Thielaviopsis basicola in San Joaquin Valley Soils and the Relationship Between Inoculum Density and Disease Severity of Cotton Seedlings

B. A. HOLTZ, former graduate student researcher, and A. R. WEINHOLD, professor, Department of Plant Pathology, University of California, Berkeley 94720

ABSTRACT

Holtz, B. A., and Weinhold, A. R. 1994. *Thielaviopsis basicola* in San Joaquin Valley soils and the relationship between inoculum density and disease severity of cotton seedlings. Plant Dis. 78:986-990.

Populations of *Thielaviopsis basicola* in naturally infested cotton field soils in the San Joaquin Valley of California were determined in 1992 with modified Specht's *T. basicola*-carrot-etridiazol-nystatin medium. In cotton fields in Kings County, CA, the pathogen was detected in 24 (88%) of the 27 fields surveyed, with a mean population density of 77.6 cfu/g of soil and a range of 1 to 220 cfu/g of soil. Black root rot was detected in 79% of the fields where plants also were sampled. Disease severity was positively correlated with inoculum density, and pathogen populations were positively correlated with the number of years fields were planted to cotton. *T. basicola* was found less frequently and at lower population densities in fields where crop rotation or summer flooding had been practiced, compared with fields planted continuously to cotton.

Additional keywords: *Chalara elegans*

Black root rot of cotton (*Gossypium hirsutum* L.), caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris (synanamorph *Chalara elegans* Nag Raj & Kendrick), is a disease of increasing importance in the San Joaquin Valley of California (B. Roberts, Kings County farm advisor, *personal communication*). *T. basicola* is a dematiaceous fungus for which no sexual state is known (25). The fungus is a common soil inhabitant, in both cultivated and noncultivated soils (44,45), causing characteristic black necrotic lesions on the main and lateral roots of over 137 plant species (8,11). *T. basicola* is most damaging to cotton plants in the early stages of seedling development, entering the plant through root hairs and successively invading all root tissues (20). Disease symptoms are characterized by a swollen taproot, internal purplish black rot of the vascular tissue, and external black rot of the central cylinder of the root (3,13,18). Microscopic examination of the diseased tissue almost invariably reveals black chlamydospores characteristic of *T. basicola* (18,21). Although the fungus colonizes the vascular tissue, under certain conditions the pericycle can remain uninjured, allowing cortical regeneration and secondary root growth (20). Seedlings may recover in the San Joaquin Valley if high temperatures prevail during late April and early May. If cool wet weather conditions exist, which are favorable to the growth of the fungus and the development of black root rot, plant growth and yields may be drastically reduced (20). The amount of disease depends on the initial inoculum, the dynamics of root growth (9), and the environmental conditions present in any given field (3,21-23,29,33).

Studies on the ecology and epidemiology of soilborne plant pathogens require methods to quantitatively determine inoculum density under natural conditions. The inoculum of *T. basicola* builds up gradually each year in the presence of a host (2). In the absence of a susceptible host, *T. basicola* must survive adverse environmental conditions. Although this fungus produces two spore types, endoconidia and chlamydospores (37), chlamydospores appear to be the main propagule responsible for the long-term survival of this fungus in soil (41). A direct quantitative assay to determine *T. basicola* inoculum densities would aid in understanding the relationship between inoculum density and black root rot.

Farm advisors and large growers in California currently monitor inoculum levels of *T. basicola* in cotton fields using several modifications of Yarwood's isolation technique (15,39,43). These procedures are time-consuming, some are not quantitative, and they require a large number of replicates for statistical accuracy (19,27,38,39,42). If *T. basicola* inoculum could be quantified quickly and accurately, assays of cotton field soil samples in early spring could be used to forecast the potential for disease development during the season and would be helpful for making management decisions. A direct quantitative assay that can be used for naturally infested San Joaquin Valley soils, which contain low inoculum levels, is required.

