Effects of Host Resistance and Soil Fumigation on *Thielaviopsis basicola* and Development of Black Root Rot on Burley Tobacco

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ABSTRACT

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Cultivars of burley tobacco (Nicotiana tabacum) with different levels of resistance to Thielaviopsis basicola were planted in fields naturally infested with the pathogen. Severity of black root rot and populations of T. basicola were determined at transplant, midseason, and after final harvest. Cultivars with monogenic resistance to T. basicola derived from Nicotiana debneyi developed little or no root rot in all tests and suppressed pathogen reproduction during the growing season. The severity of root rot and the reproduction of T. basicola were not related to the level of partial resistance. In fact, cultivars with moderate levels of partial resistance frequently had higher root rot severity and similar or greater final populations of T. basicola than did cultivars with low levels of resistance. Moderately resistant cultivars yielded well even with high root rot severity. The effects of soil fumigation on disease and pathogen population dynamics were mixed. In some tests, fumigation significantly increased yield and decreased root rot severity and pathogen population; whereas in other tests, fumigation had little effect on the disease or the pathogen and failed to increase yields. Partial and complete resistance and, in some situations, soil fumigation are effective in the short- and long-term management of black root rot on burley tobacco.

Black root rot of burley tobacco (Nicotiana tabacum L.) is caused by Thielaviopsis basicola (Berk. & Broome) Ferraris (synanamorph Chalara elegans

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Nag Raj & Kendrick). The disease occurs in about 40% of the burley tobacco fields in North Carolina and causes extensive root rot and suppression of yield in 5-10% of all fields (8). Moderate to severe levels of disease are associated with high populations of the pathogen, the use of cultivars with low levels of partial resistance, high soil pH, and con-

tinuous planting of tobacco or other susceptible crops (1,5-8,11,12).

Host resistance is the primary means of control of black root rot on burley tobacco in North Carolina. There are two types of resistance to T. basicola found in burley tobacco cultivars grown in the United States (3,13). One type is partial resistance, characterized by low to moderate levels of resistance, and is derived from N. tabacum (3,13). The resistance level is generally assigned by extension agents based on yield and quality in field trials over many locations and years, and not on disease severity ratings. About 80% of the burley tobacco grown in North Carolina has this type of resistance. About 20% of the burley tobacco planted has a very high level of monogenic resistance that is derived from Nicotiana debneyi Domin (3). Strains of T. basicola that can overcome this source of resistance have not been reported. Unfortunately, until recently, available cultivars with this source of resistance were not well adapted to the growing conditions of much of the burley area of North Carolina and had not gained wide acceptance by growers.

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Soil fumigation also is used in the control of black root rot (4,5). The effectiveness of fumigation depends on the fumigant used and the environmental conditions present at the time of fumigation. Fumigants are least effective in cool, wet soil because they fail to disperse adequately through the soil (4). These same conditions favor development of severe damage early in the growing season by T. basicola (5). Information on the effects of fumigants and host resistance on populations of T. basicola in tobacco culture are lacking. The objective of this study was to determine the effects of different levels of partial resistance and soil fumigation on the population dynamics of T. basicola and the severity of black root rot.

MATERIALS AND METHODS

Field selection, pathogen assay, and disease ratings. Tests were conducted in fields that had a history of black root rot and had been in continuous tobacco for at least 3 yr. In the first year of the study, a single field in each of three counties, and in the second year, a single field in each of two counties were selected for use. All tests were established on farms in the burley tobacco region of western North Carolina and were maintained by the grower using recommended cultural practices for burley tobacco. All fields had a moderate to high population of T. basicola (40-150 cfu/g soil) and an average pH (determined in 1:1 distilled water to soil) between 5.9 and 6.3. These conditions are conducive to the development of black root rot (5,6).

Populations of *T. basicola* were determined using a soil dilution-selective-agar medium assay. The TB-CEN medium of Specht and Griffin (10) was modified as previously described (8) and used in all assays. Each bulked soil

sample (as described below) was thoroughly mixed prior to use. A 5-g subsample was removed from the bulked sample, placed in 95 ml of deionized water, and stirred for 1 min to disperse soil particles. A 10-ml aliquot was removed and placed in about 290 ml of molten (45 C) selective-agar medium and stirred vigorously for about 30 sec. The solution was then dispensed into 10 plastic petri dishes (9-cm-diameter) and incubated in the dark for 10-14 days at 22-25 C. The number of colonies that developed was counted, and colony forming units per gram of dry soil was calculated.

