Inoculum Densities of *Thielaviopsis basicola* in Tobacco Fields and the Role of Black Root Rot in Tobacco Stunting in Virginia

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ABSTRACT

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Inoculum densities of *Thielaviopsis basicola* in 1984 were 74–166 propagules per gram of soil in five burley tobacco fields and 0–12 propagules per gram of soil in three other burley fields. Inoculum densities of *T. basicola* were 0–26 propagules per gram of soil in 12 flue-cured and two sun-cured tobacco fields and 101 and 402 propagules per gram of soil in two other flue-cured fields. Environmental factors and cultivar resistance apparently influenced disease development, because inoculum density was not necessarily related to the severity of black root rot among the fields examined. Black root rot was the primary disease associated with tobacco stunting in the burley region of Virginia. Black root rot also occurred in the flue- and sun-cured region but was not generally a significant problem there.

Additional key words: *Acaulospora* spp., *Glomus clarum*

Black root rot, caused by *Thielaviopsis basicola* (Berk. & Br.) Ferraris (=*Chalara elegans* Nag Raj & Kendrick), is an important root disease of tobacco (*Nicotiana tabacum* L.). Tobacco fields with black root rot often contain stunted plants (7); however, to our knowledge, no extensive black root rot disease surveys have been conducted. Legg et al (6) reported that black root rot causes an estimated 5–7% reduction in the yield of burley tobacco in the United States, but they did not present supporting data on disease occurrence or yield loss. The present importance of black root rot in Virginia (where flue-cured and burley are the major types of tobacco grown) is uncertain, but tobacco stunting is commonly observed in the state. Endomycorrhizae may be involved in stunting in Virginia, because a pathogenic endomycorrhizal fungus, *Glomus macrocarpum* (Tul. & Tul.) Gerd. & Trappe, causes tobacco stunt disease of burley cultivars in Kentucky (4,5,8). This paper reports the results of a statewide survey conducted to evaluate the role of *T. basicola* in tobacco stunting in Virginia. Inoculum densities of *T. basicola* in tobacco fields were examined in relation to the incidence and/or severity of black root rot and associated plant stunting. Preliminary evaluations on the importance of endomycorrhizae in tobacco stunting were also made.

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MATERIALS AND METHODS

Black root rot survey. Commercial burley, flue-cured, and sun-cured tobacco fields considered possibly to have black root rot and/or plant stunting problems were sampled 1–2 wk before transplanting of tobacco in May or early June. Fifty to 75 soil cores (2.5 cm diameter × 15–20 cm deep) were collected by uniform sampling of an area 150–200 m^2 in each field. The test area was chosen at random unless otherwise noted. The soil cores were bulked to produce one composite sample per location. All samples were passed through a 4.8-mm sieve, mixed thoroughly, and assayed for inoculum densities of *T. basicola* with TB-CEN medium (13). Twenty soil-dilution plates were used per sample. The fields were maintained by the growers and were visited again in mid-July. Five stunted and three to five normal-sized plants were sampled at random and immediately rated for mean percentage of roots with black root rot (0–100% basis). Examination of standard disease area diagrams aided accurate evaluation of root rot severity. A tobacco plant was considered stunted if its height was less than half that of normal-sized or representative larger plants in the field. The incidence or percentage of plants stunted was determined by a count of at least 100 plants. Soil was also collected from around the roots of the sampled plants. This soil was then bulked to produce two composite samples per location, one each for stunted and normal-sized plants. The soils were assayed as described for *T. basicola* and also for pH, fertility, and the presence of plant-parasitic nematodes. Soil pH was measured in 0.01 M $CaCl_2$ (10), and the other analyses were conducted by clinical laboratories at Virginia Polytechnic Institute and State University.

Isolation of endomycorrhizae. Endomycorrhizal-like spores were isolated by wet-sieving and sugar-flotation centrifugation (8) from the tobacco field soils collected from around root systems of stunted and normal-sized plants. Ten spores were selected at random from each soil sample. All of the spores were filled with protoplasmiclike contents. Each individual spore was transferred with a pasteur pipette into the root zone of a 7- to 8-wk-old tobacco seedling recently transplanted into a pot containing a steam-pasteurized, 50/50 mixture of loamy soil and sand. The tobacco cultivars were Burley 21 and NC 95, depending on whether spores were from burley or flue/sun-cured tobacco field soils, respectively. The seedlings were grown in the greenhouse for 14 wk. The plant root systems were then carefully washed, cleared in KOH, stained in trypan blue-lactophenol (11), and observed for endomycorrhizal colonization. Final plant height measurements were also taken. Spores of isolates of endomycorrhizae that colonized root systems were identified by N. C. Schenck, University of Florida.