Several selective media have been described for isolation of *T. basicola*, including rose bengal agar (RB-0, RB-M1, and RB-M2) (40,42), V8 juice-dextrose-yeast extract agar (VDYA) (28), VDYA-pentachloronitrobenzene (VDYA-PCNB) (26), and *T. basicola* medium-carrot (TBM-C) and -V8 (TBM-V8) (17). A *T. basicola*-carrot-etridiazol-nystatin (TB-CEN) medium has been described to enumerate *T. basicola* in tobacco field soils in Virginia (34). This medium has been used to obtain valuable information on the relationship between pathogen populations and disease on burley tobacco (*Nicotiana tabacum*) (1,22,23,35,36). In preliminary trials, however, none of these published media were sufficiently selective for assaying cotton field soils from the San Joaquin Valley for *T. basicola*. The TBM-C-modified TBM-2RBA medium, used to isolate *T. basicola* from highly organic (muck or peat) soils in the Fraser Valley of British Columbia, Canada (4), was not tested in this study. The objective of this research was to modify the TB-CEN medium to enhance selectivity for *T. basicola* isolation and to examine the relationship between inoculum density and disease in San Joaquin Valley soils.

MATERIALS AND METHODS

Development of modified selective medium. A combination of three selective media was used to assay for *T. basicola*. The antimicrobial ingredients initially described by Papavizas (26), modified by Maduewesi et al (17) and by the addition of the fungicide etridiazol used by Specht and Griffin (34), were combined to make the TB-CEN-pentachloronitrobenzene (TB-CENP) medium. The composition of the TB-CENP medium (per liter) consisted of 200 ml of 33% fresh carrot extract, prepared by blending (at high speed for 2 min) 100 g of fresh carrots with 200 ml of distilled water; 400 mg of etridiazol (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, added as 1.2 g of Terrazole 35 WP (Uniroyal Chemical Company, Bethany, CT); 250,000 units of nystatin; 1 g of PCNB (75% WP); 500 mg of streptromycin sulfate; 30 mg of chlortetracycline hydrochloride; 1 g of $CaCO_3$; and 17 g

Current address of first author: Department of Plant Pathology, University of California, Davis, Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier 93648.

Accepted for publication 20 July 1994.

of agar. All ingredients were added to molten water agar immediately prior to use, with the antibiotics added as solutions, as described by Papavizas (26).

Recovery from field soil. The following pour-plate procedure was used with TB-CENP medium to assay Kings County cotton field soils for *T. basicola*. One gram of field soil and 200 ml of molten (48 C) medium were mixed for 2 min in a 500-ml Erlenmeyer flask. The medium-soil suspension was poured into five 9-cm-diameter plastic petri plates. The suspension was poured while swirling the flask to keep the fungicide and soil uniformly suspended. The plates were incubated in the dark for 21 days at 16 C prior to counting colonies. To determine the effect of temperature, the plates were incubated at 16, 21–22, and 27 C and examined periodically for colonies. Populations of *T. basicola* were calculated as the mean value of three replicate 1-g subsamples per soil sample.

Recovery from artificially infested soil. Chlamydospore suspensions of *T. basicola*, prepared from 6- to 8-wk-old colonies growing on either carrot slices or 5% carrot agar plates, were harvested. The carrot slices and plates were first rinsed with water to remove endoconidia and then scraped with glass slides to release the chlamydospores. The resulting spore suspension was passed through a 400-mesh (0.038 mm) sieve, from which the chlamydospores were harvested. The chlamydospore suspension was homogenized in a blender for 1 min at high speed and passed through a 500-mesh (0.022 mm) sieve, from which the chlamydospores were harvested. A hemacytometer was used to determine spore concentrations. Each segment of the chlamydospore chain was counted as a single propagule to not underestimate the inoculum density (14,30). When the chlamydospores were obtained from cultures grown on carrot agar, they were treated with chitinase, as described by Christias and Baker (6), to break up the multicellular spores. Chlamydospores from cultures grown on nonsterile carrot disks did not require this treatment and were broken up by natural microbial activity. Predetermined spore concentrations were used to infest sterilized San Joaquin Valley cotton field soils and a greenhouse planting mix at inoculum concentrations of 100, 250, and 500 cfu/g. The infested soil was allowed to air-dry at room temperature for 5 wk. The soil was assayed for *T. basicola* with the TB-CENP selective medium as described above.

