Soils Suppressive to Black Root Rot of Burley Tobacco, Caused by *Thielaviopsis basicola*

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ABSTRACT

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Soils suppressive to black root rot were detected in fields in which the pathogen was present but in which little or no disease had developed on susceptible cultivars of burley tobacco. Suppressive soils were characterized by low base saturation, low calcium, exchangeable aluminum levels of 1 meq/100 g of soil or higher, and soil pH less than 5. Suppressiveness was confirmed under controlled environmental conditions with fumigated field soils reinfested with *Thielaviopsis basicola*. Isolates of *T. basicola* from suppressive soils caused black root rot when placed in conducive soil, indicating that the absence of disease was not due to differences in pathogen virulence. The mechanism of suppression was not biotic; autoclaving soil had no effect on suppressiveness, and transfer of suppressive soil to conducive soil (fumigated soil; 1:9, by volume) did not induce suppressiveness. Soil calcium level was not the mechanism of suppression because amending suppressive soils with calcium hydroxide nullified suppressiveness and amending soils with calcium sulfate did not. Disease developed in acidified conducive soil only if exchangeable aluminum levels were low. The acidification treatments had no effect on the inoculum density of the fungus, and the survival of the chlamydospores of *T. basicola* was not affected by the soil or soil treatments. Mechanisms of soil suppression to black root rot on burley tobacco are abiotic and dependent on the interrelationships among soil pH, base saturation, and exchangeable aluminum.

Additional keywords: soil chemistry, *Nicotiana tabacum, Chalara elegans.*

Black root rot is caused by a common fungus, *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick), which is found in both cultivated and noncultivated soils (44). It is a facultative parasite of more than 137 plant species (34), on which it forms characteristic and diagnostic black lesions on both the main and lateral roots (25). The primary infective propagules of the pathogen are the melanized, segmented chlamydospores (43); no teleomorph is known. The fungus has also been observed in nonpathogenic association with plant roots (44) and can colonize decaying residues of nonhost plant species (14).

Soils were detected in western North Carolina in which *T. basicola* was present but caused little or no black root rot on susceptible cultivars of burley tobacco (*Nicotiana tabacum* L.) (30,31), suggesting that the soils were suppressive to the disease. Suppressive soils are defined as soils in which a pathogen is present but does not cause disease on a susceptible host even though environmental factors are conducive for disease (5,17). The phenomenon is widespread and results primarily from antagonistic interactions between pathogens and saprophytic soil organisms (5,36), often influenced by soil chemical and physical properties (26). Soils suppressive to black root rot of tobacco have been reported in Switzerland (13), where the mechanism of suppression has been shown to be antagonism by a strain of the common soil bacterium *Pseudomonas fluorescens* that is favored by the type of clays in these soils (20,40,41).

Development of black root rot on burley tobacco in western North Carolina was correlated with soil factors generally associated with soil pH, including percent base saturation and amount of basic cations in the soil (31). Severe black root rot associated with high soil pH (greater than 5.6) is well documented for tobacco and other host species (1,2,8–10,18,29), although the mechanism of this effect is not yet known. Soil pH affects the distribution and activity of soil microorganisms, including plant pathogens (15,19,21). Soil pH, however, is probably not the only property of acidic soils that influences black root rot, because disease developed in strongly acidic (characterized by a pH less than 5.2) soils in western North Carolina when the base saturation surrounding individual plants exceeded approximately 70%, and disease did not develop in soils with a pH less than 5.2 when the base saturation was low (31). Because base saturation reflects the relative proportion of basic (mainly Ca^{2+}) and acidic cations (mainly ionic forms of aluminum) on soil exchange sites, we hypothesized that low soil calcium or high soil aluminum might be the mechanism of disease suppression in these soils. Many fungi, including *T. basicola* (35,39), require calcium, which is an important nutrient in reproduction of *Phytophthora* spp. (11). Aluminum is fungitoxic (12,22) and has been implicated in the suppression of two diseases caused by soilborne pathogens (32,33).

In this paper, results from a series of experiments on the nature of soils from western North Carolina suppressive to black root rot and on the mechanism of suppression are reported. Initially, the suppressiveness of certain soils to black root rot development in the field was confirmed under controlled conditions. The physical and chemical properties of naturally suppressive and conducive soils were characterized, and the effect of the soils on the survival of the fungus was determined. The hypotheses that soil calcium or aluminum affect the development of black root rot were tested by changing soil chemistry experimentally and measuring the effect on disease development. The hypothesis that biotic factors in naturally suppressive soils cause disease suppression was also tested.

MATERIALS AND METHODS

Preparation of soils. Soils from selected tobacco fields in western North Carolina (marked with an asterisk in Table 1) were collected in late fall and fumigated with methyl bromide. The soils were identified as apparently suppressive, conducive, or highly conducive to black root rot based on the inoculum density of *T. basicola* and the amount of disease present in a previous survey (31). The methyl bromide was allowed to dissipate for several days before the soil was stored, up to one year, in 20-L plastic bins. No *T. basicola* was detected in any soil after fumigation as determined in an assay on selective agar medium (36). A small amount of each soil was left unfumigated.

Preparation of inoculum of *T. basicola* and infestation of soils.

T. basicola was isolated from soil on carrot agar and stored in the dark in culture tubes of autoclaved sand. Sand from a culture tube was sprinkled onto petri dishes containing carrot agar (50 ml canned, commercial carrot juice, 18 g agar, and 950 ml deionized water), and the plates were incubated in the dark for 2 wk at room temperature. New colonies were transferred onto fresh carrot agar plates and allowed to grow for 6–8 wk for inoculum production. Suspensions of clamydospores were prepared from seven isolates: two from fields with apparent suppressive soil, three from conducive soils, and two from highly conducive soils. Chlamydospores were harvested from the carrot agar plates by flooding the medium surface with water and scraping the spores off the surface with a rubber policeman into a small beaker of water. The resulting spore suspension was washed through nested 400 (0.038 mm) and 500 (0.022 mm) mesh sieves. Most endoconidia passed through the sieves, the mycelium stayed on the top sieve, and the clamydospores were harvested from the bottom sieve. The chlamydospores were suspended in water, homogenized in a blender for 1 min at high speed, and sieved again. Spore concentration was determined with a hemacytometer. Each chlamydospore chain was counted as a single propagule, although this may underestimate the inoculum density because spores in a chain often separate at germination (4,16).

