21 × Ky 10 and Ky 14, respectively (Figs. 1 and 2). When all inoculum densities greater than zero were considered, no symptoms or only a trace of symptoms was found on 42% of the Ky 14 plants (20 of 48 in three fields) compared with only 11% of the B21 × Ky 10 plants (four of 39 in two fields). A positive relationship was observed between the inoculum density of *T. basicola* and disease severity for both cultivars. Higher levels of disease developed at low inoculum densities on B21 × Ky 10 plants than on Ky 14 plants (Fig. 2).

At inoculum densities greater than zero in fields with a low mean soil pH, either no symptoms or only a trace of symptoms was observed on 84% of the Ky 14 plants (47 of 52 in four fields)

Table 1. Populations of *Thielaviopsis basicola*, soil pH, percent base saturation, and disease resistance of burley tobacco cultivars grown in 12 fields

	Inoculum density 1988[w]		Soil[x]		Cultivar		1987[y]	
Field	Range	Mean	pH	Base saturation (%)	Name	Resistance	Inoculum density	Disease severity
1	0–88	7	4.7	65	B21 × 10	Low	2	1.0
2	0		4.2	43	B21 × 10	Low	6	1.0
3	0–51	4	4.7	55	B37	Low	8	1.8
4	0–577	58	4.7	68	Ky 14	Moderate	25	1.8
5	0–241	60	4.6	66	Ky 14	Moderate	9	1.6
6	0–913	87	4.8	74	Ky 14	Moderate	146	2.4
7	0–270	64	5.3	71	Ky 14/Ky 14 × L8	Moderate/high[z]	13	1.7
8	0–504	143	5.8	85	B21 × 10	Low	95	2.9
9	0–135	42	5.6	83	B21 × 10	Low	164	3.1
10	0–440	175	6.1	91	Ky 14	Moderate	Not sampled	···
11	0–229	57	6.3	94	Ky 14	Moderate	25	2.8
12	0–203	43	6.2	89	Ky 14	Moderate	4	1.44

[w] Colony-forming units of *T. basicola* per gram of dry soil as determined using a selective medium. Range and mean of 20 samples per field.
[x] Mean from soil around 20 plants per field. Soil pH was determined in a 1:1 soil/water suspension, and base saturation was calculated as the percentage of the cation exchange capacity occupied by Ca, Mg, and K, as determined by atomic absorption spectrophotometry or flame emission in a Mehlich-3 soil extractant.
[y] Colony-forming units of *T. basicola* per gram of dry soil as determined using a selective medium. Mean of subsamples per field from 15 bulked soil cores. Disease severity was an estimate of the percentage of the root system (six plants per field, randomly chosen) with characteristic black lesions caused by *T. basicola*, where: 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; 5 = >25% of the root system with symptoms.
[z] Field 7 had a few plants of cultivar Ky 14 × L8 that had a moderate-high resistance to *T. basicola*.

and 100% of the B21 × Ky 10 plants (11 of 11 plants in two fields). No symptoms were observed on plants in fields 1, 4, and 7 even though the inoculum density in these fields ranged from 0 to 88, 577, and 270, respectively, and was above 50 cfu/g of soil around one, five, and eight plants in these fields, respectively (Fig. 3). These soils appeared to be suppressive to black root rot.

In other fields with low soil pH (fields 3, 5, and 6), symptoms were observed (Fig. 3). The inoculum density in these fields ranged from 0 to 51, 241, and 193, respectively, and was above 50 cfu/g of soil around 1, 6, and 10 plants, respectively. The symptomatic plants were growing in parts of the fields where base saturation of the soil was > 70% and where the soil pH was not higher than the field average (Fig. 4). Black root rot developed in soils with a pH < 5.2 when the base saturation of the soil was > 70% (Fig. 5). The absence of disease in soils with a base saturation of < 70% was not due to the absence of the pathogen because the pathogen was present at inoculum levels sufficient to cause disease (Fig. 6).