Comparison of media. In preliminary studies, the TB-CENP medium was compared to the original TB-CEN medium and three published media: VDYA-PCNB, TBM-C, and TBM-V8. The selective media were evaluated by the pour-plate technique described above. Five plates of each medium were used per gram of field-soil sample taken from 27 Kings County cotton fields, which were each approximately 640 acres. The plates were incubated for 21 days at 16 C, except when temperature experiments were done. The plates were read by counting distinctive dark *T. basicola* colonies. The experiment was performed three times over a 10-wk period with the same soil samples.

Relationship of inoculum density to disease severity. Twenty-seven cotton fields in Kings County were sampled during early April 1992, approximately 12 days after planting. Each soil sample was a composite of 15 soil cores (3-cm-diameter) taken to a depth of 20 cm in a random pattern across each field. Samples were stored in open polyethylene bags at 15 C and assayed for *T. basicola* inoculum with the TB-CENP medium. Plants (15–20 per soil sample) also were sampled from 14 of the fields at the same time and from the immediate vicinity of the soil samples. The plants were rated for disease severity by estimating the percentage of the root system with characteristic lesions of black root rot, according to the following scale: 0 = no symptoms, 1 = trace (a few, small, discrete lesions), 2 = <5% of the root system with symptoms, 3 = 5–25%, 4 = 25–50%, 5 = 50–75%, and 6 = 75–100% of the root system with symptoms. The relationships between inoculum density and disease incidence/severity were tested by means of *t* tests, linear correlation analyses, and one-way analyses of variance. Soil samples also were taken from a 640-acre cotton field both before (July 1991) and after summer flooding (August 1991) was done for 4 wk. The average water depth was 15–20 cm.

RESULTS

Development of modified selective medium. After modifying the TB-CEN medium by adding PCNB, lowering the incubation temperature, and increasing the incubation period, the TB-CENP medium was highly selective for recovering *T. basicola* from San Joaquin Valley cotton field soils (Table 1). The addition of PCNB inhibited several undesired fungi, mostly *Chaetomium* and *Stachybotrys*, which commonly occur in San Joaquin Valley soils. The antibiotic solution initially described by Papavizas (26) was the most effective at reducing bacterial development on this medium. Distinct dark *T. basicola* colonies developed on the agar surface (Fig. 1). The temperatures at which TB-CENP medium plates were incubated significantly influenced recovery of *T. basicola*. Recovery from naturally infested soil was greatly reduced when the plates were incubated at or above room temperature. The highest recovery of *T. basicola*, with the fewest undesired fungi, was observed at 16 C, but an extended

Table 1. Ingredients of TB-CENP, TB-CEN, and VDYA-PCNB selective media for recovery of *Thielaviopsis basicola*

TB-CENP[x]	TB-CEN[y]	VDYA-PCNB[z]
200 mL of carrot extract (25% w/v)	80 mL of carrot extract (50% w/v)	200 ml of V8 juice
1.2 g of Terrazole (35% WP)	1.14 g of Terrazole (35% WP)	
1.0 g of PCNB (75% WP)		0.5 g of PCNB (75% WP)
46 mg of nystatin	46 mg of nystatin	30 mg of nystatin
500 mg of streptomycin-sulfate	500 mg of streptomycin-sulfate	100 mg of streptomycin-sulfate
30 mg of chlortetracycline-HCL	30 mg of chlortetracycline-HCL	2 mg of chlortetracycline-HCL
1 g of $CaCO_3$	1 g of $CaCO_3$	1 g of $CaCO_3$
17 g of agar	15 g of agar	20 g of agar
pH ≈ 5.2	pH ≈ 5.3	pH ≈ 5.2
		2.0 g of glucose
		2.0 g of yeast extract
		1.0 g of oxgall
16 C	20–22 C	25 C
21 day incubation	14 day incubation	6–7 day incubation

[x] *T. basicola*-carrot-etridiazol-nystatin-pentachloronitrobenzene (PCNB) medium developed in this study.
[y] *T. basicola*-carrot-etridiazole-nystatin medium (35).
[z] V8 juice-dextrose-yeast extract agar-PCNB medium (26).