Inoculum density associated with development of black root rot on burley tobacco generally ranges from 10 to 200 cfu/g of soil, depending on the cultivar, soil, and environmental conditions (30,31,38), and can be as low as 5–10 cfu/g of soil on susceptible cultivars in conducive soil (30,38). An inoculum density of 100 chlamydospores per gram of dry soil was found in preliminary work to be an adequate but not excessive inoculum density for phytotron studies. Test soils were infested with 100 propagules per gram of soil by adding an appropriate volume of the spore suspension to 225 cm^3 soil contained in a plastic bag. The soil and spore suspension were mixed thoroughly by hand before pots were filled. Except in the experiments in which specific isolates were used, the inoculum consisted of a mixture of field isolates.

Confirmation of suppression under controlled environmental conditions and the relative virulence of different isolates of *T. basicola*. Six-week-old seedlings of burley tobacco were transplanted into infested soil in pots 7.5 cm in diameter. Seven isolates of *T. basicola*, three cultivars of burley tobacco, and three field soils apparently suppressive, conducive, and highly conducive (collected from the Johnson, Gillespie, and Higgens farms, respectively) (Table 1) were used in a factorial design with five replicate pots per treatment. The tobacco cultivars had low (B21xKy10), moderate (Ky 14), or moderate-high (Ky14xL8) resistance to black root rot. Treatments were arranged in a completely randomized design in a walk-in growth chamber (phytotron) with 28/20 C day/night temperatures, which are similar to those in western North Carolina in the summer, and a 12-h day length. Soil temperatures were monitored by thermocouples and equilibrated to the air temperature within an hour. All subsequent experiments in the phytotron were conducted similarly.

Plant harvest procedure and determination of inoculum density of *T. basicola*. Three weeks after transplanting, the plants were removed from the pots, and the root systems were rinsed and inspected for symptoms of black root rot. The percentage of the root system with the black lesions characteristic of *T. basicola*, the fresh weight of tops and roots, and the dry weight of tops and roots (after 48 h at 70 C) were recorded. Populations of *T. basicola* in three 1-g soil samples per pot were determined using a modification of a soil plating technique (30,37). Ten milliliters of a 1:100 (w/v) soil suspension in deionized water was pipetted into 300 ml of molten 5% carrot juice agar amended with antibiotics (37). The agar:soil suspension was mixed thoroughly on a magnetic stirrer and poured into 10 plastic petri dishes, which were incubated at room temperature (22–25 C) in the dark. The number of colony-forming units of *T. basicola* per gram of soil was calculated from the number of colonies on the selective medium after 14 days. The experiment was repeated once and the results analyzed by three-way ANOVA. Harvest procedures were similar for all subsequent phytotron experiments conducted.

Survival of chlamydospores in soil. The effects of soils on the survival of chlamydospores of *T. basicola* were tested in fumigated suppressive (Johnson), conducive (Gillespie), and highly conducive (Higgens) field soils. An isolate of *T. basicola* was obtained from each of the three soils before fumigation with methyl bromide. Soil and inoculum were prepared as previously described. Soil in three replicate pots 12 cm in diameter was infested with 300 chlamydospores of one of the three isolates of *T. basicola* per gram of soil and adjusted to 20% (w/w) moisture content. Pots were covered with polyethylene (perforated to allow for some gas exchange) and arranged in a completely randomized design in a phytotron at 28/20 C day/night temperature in the dark.

One sample was taken from each pot with a 2-cm cork borer at 1, 2, 4, 8, 16, 20, and 52 wk after placement in the phytotron. Sampling times corresponded with the length of other phytotron experiments (1–4 weeks) and the length of the growing season of burley tobacco in the region where the soils were collected (4–5 months); a final sampling of the suppressive and highly conducive soils was taken after 1 yr. Propagule density was determined in three 1-g subsamples per sample on the selective

TABLE 1. Inoculum density of *Thielaviopsis basicola*, severity of black root rot, and resistance of cultivars grown in selected burley tobacco fields in western North Carolina

Grower[v]	Inoculum density[w] Mean 1987	Mean 1988	Range 1988	Disease severity[x] (%)	Cultivar resistance[y]	Site classification
*Johnson	2	7	0–88	0	low	suppressive
Buckner	25	58	0–577	0	moderate	suppressive
*Elkins	11	0	0	0	low	suppressive
Frady	9	60	0–241	0–5	moderate	suppressive
*Gillespie	219	...[z]	...	5–25	moderate	conducive
Henson	95	142	0–278	5–25	low	conducive
*Taylor	...	175	0–440	5–25	moderate	conducive
Eggers	164	42	0–135	5–25	low	conducive
*Higgens	13	...	...	5–25	low	highly conducive
Mashborn	21	...	...	5–25	low	highly conducive
Brown	25	57	0–229	5–25	moderate	highly conducive
Street	46	...	...	5–25	moderate	highly conducive

[v] Asterisks indicate soils collected and fumigated for phytotron experiments.
[w] Colony-forming units of *T. basicola* per gram of dry soil.
[x] Disease severity estimated as the percentage of the root system with symptoms characteristic of black root rot.
[y] Reported resistance to black root rot caused by *T. basicola*.
[z] Not sampled.

medium as previously described.

Testing for biotic factors involved in suppression. A suppressive soil and a conducive soil were either sterilized or left untreated. Soil was sterilized by autoclaving twice for 1 h at 121 C on successive days. All soils were infested with *T. basicola* at 100 chlamydospores per gram of soil in addition to the native inoculum density of *T. basicola*, which was 70 cfu/g in the untreated conducive soil and 7 cfu/g in the untreated suppressive soil. A single 4-wk-old burley tobacco seedling, cv B21xKy10 (low resistance), was transplanted into each of five replicate pots of autoclaved and untreated suppressive and conducive soils, grown for 3 wk in the phytotron, and harvested as described. The experiment was repeated once.

Because suppressiveness was observed in fumigated soil as well as in untreated soil, a second test was conducted in which fumigated suppressive soil was mixed with fumigated conducive soil in polyethylene bags to give 0, 10, 50, 90, and 100% suppressive soil. Soil mixtures were allowed to incubate at approximately 25% moisture in the dark for 2 wk at 28/20 C day/night temperatures. Five replicate pots 7.5 cm in diameter (225 cm^3) were filled with each soil mixture after infestation of soil with 100 chlamydospores of *T. basicola* per gram of soil. A 6-wk-old seedling of burley tobacco, cultivar B21xKy10, was planted in each pot, grown in the phytotron for 3 wk, and rated for root rot. The experiment was conducted once.