RESULTS

Black root rot survey. Ten counties were included in the survey (Fig. 1). Nineteen commercial tobacco fields were visited in 1984 (Table 1). The test areas in fields 3, 5, 8, 9, and 10 were specific locations where tobacco stunting was reported to have occurred previously. Inoculum densities of *T. basicola* in three (fields 12–14) of eight burley fields were 0–12 propagules per gram of soil. No black root rot was observed in these fields. Inoculum densities of *T. basicola* in two other burley fields (fields 15 and 16) were 74 and 76 propagules per gram of soil. Five percent or fewer of the plants in these fields were stunted, with mean percent root rot ratings on stunted vs.

normal-sized plants averaging 8 and 3.6%, respectively (for field 15), and 15 and 1%, respectively (for field 16). Another burley field (field 17) had an inoculum density of 166 propagules per gram of soil. Two percent of the plants in this field were stunted; however, *T. basicola* did not appear to be associated with the stunting, because mean percent root rot ratings on both stunted and normal-sized plants were similar (1.8 and 1%, respectively). Burley fields 18 and 19 were only sampled in early August. These two fields had large areas (about 40% for both fields) with severe tobacco stunting problems. Separate composite soil samples were collected from portions (100 m^2) of the problem and nonproblem areas in these fields. Fifty soil cores were taken by uniform sampling of the area between the rows. Soil samples were

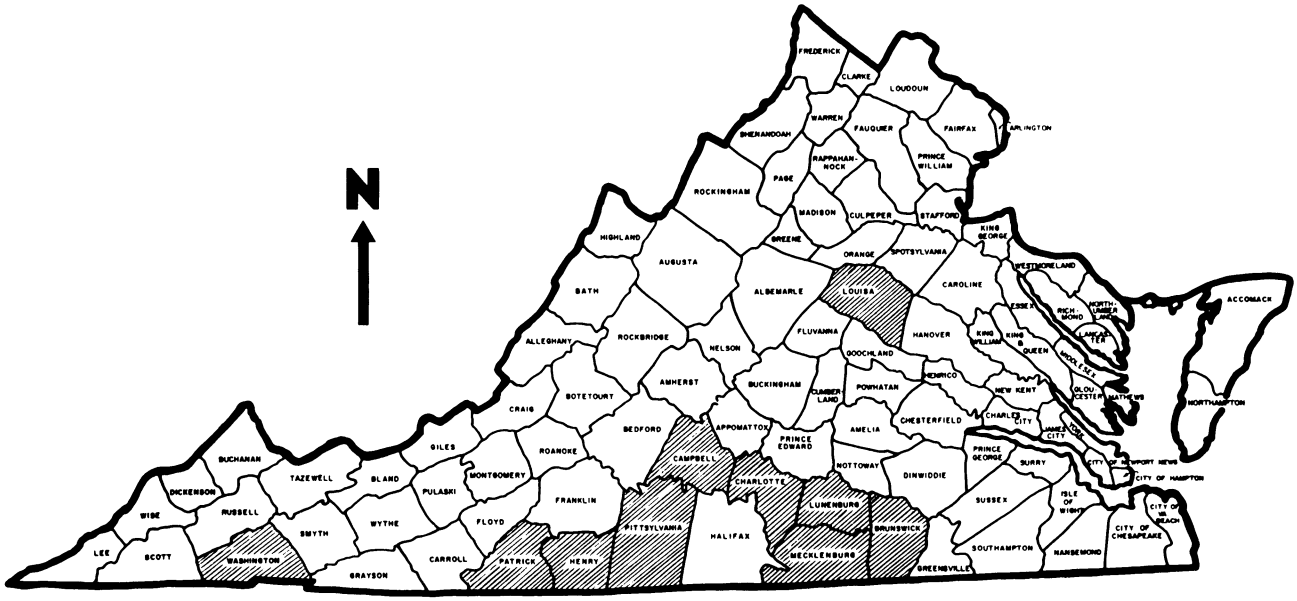

Fig. 1. Locations of counties included in the 1984–1985 Virginia black root rot/tobacco stunting survey. Eight burley fields (all in Washington County in southwestern Virginia), 16 flue-cured fields (in Patrick, Henry, Pittsylvania, Campbell, Charlotte, Mecklenburg, Lunenburg, and Brunswick counties in southcentral Virginia), and two sun-cured fields (both in Louisa County in central Virginia) were sampled.