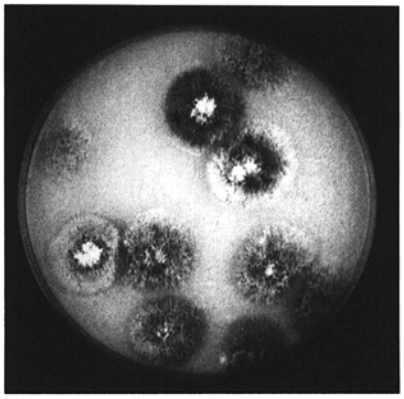

Fig. 1. Colonies of *Thielaviopsis basicola* on the *T. basicola*-carrot-etridiazol-nystatin-pentachloronitrobenzene selective medium following incubation at 15 C for 21 days.

incubation period of 21 days was required (Table 2). If more than 1 g of heavily infested field soil was assayed, the colonies were too dense to count, whereas less than 1 g of lightly infested field soil gave poor recovery.

Recovery from artificially infested soil. The mean percent recovery of *T. basicola* chlamydospores from sterilized cotton field soil and a greenhouse planting mixture infested with 100, 250, and 500 cfu/g was approximately 97, 85, and 73% respectively. Recovery was higher from the sterilized field soil.

Comparison of media. At high inoculum densities (>50 cfu/g), the recovery of *T. basicola* with the TB-CENP and TB-CEN media was similar, but at low inoculum densities (<50 cfu/g), the TB-CENP medium was more sensitive. In several field soils, the TB-CENP medium detected populations of *T. basicola* <10 cfu/g, whereas the TB-CEN medium did not (Table 3). The addition of PCNB increased the sensitivity of the TB-CEN medium for recovering *T. basicola* from San Joaquin Valley soils. Recovery was consistently higher compared to VDYA-PCNB, TBM-C, and TBM-V8 media (Table 3).

Table 2. Recovery of *Thielaviopsis basicola* from naturally infested cotton field soils using TB-CENP[x] selective medium at three temperatures

	No. of propagules/gram of soil following incubation[y,z]		
Field no.	16 C	21–22 C	27 C
10	159 a	52 b	1 c
18	122 a	22 b	3 c
47	143 a	14 b	1 c
48	25 a	1 b	0 b
63	126 a	24 b	1 c

[x] *T. basicola*-carrot-etridiazol-nystatin-pentachloronitrobenzene.
[y] Mean of three replicate 1-g samples, with five petri dishes in each replicate.
[z] Values in rows followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

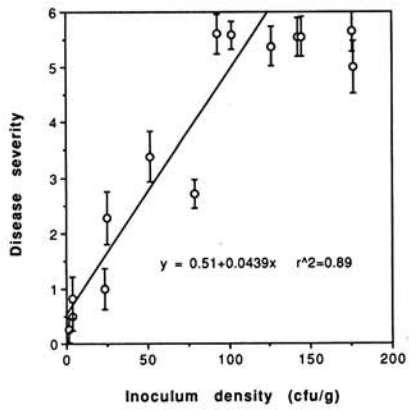

Fig. 2. Relationship between inoculum density (<125 cfu/g of soil) of *Thielaviopsis basicola* and disease severity on cotton seedlings in San Joaquin Valley cotton fields. Bars indicate standard errors.

