Evaluation of Components of Partial Resistance to Black Root Rot in Burley Tobacco

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ABSTRACT

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Cultivars with moderate (KY 14), low (Burley 21), and no resistance (Judy's Pride) to black root rot, and the F_1 of KY 14 × Burley 21 were evaluated for components of partial resistance to *Thielaviopsis basicola*. In addition, transgressive segregants from a previous generation mean analysis were intercrossed and progeny of the following crosses were evaluated: (KY 14 × Burley 21)F_2 × (KY 14 × (KY 14 × Burley 21)F_1), (KY 14 × Burley 21)F_2 × (Burley 21 × (KY 14 × Burley 21)F_1), and (KY 14 × (KY 14 × Burley 21)F_1) × (Burley 21 × (KY 14 × Burley 21)F_1). Five-week-old seedlings were transplanted into soil infested with 100 chlamydospores of *T. basicola* per gram of soil mixture and grown in the greenhouse at an average air temperature of 21°C. Disease severity (percent root necrosis), number of lesions per root, lesion length, and population density of the pathogen were estimated 3 weeks after transplanting. Significant differences were observed among genotypes for each component of partial resistance measured. Significant positive correlations were observed between lesion number and disease severity and between lesion number and lesion length. However, a significant negative correlation was observed between lesion number and population density. Selection for components of partial resistance in burley tobacco should lead to increased levels of resistance to black root rot.

Additional keywords: *Chlara elegans, Nicotiana tabacum*

Thielaviopsis basicola (Berk. & Broome) Ferraris (synanamorph *Chalara elegans* Nag Raj & Kendrick) is a soilborne fungal pathogen that causes black root rot disease on tobacco (*Nicotiana tabacum* L.). This soil inhabitant has a wide host range and is found in all major tobacco-growing regions of the world (7,13). Isolates of *T. basicola* vary widely in virulence and their ability to attack different hosts (2,7). The fungus produces two spore stages, endoconidia and chlamydospores. Chlamydospores are considered the primary infective propagules of the pathogen (21), but endoconidia also infect and cause root rot, and are probably most important in epidemics as secondary inoculum. The characteristic black lesions found on the main and lateral roots result in stunted plants and reduced yields. Black root rot is the primary disease associated with tobacco stunting in the burley region of Virginia (20) and North Carolina (8,10). Serious injury from black root rot can result from prolonged cool, wet weather after transplanting and yield losses of 5 to 7% have been reported on burley tobacco in the United States (5).

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There are two types of black root rot resistance currently utilized in commercial cultivars of tobacco. The first type, controlled by a single dominant gene from *N. debneyi,* provides immunity to black root rot (2) and has been equally effective against all isolates of the fungus from the burley tobacco growing regions of the United States. The second type of resistance is a low to moderate level of resistance found in *N. tabacum* cultivars. Resistance in the cultivar Harrow Velvet is controlled by a group of recessive genes (2), while resistance to black root rot in the flue-cured tobacco cultivars Virginia Gold, Hicks Broadleaf, and Yellow Special is controlled by three major genes (12). Additive, dominance, and epistatic effects occur in the inheritance of resistance to black root rot in the burley cultivars KY 14 and Burley 21 (22). In general, resistance to black root rot from *N. tabacum* is believed to be of a quantitative, polygenic nature (2).

Incorporation of disease resistance is a major objective in many tobacco breeding programs. One approach to the assessment of quantitative resistance is the measurement of certain components of resistance in controlled inoculation experiments. These components of quantitative or partial resistance include reduced infection frequency or lesion number, extended latent period, and decreased spore production (14,15). While these components of resistance can be readily measured for foliar pathogens, the methodology for soilborne pathogens is more complex. The objective of this study was to investigate and quantify components of partial resistance to black root rot in several burley tobacco cultivars and their progeny.

MATERIALS AND METHODS

Four genotypes were included in this experiment: KY 14 (6), moderate resistance to black root rot; Burley 21 (3), low resistance; (KY 14 × Burley 21)F_1, medium-low resistance; and Judy's Pride, susceptible. In a previous experiment that evaluated the inheritance of partial resistance to black root rot, KY 14 (parent 1) was crossed with Burley 21 (parent 2) and the F_1, F_2, BC_1P_1 (parent 1 × F_1), and BC_1P_2 (parent 2 × F_1) generations were produced (22). Transgressive segregants were identified in the segregating generations and intercrossed to produce the following populations: (KY 14 × Burley 21)F_2 × BC_1P_1, (KY 14 × Burley 21)F_2 × BC_1P_2, and BC_1P_1 × BC_1P_2.