Characterization of physical and chemical properties of suppressive and conducive soils. Soil texture was determined by the hydrometer method (7). The water-holding capacity of the soils was determined with a pressure plate apparatus set at 3, 5, 10, or 30 kPa and Büchner funnel tension plates set at 0.5 or 1 kPa. Percent moisture at each matric potential was recorded for two replicate samples of each soil. Soil calcium, magnesium, zinc, copper, manganese, potassium, and sodium were analyzed by the North Carolina Department of Agriculture (NCDA) soil testing lab by means of the Mehlich-3 extractant (28) and atomic absorption spectrophotometry (calcium, magnesium, zinc, copper, and manganese) or flame emission (potassium and sodium). Soil phosphorus was determined by the molybdate blue method. The cation exchange capacity (CEC) was calculated as the sum of basic cations (including sodium) and buffer acidity (27); the base saturation was calculated as the percent of the CEC occupied by calcium, magnesium, and potassium. Soil pH was determined in a 1:1 soil:distilled water suspension. Exchangeable aluminum was measured by titration in 1 N KCl extracts (45) on 10 g of air-dried soil. Aluminum extracted with neutral salts is trivalent (23,42).

Soil chemical factors involved in suppression: phytotron tests. To test the effect of soil calcium on disease development, a fumigated suppressive soil (Johnson) and a fumigated conducive soil (Higgens) were left untreated or were amended with 6 meq calcium as calcium hydroxide, $Ca(OH)_2$, or 6 meq calcium sulfate, $CaSO_4(2H_2O)$, per 100 g of soil. Calcium levels were chosen to be similar to those found in highly conducive soils. The reagents were added dry, and the soils were subsequently adjusted to 20% moisture and allowed to incubate for 4–6 wk. Each soil was infested with 100 chlamydospores of *T. basicola* per gram of dry soil, and seven replicate pots 7.5 cm in diameter (225 cm^3) were filled. The remaining soil was submitted to the NCDA for soil chemistry analysis. Burley tobacco seedlings with low resistance to black root rot (cultivar B21xKy10) were grown for 3 wk in each soil in the phytotron. The experiment was conducted a second time with the same soils and a third time using different suppressive and conducive soils (Elkin and Taylor, respectively).

To test the effects of soil pH and exchangeable aluminum on disease development, a suppressive and a conducive soil were left untreated or were amended with 5.5 meq of aluminum as aluminum sulfate, $Al_2(SO_4)_3(18H_2O)$, per 100 g of soil or 15 meq 85% phosphoric acid, H_3PO_4, per 100 g of soil. In all experiments, the pH of the suppressive soils was raised to that of the conducive soils (approximately 6.0) by amending with calcium hydroxide 2 wk before acidifying treatments were applied.

The acidifying compounds were chosen because preliminary work demonstrated that both amendments acidified soil (added hydrogen ions and lowered pH) but that the final amount of exchangeable soil aluminum increased with the aluminum sulfate treatment but remained low after amendment with the phosphoric acid. Thus, the effects of hydrogen ions and aluminum on disease development could be evaluated separately.

Aluminum sulfate was added dry and the phosphoric acid was diluted in deionized water and mixed into the soils. Rates were chosen that were approximately twice (aluminum sulfate) and five times (phosphoric acid, a weak acid) the value of the buffer acidity of the suppressive soil, respectively. After the soils were incubated for 4–6 wk at 20% moisture content, each was infested with 100 chlamydospores per gram of soil and used to fill seven replicate 7.5-cm pots per treatment. The remaining soil was submitted to the NCDA for soil chemical analysis. Burley tobacco seedlings with low resistance to black root rot (cultivar B21xKy10) were grown in each soil in the phytotron as described. The experiments were conducted a second time using the same soils and a third time using different suppressive and conducive soils.

To test the dosage effect of different concentrations of exchangeable aluminum on disease development, aluminum sulfate (0, 2.5, or 5.5 meq of aluminum per 100 g of soil) was added to a conducive soil (Taylor) and allowed to incubate at 20% moisture content for 2 wk. Soils were then infested with 100 chlamydospores of *T. basicola* per gram of soil as described above. Seedlings of burley tobacco, cultivar B21xKy10, were transplanted into five replicate pots (225 cm^3) per treatment and grown in the phytotron as described. The experiment was run once.

Soil chemical factors involved in suppression: field tests. The effects of soil pH and exchangeable soil aluminum on the development of black root rot were evaluated in the field by amending soil at two commercial burley tobacco farms. Soils at these sites were similar in soil chemistry (Henson farm and Taylor farm) (Table 3) and were conducive to black root rot. At each site, five replicate 0.001-ha (11.1 m^2) plots per site were left unamended or treated with aluminum sulfate, $Al_2(SO_4)_3$; phosphoric acid (85%); or elemental sulfur (95% dust). The amount of amendment added was calculated as the amount needed to lower the pH to 5.0, as determined by a buffer curve prepared for the soil at each site. The buffer curve was prepared from two replicate samples for aluminum sulfate and phosphoric acid by addition of 0.2, 0.4, 0.6, 0.8, or 1.0 meq of each amendment in 10 ml of deionized water to 10 g of dry soil, incubation for 24 h, and measurement of the pH of the supernatant with a glass electrode.

In early May, the aluminum sulfate and sulfur were broadcast and the diluted phosphoric acid (1:1) was poured directly onto the soil. Because no gradient in the distribution of the pathogen had been detected in either field in two previous years of sampling, a completely randomized design was used to assign plot treatments. Burley tobacco, cultivar B21xKy10, was transplanted about 7 wk after soil amendment at the Henson site and about 6 wk after soil amendment at the Taylor site. Plots contained 4 rows of 12 plants per row and were separated by two border rows of untreated soil. Except for the soil amendments, all soil preparation, planting, cultivation, and pest management were performed by the grower, using standard practices. Inoculum density of *T. basicola* was determined and soil chemistry analyzed in soil samples taken directly after transplanting. Each soil sample was a composite of four 3-cm-diameter soil cores taken to a depth of 20 cm around six individual plants in the two inside rows of the plot. Plants were tagged with flagging tape. In mid-July, about 6 wk after transplanting, the roots of the tagged plants were inspected for symptoms of black root rot and the percentage of the root system with symptoms characteristic of black root rot was estimated.