Disease severity ratings were made by carefully digging plants at midseason or following harvest. The percentage of the root system with necrotic lesions was rated on a scale of 1-6, where 1 = no symptoms, 2 = <5% necrotic, 3 = 5-25% necrotic, 4 = 25-50% necrotic, 5 = 50-75% necrotic, and 6 = >75% necrotic.

Yields were determined by harvesting all plants in a plot except the last plant on each end of the row. Plants were air cured prior to weighing, and yields were converted to kilograms per hectare.

Host resistance tests. Tests were conducted over a 2-yr period. Eight cultivars were tested each year and were selected based on a range in resistance level to black root rot from susceptible to highly resistant (Table 1). Two of the eight cultivars used in the first year of the study were replaced in year two to more closely reflect cultivars currently grown in North Carolina. Plots consisted of two rows, 10.7 or 13.7 m long (depending on location) and 1.2 m apart. Plants were obtained from fumigated seedbeds maintained by the grower and were transplanted 0.6 m apart. Each year in the resistance test, one row of each plot was treated 3 wk prior to transplanting with the soil fumigant Telone C17 (74% 1,3dichloropropene + 16.5% chloropicrin) at the rate of 234 L/ha. Prior to fumigation, lime and fertilizer were applied as a broadcast treatment according to soil test results. Fields were then disked, and beds (30 cm high × 91 cm wide) were formed. The fumigant was applied with a John Blue fumigation apparatus through five chisels spaced 20 cm apart and 25 cm deep. Soil temperatures ranged from 12 to 15 C at the time of fumigation; soil moisture was low to moderate. The two-row plots were arranged in a split-plot design with genotypes as whole plots and fumigant as subplots. There were four replications per treatment at each location. Soil samples were taken from each row prior to application of the fumigant, at transplant, and in some fields at midseason and after harvest, to determine populations of T. basicola. Each sample consisted of 15 soil cores (2 \times 20 cm in diameter) taken along the side and top of a row. The soil cores were bulked before assaying on selective-agar medium as previously described.

The ability of T. basicola to reproduce on a given cultivar was measured in two ways. The first method was a measure of absolute population increase obtained by subtracting the population of T. basicola at transplant, P_i , from the population at harvest, P_f . The second measure was a reproduction index (RI), obtained by dividing P_f by P_i .

Fumigation tests. In addition to the fumigation treatment in the resistance tests, several other fumigants were tested in the second year of the study to determine their effects on the population of the pathogen and development of the disease. The tests were conducted in separate areas of the same two fields as the resistance tests described above. Soil preparation, bed formation, and application equipment were the same as previously described. Fumigation materials and rates of application included the following: 40% methylisothiocyanate (SN 556) at 46.7 and 74.8 L/ha; 40% 1,3-dichloropropene + 20\% methylisothiocyanate (Vorlex) at 46.7 and 74.8 L/ha; 100% chloropicrin (Chlor-O-Pic 100) at 28 L/ha; and Telone C17 at 98.2 and 140.2 L/ha. The materials were injected 25 cm deep utilizing three chisels spaced 20 cm apart for Vorlex and SN 556, one chisel for Chlor-O-Pic, and one, three, or five chisels for each rate of Telone C17. All applications were centered on the bedded row. Eleven combinations of fumigants, rates, and chisel treatments plus a nonfumigated check were arranged in four randomized complete blocks at each location. All plots were planted with the cultivar Va 509, which has a low level of partial resistance to black root rot. Treatments were applied to single row plots 10.7 m long and 1.2 m apart. Pathogen populations

Table 1. Severity of black root rot at harvest on cultivars of burley tobacco grown in soil infested with *Thielaviopsis basicola*