Phytotron study. Either no symptoms or only a few, small, discrete lesions were observed on the burley tobacco plants grown in fumigated field soils with a pH of 4.5 under controlled environmental conditions. Black root rot severity was significantly greater on plants grown in soils with a pH of 6.0 and 6.7 (Table 2). Postharvest inoculum densities of *T. basicola* > 100 cfu/g of dry soil, however, were detected in all soils. Amendment of the strongly acidic soil (pH 4.5) with calcium hydroxide increased soil pH, base saturation, soil calcium levels, and black root rot development (Table 3), whereas symptoms of black root rot were not observed in the unamended soil. *T. basicola* was detected at inoculum densities > 100 cfu/g of dry soil in both treatments at harvest. When repeated, experiments produced similar results.

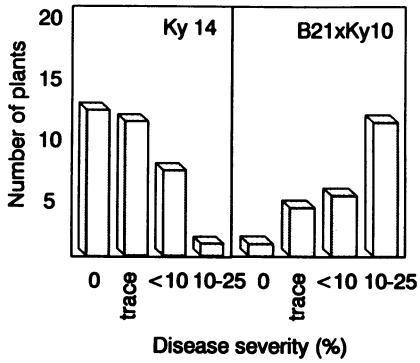

Fig. 1. Frequency distribution of black root rot severity on two burley tobacco cultivars grown in soils with an average pH of > 5.6 and an inoculum density of < 50 cfu/g of soil.