RESULTS

Confirmation of suppression under controlled environmental conditions and the relative virulence of isolates of *T. basicola*. No symptoms or only a few small, discrete lesions were observed on plants grown in the suppressive soil, regardless of the cultivar

or pathogen isolate (Fig. 1A). Disease severity was greatest in highly conducive soil (Fig. 1C) and was moderate in the conducive soil (Fig. 1B). The results presented are from the first run of the experiment and were similar when the experiment was repeated. In conducive soils, more severe symptoms developed on the cultivar with low resistance (B21xKy10) than on the cultivar with high resistance (Ky14xL8). Differences in disease development among isolates (across cultivars) were not detected in the suppressive soil ($P = 0.08$) or the highly conducive soil ($P = 0.53$). Differences in virulence among isolates in the conducive soil were apparent in the first run ($P < 0.01$) but not when the experiment was repeated. No differences in plant biomass were observed between the uninfested suppressive and highly conducive soils; dry weights of noninoculated plants growing in the suppressive and highly conducive soils were 12.0 (shoots) and 11.7 g (roots), and 12.5 (shoots) and 12.8 g (roots), respectively (n = 5). *T. basicola* was detected in all soils at harvest.

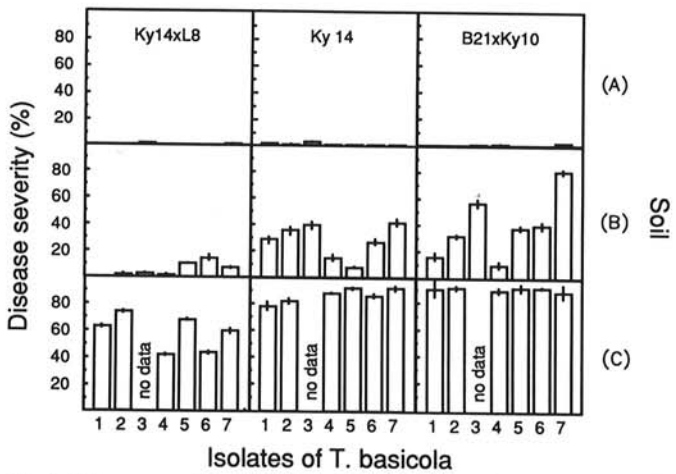

Fig. 1. Disease severity on burley tobacco cultivars with high (Ky14xL8), moderate (Ky 14), or low (B21xKy10) resistance to black root rot in fumigated field soils infested with one of seven different isolates of *Thielaviopsis basicola*. **A**, suppressive soil (Johnson); **B**, conducive soil (Gillespie); **C**, highly conducive soil (Higgens). Bars represent mean disease severity (n = 5); vertical lines indicate standard error. Disease severity was estimated as the percentage of the root system with symptoms characteristic of black root rot.

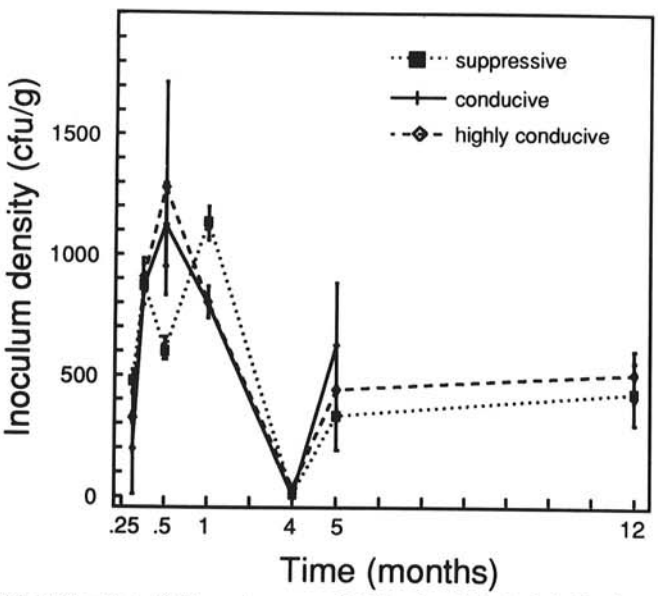

Fig. 2. Survival of chlamydospores of *Thielaviopsis basicola* in fumigated field soils suppressive, conducive, or highly conducive to black root rot. Vertical lines represent the standard error of the mean (n = 3). The isolate of *T. basicola* was collected from a highly conducive soil. Results of tests with isolates from conducive and suppressive soils were similar.

Survival of chlamydospores in soil. No differences in the survival of the chlamydospores of *T. basicola* were observed among a suppressive soil and two conducive soils (Fig. 2). Results presented are those for an isolate collected from a highly conducive soil. Results of tests with isolates from a moderately conducive and a suppressive soil were similar. During week 15 of the experiment the polyethylene covers were inadvertently removed. The soil was dry when sampled at week 16 (4 mo) and then was rewetted and covered with the plastic. The drying and rewetting cycle corresponded to a sharp drop and subsequent increase in the number of colony-forming units in all three soils (Fig. 2).

Biotic factors involved in suppression. Autoclaving suppressive soil did not change the suppressive effect of the soil on black root rot development. Disease severity decreased in proportion to the amount of suppressive soil added to the conducive soil (Fig. 3).

Characterization of physical and chemical properties of suppressive and conducive soils. No consistent differences in the physical characteristics of suppressive and conducive soils were detected (Table 2). Seven of the eight soils were classified as loams. All soils had an organic matter content of 1% or less, and the clay contents of both suppressive and conducive soils were similar. The moisture release curves of a suppressive soil and two conducive soils were similar. Because of the relatively low CEC values in relation to the clay content (Tables 2 and 3), the predominant clay type in both groups of soils was probably kaolinitic (3).

TABLE 2. Physical characteristics of western North Carolina soils suppressive or conducive to black root rot of burley tobacco

Soil/Grower	Texture class	Percent			
		Sand	Silt	Clay	Organic matter
Suppressive					
Johnson	loam	44	34	22	0.5
Buckner	loam	40	38	22	1.0
Elkins	sandy loam	56	28	15	0.8
Frady	loam	45	30	25	0.8
Conducive					
Gillespie	loam	40	33	27	0.5
Henson	loam	38	40	22	0.9
Eggers	loam	41	41	18	0.8
Higgens	loam	45	31	24	0.6

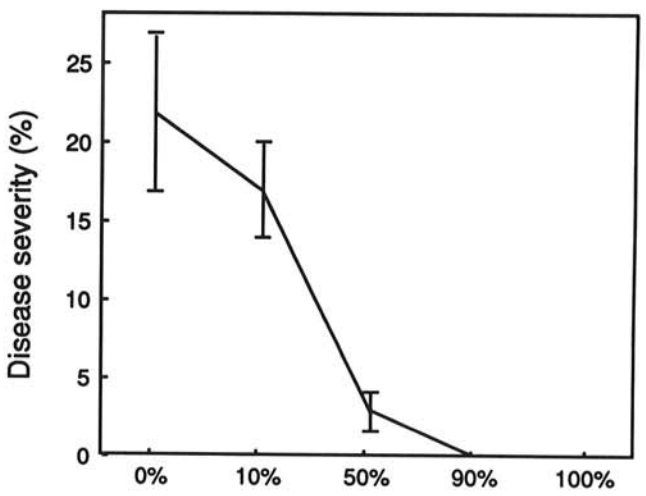

Fig. 3. Severity of black root rot on burley tobacco seedlings grown in different ratios of conducive and suppressive soil. Vertical lines indicate standard error of the mean (n = 7).