	Root rot severity ^y						
Resistance level Cultivar	Year	· 1	Year 2				
	Midseason	Harvest	Midseason	Harvest			
Susceptible							
Judy's Pride		$3.0 a^z$					
Low							
Burley 21	2.6 a	2.3 c	1.6 b	2.5 b			
Burley $21 \times \text{Ky } 10$	2.6 a	2.4 c	1.8 ab	3.2 a			
Va 509				2.4 b			
Moderate							
Ky 14	2.4 a	2.6 b	1.9 a	3.1 a			
$Ky 14 \times L 8$	2.3 b	2.7 ab	1.8 ab	2.9 a			
R7-11				3.2 a			
High							
Clay's 501		1.4 d					
Ky 15		1.3 d		1.3 c			
Tenn 86		1.2 d		1.1 c			

^yDetermined by excavating plants and rating percentage of the root system with symptoms on a 1-6 scale with 1 = no symptoms, 2 = <5% necrotic, 3 = 5-25%, 4 = 26-50%, 5 = 51-75%, and 6 = >75%.

²Numbers followed by the same letter are not significantly different at $P \ge 0.05$ according to the Waller-Duncan k-ratio test with k = 100.

and root rot severities were determined as previously described.

Data analysis. Data were analyzed by ANOVA or GLM procedures of SAS (SAS Institute, Cary, NC), and significant differences were determined by LSD or Waller-Duncan k-ratio tests (k = 100). Analysis of covariance, with initial pathogen population as the covariant, was performed to determine the effects of initial inoculum level on various disease parameters and the effects of fumigation and host resistance on yield that were not attributable to control of T. basicola.

RESULTS

Resistance tests. Similar levels of root rot developed in both years of the study. Final root rot severities across cultivars and locations were 2.6 in the first year of the study and 2.9 in the second year. These averages do not include data from cultivars with the N. debneyi source of resistance, (Ky 15, Tenn 86, and possibly Clay's 501, source of resistance not disclosed), because these cultivars had little or no root rot in all tests conducted (Table 1). Final root rot severities in these cultivars ranged from 1.1 to 1.4 (1 = nosymptoms) and were significantly less than severities for all other cultivars (Table 1).

Black root rot developed in all cultivars of burley tobacco with partial resistance to T. basicola derived from N. tabacum (Table 1). Significant differences in severity of root rot were observed at harvest among cultivars with low and moderate levels of partial resistance; severities ranged from 2.3 to 3.0 in year 1 and from 2.4 to 3.2 in year 2 (Table 1). Cultivars rated as moderate in black root rot resistance typically had higher root rot severity ratings than did cultivars considered to have low levels of partial resistance to black root rot. For example, the cultivar Burley 21, low resistance, had final root rot ratings of 2.3 in year 1 and 2.5 in year 2; whereas Ky 14, moderate resistance, had final ratings of 2.7 in year 1 and 3.1 in year 2. In year 2 of the study, the three cultivars with moderate levels of partial resistance had higher root rot ratings than two of the three cultivars with low

levels of partial resistance.

Based on root rot ratings, black root rot was active throughout the growing season (Table 1). This was especially evident in year 2 of the study, when root rot severity increased significantly between midseason and harvest on all cultivars with partial resistance. In contrast, in year 1 of the study, disease severity increased only on cultivars with moderate resistance during the last half of the growing season.

Final populations of *T. basicola* were dependent on year, cultivar, and fumigation treatment. In general, populations were higher in fields used in year 1 of the study than those used in year 2 (Table 2). For example, for cultivars with partial resistance, final populations averaged 209 and 139 propagules per gram of soil in year 1 and year 2, respectively. Final populations of T. basicola were consistently low in plots planted with highly resistant genotypes, whereas populations in plots with other genotypes varied significantly within a given resistance class (Table 2). Ky 14, moderate resistance, and Burley 21 × Ky 10, low resistance, had high final populations each year. Ky 14 × L 8, moderate resistance, had low final populations each of the 2 yr tested, and Burley 21, low resistance, had a high final population in year 1 but a low population in year 2 (Table 2). Initial population was not a significant determinant of final population.