Table 1. Population densities of *Thielaviopsis basicola* and levels of black root rot associated with stunted and normal-sized tobacco plants in Virginia fields in 1984

Field no.	Cultivar	Propagules/g soil (preplant level)	Stunted[a] (%)	Mean % root rot		Propagules/g soil around plant roots	
				Stunted	Normal-sized	Stunted	Normal-sized
1	Sun-cured[b]	0	ND[c]	0	0	0	0
2	Sun-cured[b]	5 ± 3[d]	ND	0	0	11 ± 6	16 ± 9
3	SP G-28[e]	0	1 (21)	0	0	0	0
4	McNair 944[e]	0	ND	0	0	0	0
5	McNair 944[e]	0.5 ± 1	5 (8)	0	0	0	0
6	K 326[e]	ND[e]	5 (8)	0	0	1 ± 1	0
7	Coker 319[e]	ND[e]	ND	0	0	3 ± 3	2 ± 2
8	K 326[e]	8 ± 3	3 (26)	0.4 ± 0.7	0.7 ± 1.4	3 ± 2*[f]	9 ± 3
9	Coker 319[e]	12 ± 5	ND	1.2 ± 1.0	1.0 ± 0	11 ± 5*	20 ± 5
10	NC 82[e]	26 ± 5	8 (10)	1.0 ± 0	0.7 ± 1.4	29 ± 7	28 ± 8
11	Coker 319[e]	101 ± 18	5 (10)	20 ± 8*	5.0 ± 0	139 ± 18*	265 ± 27
12	Ky 14[g]	0	8 (8)	0	0	0	0
13	B21-Ky10[g]	2 ± 2	2 (5)	0	0	1 ± 1	1 ± 1
14	B21-Ky10[g]	12 ± 5	ND	ND	0	ND	6 ± 3
15	Ky 14[g]	74 ± 16	5 (10)	8.0 ± 3.4*	3.6 ± 2.4	102 ± 32*	285 ± 71
16	Ky 14[g]	76 ± 14	1 (13)	15 ± 13*	1.0 ± 0	61 ± 27*	266 ± 76
17	Ky14-L8[g]	166 ± 17	2 (7)	1.8 ± 3.8	1.0 ± 0	186 ± 24	195 ± 14
18	B21-Ky10[g]	148 ± 38[h] 10 ± 5[i]	40 (3)	32 ± 16*	1.0 ± 0	2,040 ± 113*	234 ± 20
19	B21-Ky10[g]	158 ± 29[h] 31 ± 14[i]	40 (4)	30 ± 9*	1.0 ± 0	1,051 ± 115*	204 ± 20

[a] Percentage of plants that were stunted. Values in parentheses are the average fresh shoot weight of stunted plants expressed as a percentage of the average fresh shoot weight of normal-sized plants.
[b] Official cultivar test field.
[c] Not determined.
[d] Ninety-five percent confidence interval.
[e] Flue-cured cultivar.
[f] Asterisk indicates a significant ($P = 0.05$) difference between values for stunted and normal-sized plants as determined by a t test; t tests for mean percent root rot for fields 9–11 and 16–19 and also for propagules per gram of soil around plant roots for fields 18 and 19 were based on unequal variances. Degrees of freedom for t tests based on equal variances were 6–8 for mean percent root rot comparisons and 38 for propagules per gram of soil comparisons. Degrees of freedom for t tests based on unequal variances were 4 for stunted plants, 2 for healthy plants (mean percent root rot comparisons, $N_1 \neq N_2$), and 19 for propagules per gram of soil comparisons ($N_1 = N_2$).
[g] Burley cultivar.
[h] Propagules per gram of soil between rows in early August in an area containing mostly stunted plants.
[i] Propagules per gram of soil between rows in early August in an area containing mostly normal-sized plants.

collected from between rows to avoid pathogen reproduction on diseased roots. Before transplanting, the density of *T. basicola* between rows is not likely to be very different from that within rows (12). Population densities of *T. basicola* in the problem areas were 148 (field 18) and 158 (field 19) propagules per gram of soil, respectively, and are considered reasonable estimates of the inoculum densities.