Consistent differences in soil chemical characteristics were observed between suppressive and conducive soils (Table 3). Suppressive soils were characterized by lower calcium, magnesium, and base saturation than conducive soils; higher buffer acidity; and a soil pH near 5.0. Phosphorus levels were lower in two conducive soils than in the other soils tested. No consistent differences in manganese, zinc, or copper content were observed among the soils.

Soil chemical factors involved in suppression: phytotron tests. Postharvest inoculum densities of *T. basicola* greater than 100 cfu/g of soil were detected in all treatments (conducive and suppressive soils, amended and unamended) in each of the experiments. No disease developed in unamended, suppressive soil in any test (Tables 4 and 5). Disease developed in the suppressive soils amended with calcium hydroxide, whereas no symptoms were observed in suppressive soils amended with calcium sulfate (Table 4). Treatment of suppressive soil with calcium hydroxide increased the pH to a value similar to that of the conducive soils (from 4.6 to 6.5). Soil calcium increased from 2.8 meq to 7.4 meq/100 g of soil, and the base saturation increased from 62 to 92%, values similar to those found for unamended conducive soils (Table 4). Results for a second suppressive soil were similar (Table 4). Exchangeable aluminum decreased from about 1 meq/100 g of soil in both unamended, suppressive soils to less than 0.3 meq/100 g of soil following amendment with calcium hydroxide. Less than 0.1 meq of exchangeable aluminum per 100 g of soil was measured in unamended, conducive soils (Table 4).

In contrast, suppressive soils amended with calcium sulfate remained suppressive; no disease developed and soil pH did not change, although soil calcium increased to greater than 9 meq/100 g of soil. Exchangeable aluminum levels remained similar to those in unamended suppressive soils or increased slightly (Table 4). Results for all treatments were similar when the experiment was repeated.

Conducive soils were made suppressive to black root rot by

TABLE 3. Chemical characteristics of western North Carolina soils suppressive or conducive to black root rot of burley tobacco

Soil/Grower	pH[w]	BS[x]	Ca[y]	Mg[y]	K[y]	CEC[y]	Ac[y]	P[z]	Mn[z]	Zn[z]	Cu[z]
Suppressive											
Johnson	4.9	49	2.0	0.5	0.4	5.8	2.9	185	38	2.0	0.9
Buckner	5.1	67	3.5	0.8	0.6	8.4	2.8	337	126	6.3	2.2
Elkins	4.2	32	1.1	0.2	0.6	5.8	3.9	198	48	1.9	1.2
Bailey	4.9	47	1.8	0.7	0.8	6.7	3.6	310	78	2.3	1.3
Frady	4.7	52	1.9	0.5	1.2	6.2	2.9	316	206	4.1	2.7
Conducive											
Gillespie	5.9	80	3.5	1.7	0.7	7.4	1.5	180	85	4.7	1.7
Henson	5.7	79	4.3	1.7	1.3	9.2	1.9	290	139	6.8	1.6
Taylor	6.1	90	4.2	1.7	0.3	7.0	0.6	35	50	6.0	2.5
Eggers	6.0	82	5.0	2.3	0.6	9.8	0.8	80	62	2.0	1.4
Higgens	6.7	92	5.5	1.9	1.1	9.2	0.8	208	107	15.3	4.0

[w]Measured in a 1:1 soil:distilled water suspension.
[x]Percent base saturation.
[y]Cations, exchangeable acidity (Ac), and cation exchange capacity (CEC) in meq/100 cm^3 soil measured by atomic absorption spectrophotometry in a Mehlich-3 extractant.
[z]P, Mn, Zn, and Cu in mg/dm^3.

TABLE 4. Effect of calcium soil amendments on soil chemistry and development of black root rot on burley tobacco seedlings grown in the phytotron in suppressive and conducive soils

Soil/Treatment (Grower)	Rate[t]	DS[u]	pH	Al[v]	BS[w]	Ca[x]	P[y]	DW[z]
Suppressive								
(Johnson)								
Calcium sulfate	6.0	0	4.4	1.1	78	9.7	200	2.0
Calcium hydroxide	6.0	23	6.5	0.1	92	7.4	192	1.5
Unamended	···	0	4.6	1.0	62	2.8	301	2.3
(Elkins)								
Calcium sulfate	6.0	0	4.3	1.4	81	9.9	420	3.8
Calcium hydroxide	6.0	6	5.8	0.3	88	7.8	456	4.2
Unamended	···	0	4.4	0.9	65	3.2	456	3.3
Conducive								
(Higgens)								
Calcium sulfate	6.0	27	6.4	0.1	97	16.9	200	1.3
Calcium hydroxide	6.0	29	4.7	0.05	100	11.3	200	1.0
Unamended	···	33	6.2	0.1	92	6.9	184	1.2
(Taylor)								
Calcium sulfate	6.0	21	5.8	0.05	93	13.3	47	2.0
Calcium hydroxide	6.0	20	6.9	0.05	100	10.2	57	1.4
Unamended	···	15	5.8	0.05	89	6.4	50	2.1

[t]Meq of Ca added per 100 g of soil.
[u]Disease severity estimated as the percentage of the root systems with symptoms characteristic of black root rot caused by *Thielaviopsis basicola*.
[v]Exchangeable aluminum in meq/100 g of soil.
[w]Percent base saturation.
[x]In meq/100 g of soil.
[y]In mg/dm^3.
[z]Total plant dry weight in grams.