Significant differences were observed in the reproductive ability of T. basicola among cultivars (Table 2). Highly resistant cultivars and the moderately resistant cultivar Ky 14 × L 8 resulted in a net reduction in populations of T. basicola in year 1 and only a small increase in year 2. Among the other cultivars tested, mean populations of T. basicola increased an average of about 100 cfu/g soil over the 2-yr period. An exception to this was the cultivar Burley 21, which had a mean increase of 68 cfu/g soil (Table 2). In the nonfumigated rows, the final population of T. basicola was higher in year 1 than in year 2, but reproduction index and root rot severity at harvest were higher in year 2 (Table 3).

The population of T. basicola at

Table 2. Final (Pf) population and reproduction index (RI) of Thielaviopsis basicola on cultivars of burley tobacco

Resistance level Cultivar		Year 1	Year 2			
	P _f ^w	Increasex	RI	P_{f}	Increase	RI
Susceptible						
Judy's Pride	275 a ^z	168 a	2.6			
Low						
Burley 21	215 ab	90 ab	1.7	78 c	45 bc	2.4
Burley $21 \times \text{Ky } 10$	265 a	100 ab	1.6	170 ab	104 ab	2.6
Va 509				122 abc	94 ab	4.4
Moderate						
Ky 14	210 abc	51 ab	1.3	199 a	153 a	4.3
Ky $14 \times L 8$	80 bc	−73 b	0.5	98 bc	41 bc	1.7
R7-11				168 ab	108 ab	1.6
High						
Clay's 501	96 abc	-15 b	0.9			
Ky 15	75 c	−23 b	0.8	64 c	28 c	1.8
Tenn 86	85 bc	−73 b	0.5	48 c	−8 c	0.9

[&]quot;Colony forming units per gram of soil at harvest as determined by soil assay on selectiveagar medium. Each value is the mean of four replications per cultivar in each of three fields in year 1 and two fields in year 2.

Table 3. Reproduction of Thielaviopsis basicola and severity of black root rot on burley tobacco grown under field conditions in untreated soil and in soil treated with the fumigant Telone C17

		Year 1			Year 2			
Treatment P _f w	P _f ^w	Increase ^x	RI ^y	Disease severity ^z	$\mathbf{P_f}$	Increase	RI	Disease severity
Unfumigated Fumigated	268** 52	56** 4	1.3* 1.1	2.2* 1.9	162** 74	106** 35	2.9* 1.9	2.6* 2.2

^v Soil was fumigated with 234 L/ha of Telone C17 approximately 3 wk prior to transplanting.

 $^{^{}x}$ Increase = $P_f - P_i$.

y Reproduction index = P_f/P_i .

² Values within columns followed by the same letter are not significantly different at $P \ge 0.05$ according to the Waller-Duncan k-ratio test with k = 100.

[&]quot;Colony forming units of T. basicola per gram of dry soil at harvest. Values for year 1 are means across eight cultivars planted at each of three locations (N = 88). Values for year 2 are means from eight cultivars and two locations (N = 64). Values within columns are significantly different at P < 0.01 (**) or P < 0.05 (*) according to LSD test.

^x Increase is $P_f - P_i$.

 $^{^{}y}$ RI is P_{f}/P_{i} .

² Disease is the final root rot severity rating taken at harvest and is on a 1-6 scale with 1 = no symptoms, 2 = <5% root rot, 3 = 5-25%, 4 = 26-50%, 5 = 51-75%, and 6 = >75%. Values include all cultivars.

transplant was a significant covariant for several of the parameters measured. In year 1, P_i was a significant determinant of root rot at harvest, reproduction index, and yield, but not of final P_f . In year 2, P_i was a significant determinant of yield but not of root rot at harvest, P_f , or reproduction index.

Severity of root rot at harvest and reproduction of *T. basicola* were suppressed by fumigation of subplots with high rates of Telone C17 (Table 3). Final population, reproduction index, and root rot at harvest were higher in year 2 of the study. Populations of *T. basicola* were similar at transplanting in year 1 (48 cfu/g soil) and year 2 (39 cfu/g soil).