Experiments were conducted in the greenhouse at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Va., during the winter of 1990–91. The experimental design was a randomized complete block with three replications and the experiment was repeated three times. There were 10 tobacco seedlings of each genotype in each replication. A virulent isolate of *T. basicola*, obtained from burley tobacco in Madison Co., N.C., was used to infest soil. The fungus was maintained on 5% carrot agar in 9-cm petri dishes in the dark at room temperature. Inoculum was prepared from 3-week-old plates as previously described (22) and the spore suspension was calibrated with a hemacytometer. Soil was infested to establish an inoculum density of 100 chlamydospores per gram of soil mix.

Five-week-old tobacco seedlings were transplanted into 10 × 10 cm plastic pots containing a 1:1:1 (vol/vol/vol) mixture of steam-sterilized soil, sand, and vermiculite. The soil mixture pH was 6.0, 5.9, and 5.9 for the three repetitions of the experiment. Seedlings were fertilized every 6 days with 25 ml of Peters solution (20-20-20; W. R. Grace & Co., Fogelsville, Pa.)

per pot. The pH of the Peter's solution was 5.6. Plants were rated for disease severity 3 weeks after transplanting by estimating the percentage of the root system with characteristic black lesions caused by *T. basicola*, using a Horsfall-Barratt rating scale (4) as follows: 0 = no symptoms, 1 = 1 to 3% of the root area with symptoms, 2 = 4 to 6%, 3 = 7 to 12%, 4 = 13 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, 7 = 76 to 88%, 8 = 89 to 94%, 9 = 95 to 97%, 10 = 98 to 99%, and 11 = 100% of the root area with symptoms. In addition, five roots were selected at random from each plant and the number of lesions per root were counted. Lesion length (mm) was determined on five randomly chosen lesions per root. Fresh weight (g) of shoots and roots were determined for all plants. Population density, measured as number of propagules of *T. basicola* per gram of soil, was determined as described by Meyer and Shew (9). One soil core was taken from each pot with a 1.2-cm cork borer just prior to the root ratings. Three 1-g subsamples per sample were used to determine population density.

Data were analyzed by analysis of variance and Fishers's least significant difference test was used to compare the differences among means. Pearson product moment correlations among resistance components were computed.

RESULTS

An analysis of variance was conducted for each repetition and combined analyses were performed over repetitions. The repetition by genotype interaction was not significant for any of the characters measured, therefore, a combined analysis was justified. Significant differences ($P = 0.05$) were observed among genotypes for disease severity, lesion number, lesion length, population density, shoot weight, and root weight measured in a combined analysis over runs.

Means for each character measured for each genotype are presented in Table 1. The disease severity rating for KY 14 was significantly higher than Burley 21 and was similar to Judy's Pride, the susceptible check. The F_1 hybrid was intermediate between the parents and had a significantly higher disease severity rating than Burley 21. No differences were observed among Burley 21 and the three genotypes generated by intercrossing the transgressive segregants.

There were no differences between KY 14 and Judy's Pride for number of lesions per root; both had significantly more lesions than Burley 21 and the three genotypes created by intercrossing the transgressive segregants. The F_1 hybrid was intermediate between the two parents for lesion number. There were no significant differences among genotypes for lesion length with the exception of (KY 14 × Burley 21)F_2 × BC_1P_1 which had significantly smaller lesions than KY 14, Judy's Pride, and (KY 14 × Burley 21)F_2 × BC_1P_2. Lesion lengths observed on the (KY 14 × Burley 21) × BC_1P_1 genotype were not significantly different from Burley 21 or BC_1P_1 × BC_1P_2.

The population density of *T. basicola* associated with Burley 21 was significantly lower than for all the other genotypes. In contrast, (KY 14 × Burley 21)F_2 × BC_1P_2 had the highest population density but was not significantly different from KY 14 or (KY 14 × Burley 21)F_2 × BC_1P_1. Although Judy's Pride is very susceptible to black root rot, it had one of the lower population densities in this test. The F_1 hybrid was again intermediate between the two parents.