Inoculum densities of *T. basicola* in two of two sun-cured fields (fields 1 and 2) and eight of nine flue-cured fields (fields 3–10) were 0–26 propagules per gram of soil. Tobacco stunting occurred to some extent in all of these fields; however, *T. basicola* was not associated with the stunting, because mean percent root rot ratings on both stunted and normal-sized plants were low (range 0–1.2%). One flue-cured field (field 11) had an inoculum density of 101 propagules per gram of soil. Five percent of the plants in this field were stunted, with mean percent root rot ratings on stunted vs. normal-sized plants averaging 20 and 5%, respectively.

For five of the fields (8, 9, 11, 15, and 16), population densities of *T. basicola* in July were significantly higher in soils taken from around root systems of normal-sized than of stunted plants (Table 1). This occurred even in three fields (11, 15, and 16) where black root rot was more severe on stunted plants. The root systems of normal-sized tobacco plants were always much larger than those of stunted plants. These larger root systems, though less diseased, in some instances apparently provided sufficient substrate for pathogen colonization and reproduction. In the two burley fields that had the most severe levels of black root rot and plant stunting (18 and 19), however, significantly higher population densities of *T. basicola* were found in soils taken from around root systems of stunted plants.

Five additional flue-cured fields were visited in late June 1985. Inoculum densities of *T. basicola* were estimated at these locations by assaying soil taken from between rows. Estimated inoculum densities in four of the fields (fields 20–23) were 0–6 propagules per gram of soil. The other field (field 24) had 402 propagules per gram of soil; the cultivar planted in this field was K 326, which has low to medium resistance to black root rot. Seven percent of the plants in this field were stunted, with mean percent root rot ratings on stunted vs. normal-sized plants averaging 32 and 1.3% (significantly different at $P = 0.05$), respectively. Population densities of *T. basicola* in soils taken from around root systems of stunted vs. normal-sized plants were 2,150 and 825 propagules per gram of soil (significantly different at $P = 0.05$), respectively. With TB-CEN medium (13), *T. basicola* was consistently isolated from tobacco root segments with representative lesions. For fields where *T. basicola* was detected in soil but no black root rot was observed, the pathogen also was occasionally isolated from asymptomatic roots.

Within fields, there were no appreciable differences in pH, percent organic matter, and P, K, Ca, Mg, Zn, and Mn levels between soil samples collected from around roots of stunted vs. normal-sized plants. Soil pH ranged from 4.5 in field 13 to 6.8 in field 1. Black root rot was severe over a range of pH levels, because the values in mid-July in field 18 were both 6.5 but only 5.2 (soil around stunted plants) and 5.4 (soil around normal-sized plants) in field 19. *Pratylenchus* and *Tylenchorhynchus* spp. were present in a couple of fields, but their population densities were well below economically damaging levels. A complicating factor in field 24 was the presence of *Globodera solanacearum* (tobacco cyst nematode). There were 280 and 540 larvae of *G. solanacearum* per 500 cm^3 in soil taken from around root systems of stunted and normal-sized plants, respectively. Ten larvae or one cyst per 500 cm^3 of soil is considered by the VPI&SU Nematode Assay Clinic to represent an economically damaging population density.

Isolation of endomycorrhizae. Four of the fields sampled for black root rot were selected for the endomycorrhizal study. Tobacco stunting was not associated with black root rot in two of the fields (burley field 17 and sun-cured field 2), but it was in the other two (burley field 19 and flue-cured field 11). Endomycorrhizal-like spores were found in all soils assayed; however, endomycorrhizae successfully colonized root systems of only five of 40 burley and zero of 40 flue-cured tobacco plants grown in the greenhouse. The endomycorrhizal fungus *Glomus clarum* Nicolson & Schenck (9) was isolated from soils taken from around root systems of both stunted and normal-sized plants in field 19. *G. clarum* was isolated also from soil taken from around root systems of stunted plants in field 17. Two *Acaulospora* spp. were isolated from soil taken from around root systems of normal-sized plants in field 17. The heights of greenhouse-grown tobacco plants not colonized by endomycorrhizae (range 56–89 cm) were not significantly different from those of plants that were colonized (range 56–81 cm).