Relationship of inoculum density to disease severity. *T. basicola* was detected in 24 of the 27 fields (88%) sampled, with a range of inoculum density of 1 to 221 cfu/g of dry soil (Table 3). Black root rot was found in 11 of 14 fields (79%) where plants also were sampled. It was possible to derive an inoculum density-disease severity relationship for *T. basicola* in these San Joaquin Valley cotton fields. Disease severity was positively correlated with inoculum level ($r^2 = 0.89$) (Fig. 2). Inoculum densities of <25 cfu/g of soil were associated with trace symptoms, whereas with densities >100 cfu/g almost the entire root was covered with characteristic black lesions. The pathogen was recovered more frequently and at higher inoculum densities when fields were planted continuously to cotton. Fields that had been planted for three or more years had significantly ($P < 0.01$) higher inoculum levels than fields in which an alternate crop had been planted or the field had been summer flooded. Mean inoculum density following continuous cotton was 116.4 ± 15.6 (standard error) colony forming units per gram of soil ($n = 17$ fields), while mean inoculum density in rotated or flooded fields was 15.7 ± 7.6 cfu/g of soil ($n = 10$). A significant difference (t value at $P < 0.01$) was found in inoculum level in a Kings County cotton field sampled both before (sample mean = 69.0 ± 10.7 cfu/g) and after (sample mean = 23.3 ± 3.9 cfu/g) summer flooding.

Table 3. Comparison of five selective media for recovery of *Thielaviopsis basicola* from naturally infested cotton field soils

	No. of propagules/gram of soil[u]				
Field no.	TB-CEN-PCNB[v]	TB-CEN[w]	TBM-C[x]	TBM-V8[y]	VDYA-PCNB[z]
10	159 a	137 a	35 b	5 c	0 c
18	113 a	55 b	12 c	0 c	0 c
38	174 a	100 b	30 c	2 d	0 d
41	193 a	125 a	62 b	19 c	2 c
42	221 a	167 b	72 c	13 d	2 d
43	52 a	62 a	12 b	5 b	2 b
44	0 a	0 a	0 a	0 a	0 a
45	3 a	0 a	0 a	0 a	0 a
46	0 a	0 a	0 a	0 a	0 a
47	143 a	95 b	27 c	5 d	1 d
48	25 a	18 a	2 b	0 b	0 b
49	0 a	0 a	0 a	0 a	0 a
50	2 a	0 a	0 a	0 a	0 a
51	8 a	0 b	0 b	0 b	0 b
52	76 a	33 b	2 c	1 c	1 c
53	33 a	6 b	0 c	0 c	0 c
54	10 a	0 b	0 b	0 b	0 b
55	93 a	83 a	11 b	0 b	1 b
56	142 a	113 a	18 b	6 c	2 c
57	52 a	37 a	2 b	0 b	0 b
58	176 a	118 b	29 c	5 d	0 d
59	176 a	178 a	45 b	10 c	8 c
60	79 a	35 b	2 c	0 c	0 c
61	4 a	0 a	0 a	0 a	0 a
62	23 a	3 b	0 b	0 b	1 b
63	126 a	45 b	14 c	2 d	0 d
64	102 a	34 b	7 c	0 c	0 c

[u] Means are an average of three replicate 1-g samples, with five petri dishes per replicate. Values in rows followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[v] *T. basicola*-carrot-etridiazol-nystatin-pentachloronitrobenzene (PCNB) medium.
[w] *T. basicola*-carrot-etridiazol-nystatin medium.
[x] *T. basicola* medium-carrot.
[y] *T. basicola* medium-V8 juice.
[z] V8 juice-dextrose-yeast extract agar-PCNB medium.

DISCUSSION

The TB-CENP selective medium provided a direct quantitative method to determine the inoculum densities of *T. basicola* in naturally infested San Joaquin Valley soils. This medium made it possible to survey fields for *T. basicola* and to examine the relationship between inoculum density and disease severity. The pathogen was widely distributed in cotton field soils in Kings County. Disease severity was positively correlated with inoculum density of *T. basicola*. Significant root rot developed in most fields only when populations of *T. basicola* were >50 cfu/g of soil. This observation agrees with pathogenicity tests in the greenhouse (21,33,38) and reports of black root rot on tobacco (23,35,36), where a positive linear correlation between inoculum density of *T. basicola* and black root rot disease has been reported. Therefore, an inoculum density of 50 cfu/g of soil may be considered optimal for disease in San Joaquin Valley cotton field soils. Similar recovery rates, from tobacco field soils, were reported for *T. basicola* assayed

with the TB-CEN medium (1,22,34).