TABLE 5. Effect of acidifying soil amendments on soil chemistry, plant biomass, and development of black root rot on burley tobacco seedlings grown in the phytotron in suppressive and conducive soils

Soil/Treatment (Grower)	Rate[t]	DS[u]	pH	Al[v]	BS[w]	Ca[x]	P[y]	DW[z]
Suppressive								
(Johnson)								
Al-sulfate	5.5	0	3.9	5.5	62	7.9	200	0.2
Phosphoric acid	15.0	2	4.0	1.5	58	3.1	1,550	2.0
Unamended	···	0	4.6	1.0	62	2.8	301	2.3
(Elkins)								
Al-sulfate	5.5	0	4.2	3.4	81	12.7	357	1.6
Phosphoric acid	15.0	3	3.8	1.0	60	6.2	432	3.0
Unamended	···	0	4.4	0.9	65	3.2	456	3.3
Conducive								
(Higgens)								
Al-sulfate	5.5	0	4.3	2.0	76	9.1	229	2.0
Phosphoric acid	15.0	0	3.8	5.0	68	9.2	215	0.2
Unamended	···	33	6.2	0.1	92	6.9	184	1.2
(Taylor)								
Al-sulfate	5.5	0	4.2	3.1	72	9.0	69	1.6
Phosphoric acid	15.0	9	4.6	0.5	74	6.7	1,185	3.8
Unamended	···	15	5.8	0.05	89	6.4	50	2.1

[t]Meq of Al or phosphoric acid added/100 g of soil.
[u]Disease severity estimated as the percentage of the root system with symptoms characteristic of black root rot caused by *Thielaviopsis basicola*.
[v]Exchangeable aluminum in meq/100 g of soil.
[w]Percent base saturation.
[x]In meq/100 g of soil.
[y]In mg/dm^3.
[z]Total plant dry weight in grams.

the addition of aluminum sulfate. The pH decreased from 6.2 to 4.7, and exchangeable aluminum increased from 0.1 to 2.0 meq/100 g of soil. Exchangeable aluminum levels were higher than those found in unamended suppressive soils (Table 5). Results for a second conducive soil were similar (Table 5). Amendment with 15 meq phosphoric acid increased soil acidity in both conducive soils to values lower than those in the suppressive soils, but had a variable effect on disease development and on soil aluminum (Table 5). Disease was suppressed in one conducive soil (Higgens) and a high level of exchangeable aluminum (5 meq/100 g) was measured in the titration assay. In contrast, significant amounts of disease developed in a second conducive soil (Taylor), and aluminum levels were moderate (0.5 meq/100 g). High levels of phosphorus were also measured.

Amendment of conducive soil with 2.5 meq aluminum or 5.0 meq/100 g soil as aluminum sulfate resulted in 0.6 meq and 1.6 meq exchangeable aluminum per 100 g of soil, respectively, and less black root rot development than in unamended soil (Table 6). However, low plant dry weight at the high aluminum concentration indicated phytotoxicity.

Soil chemical factors involved in disease suppression: field tests. Disease severity at the Henson site was significantly less in plots amended with aluminum sulfate than in plots treated with phosphoric acid, treated with sulfur, or left untreated, although no differences in inoculum densities among the treatments were detected (Table 7). At the Taylor site (data not shown in table form), the mean disease severity in plots treated with aluminum sulfate was also significantly lower ($P = 0.05$) than in those treated with phosphoric acid or sulfur or left untreated, with mean disease severity (n = 5) of 1, 5, 4, and 16%, respectively, and the amendments had no effect on the inoculum density among plots (92, 80, 141, and 166 cfu of *T. basicola* per gram of soil, respectively; no significant differences [$P < 0.05$] according to the Tukey honest significant difference procedure). After 7 wk, all treated soils at the Henson site were significantly more acidic than the untreated controls (Table 7). Unfortunately, due to very wet soil conditions, no changes in soil chemistry were measured 7 wk after soil amendment at the Taylor site. However, by 12 wk after amendment at the Taylor site, when the plants were rated for disease, soil pH was 5.0, 5.1, 5.4, and 6.4 for the plots treated with aluminum sulfate, phosphoric acid, or sulfur or nontreated, respectively. Thus, the amendments had affected soil chemistry by this time. Too few samples (2 plots per treatment), however, were collected at this later time to reliably evaluate the effects of soil chemistry on disease response at the Taylor site. Therefore, only the data from the Henson site were further analyzed.

The amount of exchangeable aluminum around individual plants at the Henson site increased sharply with decreasing base saturation and exceeded 1 meq/100 g of soil. These aluminum levels were associated with a percent base saturation of approximately 70% or less (Fig. 4). Suppression of disease was associated with exchangeable aluminum levels of 1.0 meq aluminum or higher per 100 g soil (Fig. 5), similar to the level of exchangeable aluminum found naturally in suppressive soils (Tables 4 and 5). When exchangeable aluminum exceeded 1.4 meq/100 g of soil, base saturation was less than 70% and disease severity was near zero (Fig. 6). Disease developed at all pH levels unless exchangeable aluminum exceeded 1.4 meq/100 g of soil (Fig. 7).

TABLE 6. Effect of aluminum sulfate amendments on severity of black root rot of burley tobacco, pH, and exhangeable aluminum in fumigated field soil conducive to black root rot

Al added[w]	Disease[x] severity (%) (s.e.)	Al[y] (s.e.)	pH	Dry wt.[z] (s.e.)
0	13 (0.3)	0.2 (0.02)	6.0	0.95 (0.10)
2.5	3 (1.5)	0.6 (0.07)	5.0	0.85 (0.17)
5.0	0 (0.0)	1.6 (0.29)	4.6	0.32 (0.08)

[w]Al in meq/100 g of soil. Added as reagent grade aluminum sulfate ($Al_2(SO_4)_3$).
[x]Disease severity estimated as the percentage of the root system with symptoms of black root rot caused by *Thielaviopsis basicola* (mean and standard error; n = 5).
[y]Exchangeable Al in meq/100 g of soil (mean and standard error; n = 5).
[z]Total plant dry weight (mean and standard error; n = 5).

TABLE 7. Effect of acidifying soil amendments on severity of black root rot on burley tobacco, inoculum density of *Thielaviopsis basicola*, and soil chemical variables in a commercial burley tobacco field (Henson site)

Treatment	DS[v] (%)	ID[w] (cfu/g)	pH	Al[x]	BS[y]	P[z]
Al-sulfate	7 a	61 a	5.0 a	1.0	73 a	420 a
Phosphoric acid	12 b	72 a	5.3 a	0.5	77 a	413 ab
Sulfur	14 b	94 a	5.4 a	0.5	79 b	411 a
Unamended	20 b	30 a	6.1 b	0.2	88 c	394 a

[v]Disease severity estimated as the percentage of the root system with symptoms of black root rot. Means followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls procedure (n = 5).
[w]Inoculum density of *T. basicola* (cfu/g of soil).
[x]Exchangeable aluminum in meq/100 g of air-dried soil.
[y]Percent base saturation.
[z]Extractable phosphorus in mg/dm^3.