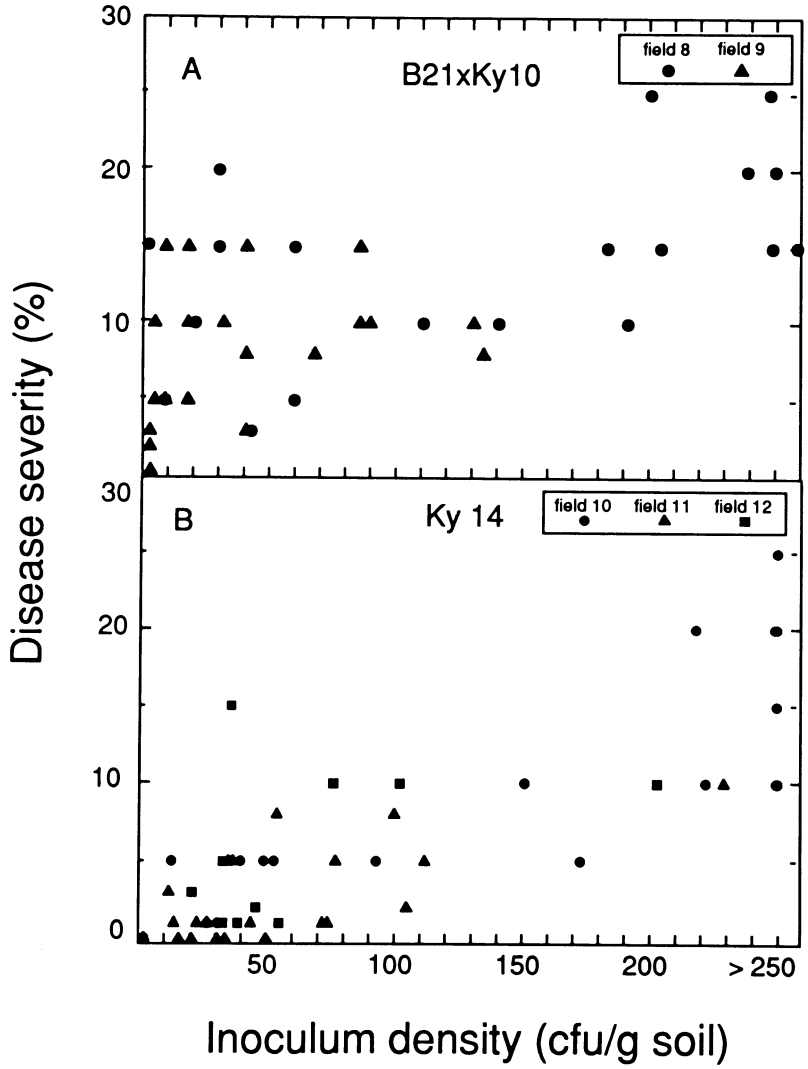

Fig. 2. Relationship between the inoculum density of *Thielaviopsis basicola* around individual plants and disease severity on burley tobacco **(A)** cv. B21 × Ky 10 (low resistance) in two fields (39 plants) with an average soil pH of > 5.6 and **(B)** cv. Ky 14 (moderate resistance) in three fields (55 plants) with an average soil pH of > 5.6.

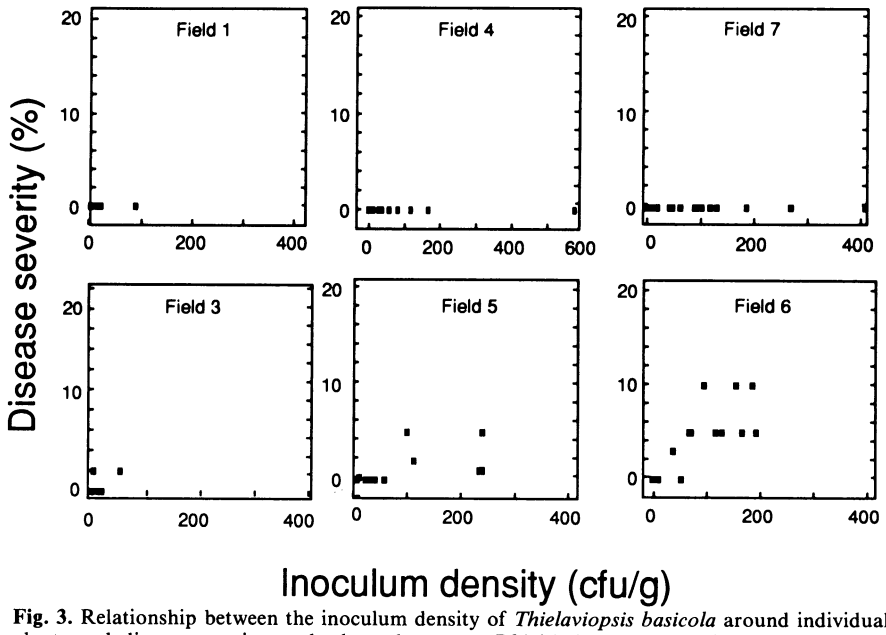

Fig. 3. Relationship between the inoculum density of *Thielaviopsis basicola* around individual plants and disease severity on burley tobacco cv. B21 × Ky 10 (low resistance) in fields 1 and 3 (40 plants) and cv. Ky 14 (moderate resistance) in fields 4–7 (75 plants) with an average soil pH of < 5.2.

DISCUSSION

Host resistance modified the relationship between inoculum density of *T. basicola* and the severity of black root rot, confirming observations from a previous survey (18). At low populations of *T. basicola* (< 50 cfu/g of soil), disease development was significant on cultivars with low resistance but not on cultivars with moderate resistance. Most tobacco fields in regions where black root rot occurs are characterized by low populations of *T. basicola*. For example, in 80 randomly surveyed tobacco fields in western North Carolina, 74% of the fields in which *T. basicola* was present had populations < 50 cfu/g of soil (J. R. Meyer, *unpublished*). Anderson and Welackey (2) reported that about 72% of 195 tobacco fields surveyed in Ontario, Canada, infested with *T. basicola* had < 60 cfu/g of soil. The low pathogen threshold level for cultivars with low resistance indicates a potential for significant disease development whenever the pathogen is present. The relationship between inoculum density and development of black root rot on burley tobacco cultivar B21, which has low resistance to black root rot, in a temperature tank study (21) was similar to that found in the field in this study. The higher threshold level on cultivars with moderate resistance demonstrates the effectiveness of host resistance as part of a management strategy for this disease.