Cultivars responded differently to the fumigation treatment. Yield of the highly resistant cultivars and the moderately resistant Ky $14 \times L$ 8 did not increase significantly following fumigation, whereas yield increased 18-33% in other cultivars with partial resistance (Table 4). In the fumigation treatment, no significant differences in yield occurred among cultivars, except with the highly suscep-

tible cultivar Judy's Pride, where yield was very low (Table 4).

Fumigation tests. Populations of T. basicola were reduced 0-93% with the fumigation treatments, but none of the treatments was different from the control, where populations declined by 34% during the same time period (Table 5). Final population and reproduction index of T. basicola also varied with treatment, but only one treatment, Telone C17 at the highest rate of application through one chisel, significantly reduced the final population; none of the treatments reduced reproduction index compared to the control (Table 5). Root rot at harvest was reduced only by the high rate of Telone C17 (Table 5).

DISCUSSION

The level of host resistance significantly affected the population dynamics of *T. basicola* and the severity of black root rot on burley tobacco. The most dramatic effects on the pathogen and disease were observed with cultivars that have the single gene for resistance to *T.*

Table 4. Yield response of burley tobacco cultivars to fumigation of soil infested with *Thielaviopsis* basicola

	Yield (kg/ha) ^x					
Cultivar	Nonfumigated	Fumigated ^y	Increase (%			
Judy's Pride	632	1,262	100* ^z			
Burley 21	2,654	3,530	33*			
Burley 21 × Ky 10	3,121	3,931	26*			
Ky 14	2,992	3,537	18*			
Ky 14 × L 8	3,510	3,666	4			
Clay's 501	3,552	3,695	4			
Ky 15	3,552	3,882	9			
Tenn 86	3,767	4,032	7			
LSD $(P = 0.05)$	558	624				

^{*}Mean of four replicate plots.

Table 5. Population of *Thielaviopsis basicola* and development of black root rot following soil fumigation with different materials and methods of application

Material and rate (L/ha)		Populations of T. basicola and root rot severity						
	No. chisels	Population reduction (%)"	P _f ^x	Increase	RI	Disease severity ^z		
Vorlex (75)	3	70	168	150	9.3	2.4		
Vorlex (47)	3	0	116	72	2.6	2.4		
SN 556 (75)	3	49	195	161	5.7	2.8		
SN 556 (47)	3	0	178	122	3.2	2.6		
Chlor-O-Pic (28)	1	56	124	96	4.4	2.6		
Telone C17 (98)	1	74	70	55	4.7	2.3		
Telone C17 (98)	3	0	93	52	2.3	2.5		
Telone C17 (98)	5	57	153	110	3.6	2.8		
Telone C17 (140)	1	93	39	33	6.5	2.0		
Telone C17 (140)	3	64	118	102	7.4	2.0		
Telone C17 (140)	5	58	119	94	4.8	2.0		
Control		34	125	90	3.4	2.8		
LSD $(P = 0.05)$		67	72	73		0.6		

[&]quot;Percent reduction in population of T. basicola following fumigation.

basicola derived from N. debneyi (3). Very little root rot was observed on these cultivars in our tests. T. basicola was infrequently isolated from lesions on roots of cultivars such as Tenn 86, but none of the isolates caused root rot when reinoculated onto Tenn 86 or Ky 15. The isolates were highly pathogenic on other cultivars (H. D. Shew, unpublished). T. basicola probably colonized the highly resistant cultivars after roots were already infected or wounded by other fungi, nematodes, or insects. The interaction of these factors has not been investigated for black root rot of tobacco, but interactions are very important in other host-pathogen systems that involve root pathogens (9).

There are no reported races of *T. basicola* that can overcome the resistance gene from *N. debneyi* (3,5,8,13). However, until recent years, few cultivars that carry this gene gained wide acceptance by growers, so selection pressure on the pathogen to develop races that can overcome this gene probably has been minimal. As new cultivars with the *N. debneyi* source of resistance are released, the probability of the development a new race of *T. basicola* that can attack cultivars with this resistance will increase. The mechanism of resistance is not known.