DISCUSSION

Lack of disease development on burley tobacco grown in certain fumigated field soils infested with *T. basicola* and maintained under controlled environmental conditions indicates that inhibition of black root rot was mediated by factors in the soil. Although genetic variability occurs in *T. basicola* (6), no differences among isolates from suppressive and conducive soils were detected that could explain the range in disease response observed among these field soils.

None of the results support a biotic mechanism of suppression of black root rot of burley tobacco. Autoclaving and fumigating soil did not nullify suppression, which indicates that native soil organisms are not the primary factors involved. The suppressive factor was transferable, but it was proportional to the amount of suppressive soil added, also suggesting that the factor is abiotic. The disease suppression in western North Carolina soils appears

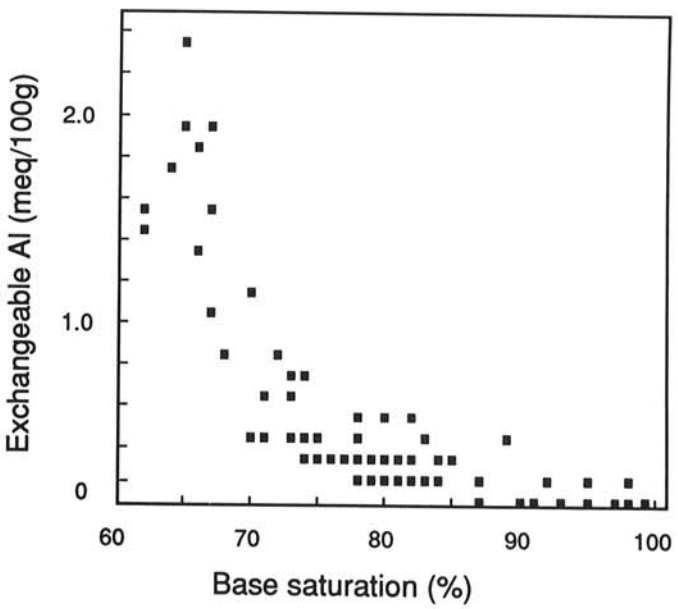

Fig. 4. Relationship between exchangeable soil aluminum and percent base saturation. Data points are from soil samples taken around 100 individual plants in 20 plots (5 plants per plot) amended with aluminum sulfate, phosphoric acid, or sulfur or left unamended.

to have a different mechanism than that identified in suppressive soils in Switzerland (40). In the Swiss soils studied, black root rot appears to be suppressed via antagonism by a strain of *P. fluorescens* favored by montmorillonitic and vermiculite clays in the suppressive soils (20,40,41).

Soil physical factors such as soil texture were not correlated with disease severity in the 1987 survey of North Carolina (31) and were found to be very similar in both suppressive and conducive soils. Although the clay type was not determined analytically, the low cation exchange capacity per unit clay strongly suggests 1:1 kaolinitic type clays in both suppressive and conducive soils in this region. The similar water-holding capacity of a suppressive and two conducive soils is probably the result of a similar quantity and type of clay and amount of organic matter in these soils and suggests that the differences in disease response were not due to differences in water relations.

The consistent differences in soil chemical characteristics between suppressive and conducive soils suggest that the mechanism of suppression is abiotic and related to soil acidity. This is supported by observations on tobacco for many years (1,9,10). Suppressiveness could be nullified by raising the soil pH in pot tests but it could not be nullified by raising soil calcium only. This indicates that calcium deficiency was probably not the mechanism of disease suppression in these soils.

Effects of pH on biological systems in soil are difficult to separate from the effects of aluminum ions. Aluminum becomes more soluble as pH decreases and, conversely, the pH decreases when aluminum is added because of aluminum hydrolysis and release of protons. Evidence to support a direct suppressive effect of pH on the development of black root rot would be a combination of low pH and low exchangeable aluminum concentration with no disease development. This was tested by amendment of conducive soils with phosphoric acid, which lowered pH without a subsequent increase in aluminum. Disease was not suppressed when pH was lowered with phosphoric acid and aluminum levels were moderate (Table 5, Taylor soil). Therefore, low pH alone was not effective in suppressing black root rot. This conclusion was supported in the field trials when black root rot developed in acidic soil (pH 5.2 or less) when exchangeable aluminum concentrations remained low (Fig. 7). The ability of *T. basicola* to grow in vitro in media with a range in pH values (24) also suggests that soil pH does not affect the activity of the fungus directly.

Interpretation of the results in suppressive soils amended with phosphoric acid is complex because high aluminum was measured after the addition of phosphoric acid. This could be due to aluminum oxides in the clays that had become soluble. Alternatively, the titration assay may have overestimated aluminum in these soils because this technique measures both H^+ and Al^{3+} when the pH of the extract is less than 4.0 (45), as it was in some cases.

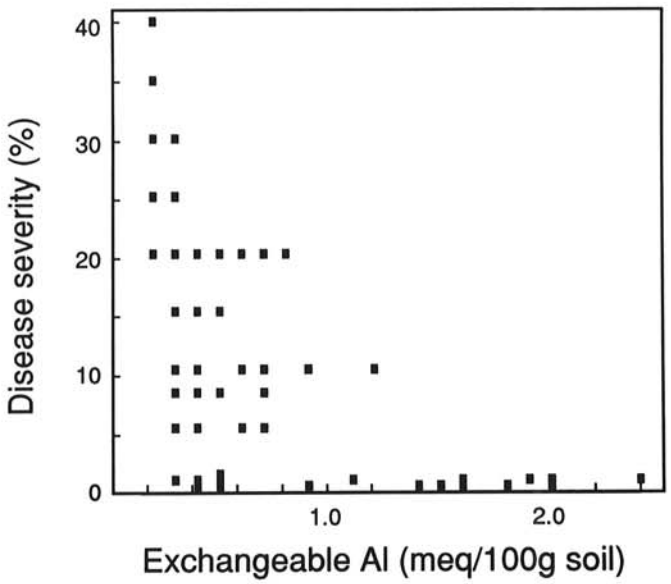

Fig. 5. Relationship between severity of black root rot and exchangeable aluminum. Data points are from soil samples taken around 100 individual plants in 20 plots (5 plants per plot) amended with aluminum sulfate, phosphoric acid, or sulfur or left unamended.