The shoot weight of KY 14 was significantly greater than for all other genotypes with the exception of (KY 14 × Burley 21)F_2 × BC_1P_1 (Table 2). No differences in shoot weight were observed for Burley 21, Judy's Pride, and BC_1P_1 × BC_1P_2. The F_1 hybrid was intermediate between the two parents. In contrast, Judy's Pride had a significantly lower root weight than all of the other genotypes. No differences in root weight were observed for KY 14, Burley 21, the F_1 hybrid, (KY 14 × Burley 21)F_2 × BC_1P_1, and (KY 14 × Burley 21)F_2 × BC_1P_2. BC_1P_1 × BC_1P_2 had significantly lower shoot and root weights than the other two genotypes created by intercrossing the transgressive segregants.

Correlation coefficients among the characters measured are presented in Table 3. A positive correlation was observed between disease severity and lesion number, but no correlation was observed between disease severity and lesion length or population density. Lesion number was positively correlated with lesion length and negatively correlated with the population density. Lesion length and population density were not significantly correlated.

DISCUSSION

Evidence of partial resistance to black root rot in burley tobacco was observed. Differences in lesion number and lesion size have been observed in many host-pathogen systems (15). Lesion size refers to the area showing disease symptoms and is assumed to reflect the growth rate of the pathogen in the host. The number of lesions and spore production are dependent on many different factors, including host genotype, developmental stage of the host, and environmental conditions. Lesion number is frequently not considered a component of partial resistance because it

Table 1. Means for components of partial resistance to black root rot (*Thielaviopsis basicola*) in burley tobacco

Genotype[a]	Disease severity rating[b]	Lesion number per root[c]	Lesion length	Propagules per gram of soil[d]
KY 14	6.48	47.5	0.47	431
Burley 21	4.61	38.0	0.46	343
Judy's Pride	6.62	47.9	0.48	366
(KY 14 × Burley 21)F_1	5.52	42.9	0.45	390
(14 × 21)F_2 × BC_1P_1	4.64	35.7	0.42	424
(14 × 21)F_2 × BC_1P_2	5.12	39.3	0.47	445
BC_1P_1 x BC_1P_2	5.06	40.3	0.46	417
LSD ($P = 0.05$)[e]	0.63	5.2	0.04	22

[a] BC_1P_1 = KY 14 × (KY 14 × Burley 21)F_1; BC_1P_2 = Burley 21 × (KY 14 × Burley 21)F_1.
[b] Disease severity was an estimate of the percent root system with characteristic black lesions caused by *Thielaviopsis basicola* according to the following rating scale: 0 = no symptoms, 1 = 1–3% of the root system with symptoms, 2 = 4–6, 3 = 7–12, 4 = 13–25, 5 = 26–50, 6 = 51–75, 7 = 76–88, 8 = 89–94, 9 = 95–97, 10 = 98–99, and 11 = 100% of the root system with symptoms. Data represents mean of 10 plants per replication, three replications per run, and three runs.
[c] 5 roots × 10 plants = 50 observations per replication.
[d] Determined using a soil dilution:selective-agar medium assay on samples taken at the conclusion of the experiment.
[e] According to Fisher's least significant difference (LSD) test.

Table 2. Relative ranking of each genotype for components of partial resistance to black root rot (*Thielaviopsis basicola*) in burley tobacco[a]

Genotype[b]	Disease severity rating	Lesion number per root	Lesion length	Propagules per gram of soil	Plant weight	Root weight
KY 14	2	2	2.5	2	1	3
Burley 21	7	6	4.5	7	5	4.5
Judy's Pride	1	1	1	6	7	7
(KY 14 × Burley 21)F_1	3	3	6	5	4	4.5
(14 × 21)F_2 × BC_1P_1	6	7	7	3	2	2
(14 × 21)F_2 × BC_1P_2	4	5	2.5	1	3	1
BC_1P_1 × BC_1P2	5	4	4.5	4	6	6

[a] Genotypes are ranked from highest to lowest value for each parameter.
[b] BC_1P_1 = KY 14 × (KY 14 × Burley 21)F_1; BC_1P_2 = Bu 21 × (KY 14 × Burley 21)F_1.

does not directly affect the reproduction rate of the pathogen and is similar in genotypes with low and high levels of partial resistance (16,18). However, lesion number and population density were significantly correlated. Spores produced from lesions on some black root rot resistant tobacco genotypes were not viable, which reduces secondary inoculum (M. E. Hood and H. D. Shew, *unpublished*). Even though differences in population densities were significant statistically, the numbers were all very high and all would be sufficient to initiate severe levels of disease under field conditions (9).