DISCUSSION

Black root rot appeared to be a major cause of tobacco stunting in the burley region of Virginia but not in the flue and sun-cured regions; however, burley fields 18 and 19 were the only two tobacco fields in the survey that had severe stunting problems associated with black root rot. To our knowledge, the data presented here are the first to document the relative importance of black root rot in stunting in commercial tobacco fields. We were unable to find evidence to indicate that endomycorrhizae were associated with burley stunting, as found in Kentucky (4,5,8); however, the results of the endomycorrhizal study were inconclusive, and a more complete study would be required to determine the unfavorable or favorable effects of endomycorrhizae on tobacco in Virginia.

T. basicola generally occurred more frequently and was present at higher inoculum densities in soils of burley than in soils of flue- and sun-cured tobacco fields. Most of the flue- and sun-cured cultivars have low or low to medium levels of resistance, so other factors are probably responsible for the low incidence and severity of black root rot in these types. For instance, black root rot is most severe when soil temperatures are 23 C or lower (7). The flue- and sun-cured cultivars are grown in the low-elevation Piedmont region in central Virginia, whereas the burley cultivars are grown in the higher elevation Appalachian region in southwestern Virginia. Air temperatures at the Virginia Tech Southern Piedmont Agricultural Experiment Station at Blackstone averaged 18.4, 23.8, 23.2, and 23.7 C during May, June, July, and August of 1984, respectively. Air temperatures at the Abingdon weather station in Washington County in southwestern Virginia during these months averaged 15.6, 22.2, 21.7, and 22.3 C, respectively. Soil temperatures (10 cm deep) at Blackstone during these months averaged 20.5, 26.9, 25.9, and 26.9 C, respectively. Because the flue- and sun-cured cultivars were transplanted in early to mid-May, soil temperatures in central Virginia were apparently only favorable for black root rot development for the first few weeks after transplanting. No corresponding soil temperature data were available for southwestern Virginia; however, soil temperatures throughout most of the growing season in the burley tobacco fields, which were transplanted in late May to early June, were probably close to the upper limit of the range (17–23 C) considered most favorable for black root rot to occur. Other environmental factors also may have contributed to the greater severity of black root rot in the burley fields. In Virginia, soils in burley fields are generally finer textured and slightly higher in pH than soils in flue- and sun-cured fields (*unpublished*). Fine soil texture and high soil pH (generally 6.0 or greater) also favor black root rot (7).

The cultivars B21-Ky10, Ky 14, and Ky14-L8 have low to medium, high, and high levels of resistance to black root rot, respectively (1–3). Low levels of host resistance and relatively high inoculum densities (148 and 158 propagules per gram of soil in areas containing mostly stunted plants) would explain why severe tobacco stunting problems occurred in

fields 18 and 19. On the other hand, high levels of host resistance of Ky 14 and Ky14-L8, even in the presence of medium to high inoculum densities (74–166 propagules per gram of soil), is apparently the major reason black root rot was not a significant problem in burley fields 15–17. Ky14-L8, the most popular burley cultivar in Virginia, may have an even higher level of resistance to black root rot than Ky 14, because mean percent root rot ratings on Ky14-L8 (field 17) were very low despite a high inoculum density. Some burley cultivars, such as Ky15 and Ky17, are practically immune to black root rot but are not widely grown because they do not yield as well as other cultivars under disease-free conditions and because they have poor agronomic characteristics (6).

The population density of *T. basicola* in one of five flue-cured fields (field 24) sampled in 1985 was very high (402 propagules per gram of soil). Additional studies conducted in 1985 (*unpublished*) showed that the inoculum density of *T. basicola* in field 19 increased over 300% (from 148 to 649 propagules per gram of soil) compared with the previous year. The generally higher inoculum densities of *T. basicola* observed in 1985 were probably due to a large buildup of inoculum on diseased root systems in 1984. Field 12 was the only burley location where *T. basicola* was not found. This was a new field that had been planted to tobacco for only a couple of years, and the pathogen was apparently not yet established. *T. basicola* was also detected in many of the flue- and sun-cured fields. The presence of the pathogen in so many fields indicates that the potential for economically important black root rot and tobacco stunting exists, given that susceptible cultivars are grown and that environmental conditions are favorable for several years to allow for sufficient inoculum buildup. The development of a practical black root rot disease-prediction program would need to consider not only pathogen inoculum density but also cultivar susceptibility and environmental factors.

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