Development of black root rot was also modified by soil chemistry. Disease did not develop in fields characterized by low soil pH even though the pathogen was present at inoculum densities that caused disease in other soils. Suppression of disease also was observed in fumigated acidic field soils where the inoculum density of *T. basicola* was controlled. The suppressive effect was reversed when soil chemistry was experimentally altered by liming to increase soil pH and base saturation. The field data suggested that H^+ activity (pH) was not the sole mechanism of suppression of black root rot development. These data are the first indication that black root rot can develop under field conditions in acidic soils if the base saturation is high enough. It is known, however, that *T. basicola* can grow at pH 5 in vitro (12). Because base saturation is an indicator of the proportions of base cations (primarily Ca) and acidic cations (primarily Al) on the cation exchange sites (4), the lack of disease development in soils with low base saturation in the presence of *T. basicola* suggests that low Ca or high Al levels may be part of the mechanism influencing host susceptibility or pathogen virulence. These hypotheses are currently being tested.

Populations of *T. basicola* were generally, but not consistently, higher around plants growing in soils with a high percent base saturation than around plants growing in soils with a low percent base saturation. The higher inoculum densities, which were detected in soil around the individual plants before any disease had developed during the current season, were probably the result of disease development during the previous year and may indicate an increase of

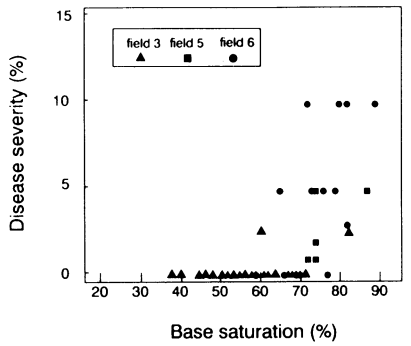

Fig. 4. Relationship between the severity of black root rot and the base saturation of the soil surrounding burley tobacco plants in three fields (55 plants) with symptomatic plants and an average soil pH of < 5.2.

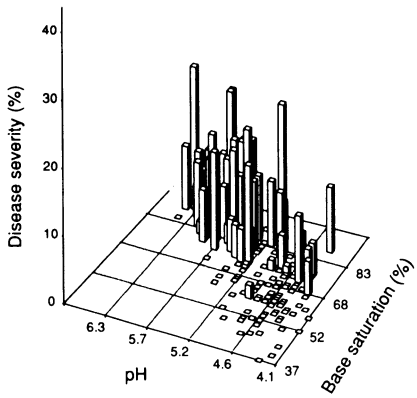

Fig. 5. Relationship between the severity of black root rot on burley tobacco plants and the soil pH and percent base saturation in 11 fields (209 plants). Each bar represents data from an individual plant.

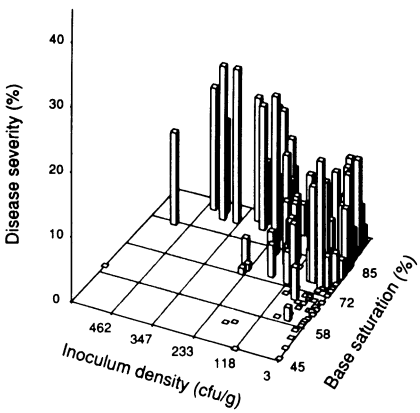

Fig. 6. Relationship between the severity of black root rot on burley tobacco, the inoculum density of *Thielaviopsis basicola* in early season, and the percent base saturation of the soil in 11 fields (153 plants). Each bar represents data from an individual plant exposed to an inoculum density greater than zero.