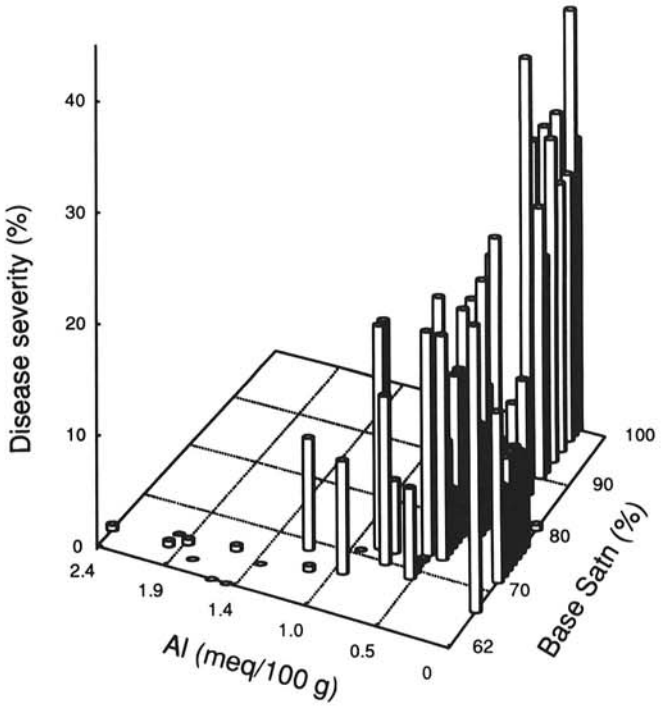

Fig. 6. Severity of black root rot on burley tobacco as a function of exchangeable soil aluminum and percent base saturation. Data points are from soil samples taken around 100 individual plants in 20 plots (5 plants per plot) amended with aluminum sulfate, phosphoric acid, or sulfur or left unamended.

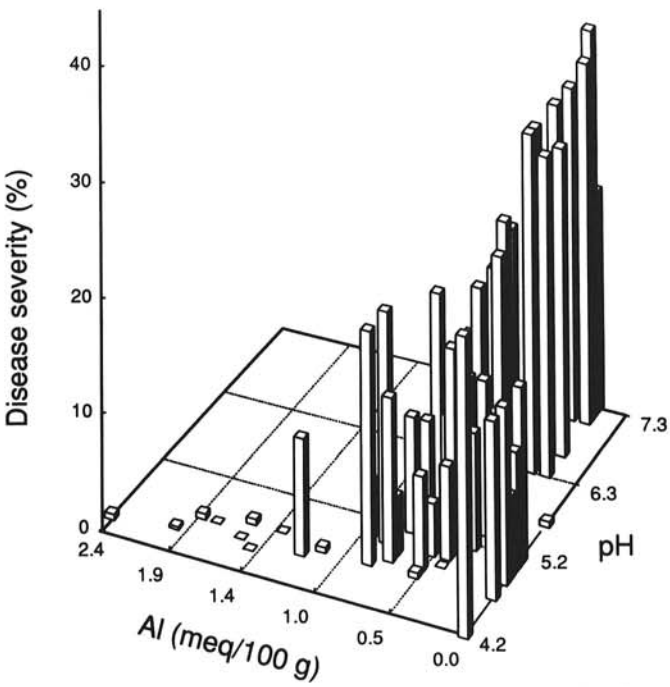

Fig. 7. Severity of black root rot on burley tobacco as a function of soil pH and exchangeable aluminum. Data points are from soil samples taken around 100 individual plants in 20 plots (5 plants per plot) amended with aluminum sulfate, phosphoric acid, or sulfur or left unamended.

A dose-response relationship between exchangeable aluminum and disease development appears to occur. The amount of exchangeable aluminum associated with disease suppression was approximately 1.0 meq aluminum per 100 g of soil in naturally suppressive soils, in pot tests with amended conducive soils, and in the field trial. Disease severity was reduced at about 0.5 meq aluminum per 100 g of soil and was severe at concentrations less than 0.3 meq aluminum per 100 g of soil that was found naturally in all conducive soils assayed and in suppressive soils treated with calcium hydroxide.

Base saturation appears to be a good predictive variable of conditions favorable or unfavorable for black root rot (31) because it is a good indicator of the levels of exchangeable aluminum. For example, when the base saturation was less than 70%, the amount of available aluminum in the Henson soil was considerably greater than when base saturation was greater than 70%. This corresponds with observations that inoculum densities of *T. basicola* in the field were adequate to cause disease but that black root rot developed only in the parts of the field in which the base saturation was less than 70% (30). Although the exchangeable aluminum levels in the soil surrounding those plants were not measured, our data predict that aluminum levels were high enough to suppress disease when base saturation was less than about 70%.

Results from this study can be explained by acid-dependent effects of the soil on black root rot development that are determined by the particular chemistry of a soil. For example, if a soil high in basic cations with high percent base saturation is fertilized with an acidic fertilizer, the pH may decrease to values considered unfavorable for black root rot, but the disease will still develop if the decrease in pH is not accompanied by an increase in aluminum. If the decrease in pH, however, results in the leaching of basic cations and decrease in base saturation, which is the process of natural soil acidification, H^+ and particularly ionic forms of aluminum on the soil exchange sites increase and the soil will become increasingly suppressive to the development of black root rot. This process has probably taken place in the suppressive soils found in North Carolina. Similarly, an acidic soil high in aluminum will continue to be suppressive to black root rot even when basic cations are added (e.g., in the form of calcium sulfate), unless they are in the form of a liming material, like calcium hydroxide, that will precipitate aluminum.

The mechanisms of suppression of certain soils in western North Carolina to black root rot of burley tobacco appear to be dependent upon interrelationships of soil pH, base saturation, and exchangeable aluminum. Survival and some growth of the fungus apparently occurs in suppressive soils because *T. basicola* was detected in fields in which no disease developed under conducive environmental conditions (30); and inoculum of three isolates of *T. basicola* survived at least one year in both suppressive and conducive soils and increased after drying and rewetting. The ability of the fungus to colonize decaying residues of both host and nonhost plants (14) may be providing enough protection from adverse soil chemical conditions to maintain some inoculum in suppressive soils. The suppression of disease may occur in an active, sensitive stage of the fungal life cycle, such as germ tube growth through the rhizosphere. Although no differences in plant biomass production between suppressive and conducive soils were observed, soil chemistry may be affecting host resistance directly. Suppression of black root rot by aluminum in soil may be widespread since vast crop-producing areas of the world, especially in the humid tropics and subtropic zones, the eastern United States, and Western Europe, are affected by soil acidity and aluminum mobilization.

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