Shew and Shoemaker (17) evaluated several burley tobacco cultivars in fields naturally infested with *T. basicola*. They reported that while host genotype was important in determining pathogen reproduction, the severity of root rot and reproduction of *T. basicola* were not related to the level of partial resistance of a cultivar. They observed higher root rot severities and greater reproduction on moderately resistant cultivars (including KY 14) than on cultivars rated low in resistance. Similar results were observed for KY 14 and Burley 21 in our experiments. The resistance level of a cultivar is not based on disease severity ratings but is generally based on the overall agronomic performance of cultivars in field experiments conducted over many locations and years.

Although the susceptible check, Judy's Pride, had the highest disease severity rating, it also had one of the lowest population densities (Table 2). This observation supports previous reports on the highly susceptible nature of Judy's Pride. The lower population also may have resulted from the smaller root system of Judy's Pride. For a near-equivalent disease severity rating for KY 14, a significantly higher population density was observed, indicating the pathogen reproduced more readily on KY 14. Burley 21 appears to have a higher level of resistance to *T. basicola* based on the population density and disease severity rating, but field experiments indicate that each lesion on Burley 21 may cause greater detriment to plant yield than on KY 14 (17). Burley 21 thus appears to be intolerant of *T. basicola*. Burley 21 is in the pedigree of KY 14 and is thought to contribute to the level of black root rot resistance in KY 14.

Resistant cultivars are the primary method for control of black root rot in burley tobacco. Black root rot symptoms are likely to occur in all burley cultivars with partial resistance to *T. basicola* (10,17,19). However, cultivars with the *N. debneyi* source of resistance show few or no black root rot symptoms when grown in fields with high inoculum densities (10, 17). Resistance to black root rot in TN 86 is controlled by a single dominant gene from *N. debneyi,* which may or may not be durable (11). Several recently released burley tobacco cultivars have TN 86 in their background and, therefore, the same source of resistance to black root rot. Reported yield losses due to black root rot in the burley region may decrease slightly as the percent acreage planted to TN 86 and these other new cultivars increases. The increased use of these new cultivars could impose extreme selection pressure on the pathogen population to develop new races that are more virulent. Development of cultivars with high levels of partial resistance is worthwhile in the event that races of *T. basicola* develop that are capable of overcoming the dominant resistance gene currently being employed.

Efficient crop production is dependent on the development of disease resistant cultivars. Incorporation of resistance from different sources is essential for developing stable cultivars that are not easily overcome by pathogens (16). Although polygenic resistance usually does not inhibit pathogen reproduction as completely as monogenic resistance, its durability is advantageous (16). Nevertheless, the quantitative nature of polygenic resistance and the difficulty of transferring it to commercial cultivars through conventional breeding methods are disadvantages. In this study, the level of partial resistance in KY 14 was significantly lower than in Burley 21 and was not significantly different from that of Judy's Pride, the susceptible check. However, KY 14 consistently yields well in the presence of black root rot (17) and may have tolerance to *T. basicola* (1). The F_1 hybrid was intermediate in disease severity rating between the two parents. These results differ from a previous study in which there were no significant differences between KY 14 and Burley 21 and the F_1 was significantly more susceptible than either parent (22). Overall, the genotype with the highest level of partial resistance was (KY 14 × Burley 21)F_2 × BC_1P_1. It had the fewest number of lesions, the smallest lesions, the second to lowest disease severity rating, but was intermediate for propagules per gram of soil. These results indicate that recurrent selection may be successful in identification of genotypes with increased levels of partial resistance.

Table 3. Simple correlation coefficients among components of partial resistance to black root rot in burley tobacco

	Lesion number	Lesion length	Propagules per gram of soil
Disease severity	0.51**[a]	0.04	0.06
Lesion number		0.31*	–0.31*
Lesion length			0.18

[a] *,**Significant at the 0.05 and 0.01 probability level, respectively.

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