Black root rot developed on all cultivars with partial resistance to T. basicola. The high root rot severity ratings observed on moderately resistant cultivars compared to several cultivars rated as low in resistance were somewhat unexpected, but are in agreement with a previous greenhouse study on partial resistance to T. basicola in burley tobacco (13). Nominal disease ratings are based on overall performance of cultivars in tests conducted over a period of years and locations, and not on root rot ratings and pathogen populations. Cultivars could therefore be rated as moderate in resistance based on yield and quality but have similar or higher levels of root rot than cultivars rated low in resistance; i.e., nominal ratings may confound resistance and yield factors, including tolerance (2). Examples of this can be seen in Ky 14 and R7-11, cultivars designated moderately resistant, and Burley 21 and Va 509, cultivars rated as having low levels of partial resistance.

In a previous study, it was determined that resistance to *T. basicola* in the cultivar Burley 21 is inherited as a dominant trait, while resistance in Ky 14 is inherited as an additive trait (13). Because resistance to *T. basicola* in Burley 21 and Ky 14 is derived from *N. tabacum* (13), it is probable that multiple mechanisms of resistance are present in *N. tabacum*, and that combining these mechanisms may enhance resistance to *T. basicola*.

Differences in reproduction of T. basicola were observed among cultivars.

^ySoil fumigated 3 wk prior to transplant with 234 L/ha of Telone C17.

² Numbers followed by an asterisk represent a significant increase in yield due to fumigation (P = 0.05) according to LSD test.

x Population of T. basicola per gram of air-dried soil at end of growing season.

y Reproduction index for T. basicola is P_f/P_i .

² Root rot severity at end of growing season based on 1-6 scale with 1 = healthy, 2 = <5% root rot, 3 = 5-25%, 4 = 26-50%, 5 = 50-75%, and 6 = >75%.

In general, populations were highest on cultivars that had high root rot severities at harvest. A notable exception to this trend was observed with the cultivar Ky 14 × L 8. Root rot severities on Ky 14 × L 8 were among the highest each year of the test, but the final population and reproduction index of T. basicola were similar to those on cultivars with resistance from N. debneyi. These results differ from several greenhouse and growth chamber tests that used Ky 14 \times L 8 as one of the test cultivars (7). In these tests, Ky 14 \times L 8 had lower root rot ratings than Ky 14 and the lowresistance cultivar Burley $21 \times Ky 10$. It appears that the L 8 parent contributes significantly to the resistance of the hybrid to T. basicola, and that this resistance suppresses reproduction by the pathogen. Since hybrids are frequently used in burley tobacco, selection of certain cultivars or lines for development of a hybrid may enhance disease resistance.

Black root rot increased in severity throughout the growing season during each of the 2 yr of the study. This disease is often considered important only during the early part of the growing season (5). Stunting of infected plants is most prominent early in the season, but it is likely that yield suppression and increases in pathogen populations result from infections that occur over the entire growing season, as was observed in this study. The importance of secondary inoculum in the development of black root rot and the suppression of yield has not been quantified.

Attempts to control black root rot with soil fumigation have given mixed results (4; P. B. Shoemaker, *unpublished*). In

this study, the very high rate of Telone C17 used in the resistance tests resulted in decreased initial inoculum levels. reduced reproduction by T. basicola, and increased yields on cultivars with low to moderate resistance. Results were not as clear with the range of fumigants tested in year 2 of the study, but the highest rate of Telone C17, which was only 60% of the rate used in the resistance tests, reduced several of the disease and pathogen parameters measured. None of the fumigant treatments used in year 2 increased yield, even though a cultivar with low resistance to T. basicola was used. Effectiveness of soil fumigation for increasing yield thus appears to be dependent on cultivar, location, and year. These results agree with a previous study on soil fumigation and management of black root rot (4), and indicate that control practices in addition to fumigation are necessary to consistently reduce losses to black root rot.

Initial inoculum was not a good predictor of final population of T. basicola. This is not surprising, in that host resistance was so important in determining pathogen reproduction; reproduction was very low or nonexistent on cultivars with resistance from N. debneyi. Initial inoculum level was a significant factor in determining yield loss on cultivars with partial resistance to T. basicola and could possibly be used in conjunction with knowledge of soil factors that affect disease development (6,7) to predict the best practices for management of black root rot for a particular grower.

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