Table 2. Severity of black root rot disease on burley tobacco in relation to host resistance and soil pH[y]

Soil pH	Test 1			Test 2		
	Low resistance	Moderate resistance	High resistance	Low resistance	Moderate resistance	High resistance
4.5	<1 a[z]	<1 a	<1 a	<1 a	<1 a	<1 a
6.0	38 b	27 b	6 b	10 b	6 b	<1 a
6.7	90 c	86 c	58 c	36 c	37 c	15 b

[y] Disease severity was estimated as percentage of root system with characteristic black lesions caused by *Thielaviopsis basicola*. Values represent the mean of five replicate pots for each of seven isolates of *T. basicola* ($n = 35$). Plants were grown in field soil that had been fumigated and reinfested with 100 chlamydospores per gram of soil. Cultivars were B21 × Ky 10 (low resistance), Ky 14 (moderate resistance), and Ky 14 × L8 (high resistance).

[z] Means followed by the same letter are not significantly different at $P = 0.05$, according to the Tukey multiple range test.

Table 3. Effect of soil amendment with calcium hydroxide [$Ca(OH_2)$] on soil chemistry and severity of black root rot on burley tobacco cv. B21 × Ky 10 in acidic field soil fumigated and reinfested with *Thielaviopsis basicola*

Treatment[w]	Disease severity[x]	Soil pH[y]	Base saturation[z] (%)	Elements (meq/100 cm³)			Cation exchange capacity	Buffer acidity	Zn (mg)
				Ca	Mg	K			
Unamended	0	4.6	62	2.8	0.6	0.6	6.7	2.6	2.0
$Ca(OH_2)$	23	6.5	92	7.4	0.7	0.5	9.4	0.7	3.0

[w] Dry test soil was treated with 2.2 mg of $Ca(OH_2)$ per gram of soil, brought to 25% moisture holding capacity, and allowed to incubate for 4–6 wk. Values represent the mean of eight replicate plants.

[x] Percentage of root system with characteristic black lesions caused by *T. basicola*.

[y] Determined in a 1:1 soil/water suspension.

[z] Calculated as percentage of cation exchange capacity occupied by Ca, Mg, and K, as determined by atomic absorption spectrophotometry or flame emission in a Mehlich-3 soil extractant.

inoculum under conditions of high base saturation.

Natural processes and agricultural practices may play a role in development of suppressive properties of some fields. The soils in the Appalachian region of North Carolina, where burley tobacco is grown, are generally highly weathered mineral soils (typic Hapludults) and have been cultivated for many decades. Because of the humid climate, continuous cultivation, and strong weathering conditions of the region, the soils are typically very acidic. The parent materials of the soils in the northeastern region of the mountains, where most of the highly conducive soils were detected, are often schists and hornblende gneiss metamorphic rocks high in iron and base cations (3). Soils in the central part of the mountains, where most of the suppressive soils were detected, were formed from more acidic rocks such as granitic gneiss low in base cations and high in acidic cations such as aluminum. The differences in soil pH and base saturation observed among fields in this region may be due to the different fertilization and liming practices adopted by individual growers. The soil chemistry that results from the natural soil conditions, coupled with fertilization and liming practices, may determine the conduciveness of a particular field soil to black root rot.

Analysis of soil and pathogen characteristics around individual plants, rather than random field samples or means, was an effective way of determining the soil chemical factors that affect the disease and the different combinations of soil conditions and inoculum densities that can affect disease development. Soil pH can be a general indicator of soil conditions favorable for development of black root rot. This study, however, indicated that the most accurate prediction of disease potential should also include a measurement of the percent base saturation. In regions where there is a range in soil acidity, the relative potential for development of black root rot can be predicted when the percent base saturation, soil pH values, populations of *T. basicola*, and resistance level of the cultivar are known. This study provides estimates of the values needed for such predictions for the Appalachian region of North Carolina.

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