Crop rotation and summer flooding are cultural practices aimed at reducing black root rot development on cotton in the San Joaquin Valley. The most common rotation crops in Kings County are safflower, wheat, and barley. There was a positive correlation between inoculum densities of *T. basicola* and the number of years in continuous cotton. A similar relationship was found in field soils planted continuously to tobacco (23,35). Populations were lower in fields where rotation or summer flooding had been practiced. Populations of *T. basicola* increase only in the presence of a host plant and decrease in soil planted with nonhosts or in fallow soil (2,23,32).

Summer flooding of cotton fields has become a fairly common practice for farmers in the Tulare Lake Basin area of the San Joaquin Valley. As much as 40,000 acres of land have been flooded to reduce black root rot development (B. Roberts, Kings County farm advisor, *personal communication*). This practice was initiated after growers observed increased yields in cotton fields that were naturally flooded after heavy winter rainfall. Because the soil in this area is finely textured and does not drain easily, it can be flooded for several weeks without using excessive amounts of water. A significant difference (t value at $P < 0.01$) was found in inoculum levels of *T. basicola* in a Kings County cotton field sampled before and after 4 wk of summer flooding. High temperatures (>30 C) while flooding have been shown to reduce *T. basicola* phialospores in the high organic soils of British Columbia, Canada (5). Further investigation is needed to determine the effects of summer flooding on survival of *T. basicola* inoculum in the San Joaquin Valley. Flooding of fields also has reduced inoculum of two other soilborne plant pathogens that can cause disease of cotton, *Verticillium dahliae* (10,31) and *Fusarium oxysporum* f. sp. *vasinfectum* (7).

Other factors affect the severity of black root rot and play an important role when estimating potential disease in any given field. Soil environmental conditions, such as temperature and moisture, influence the development of black root rot of cotton and several other crops (16, 21,33). Cool soil temperatures (16–18 C) increase disease severity on cotton (3) and tobacco (12). When soil temperatures are above 20 C, black root rot on tobacco is reduced, and at temperatures above 26 C, the disease is absent. Black root rot also can be more severe if cotton is grown on wet, poorly drained soils (13). Soil chemical factors affect the development of black root rot of tobacco in North Carolina. Low base saturation, low calcium, high exchangeable aluminum, and low pH all suppress disease (22–24). Further investigation is needed on the effects of soil temperature and soil chemistry on *T. basicola* and black root rot of cotton. Measurements of these variables and an understanding of their interactions should be useful in the prevention and management of black root rot of cotton in the San Joaquin Valley.

The results from this study suggest that inoculum density of *T. basicola* may be a good indicator for potential black root rot development. This information could be useful in establishing pathogen threshold levels and in predicting disease losses under various field conditions. Knowledge of the relationship between inoculum levels and black root rot will aid growers in deciding when to implement control practices, such as summer flooding or crop rotation, which are commonly used in the San Joaquin Valley.

ACKNOWLEDGMENTS

Supported in part by a grant from the California Statewide Integrated Pest Management Project 91FE013. We thank B. A. Roberts, Kings County farm advisor, for assistance in locating and sampling cotton fields, and J. G. Hancock for his advice during the project and careful review of the manuscript.

LITERATURE CITED

1. Anderson, T. R., and Welacky, T. W. 1988. Populations of *Thielaviopsis basicola* in burley tobacco fields and the relationship between soil inoculum concentration and severity of disease on tobacco and soybean seedlings. Can. J. Plant Pathol. 10:246-251.
2. Bateman, D. F. 1963. Influence of host and nonhost plants upon populations of *Thielaviopsis basicola* in soil. Phytopathology 53:1174-1177.
3. Blank, L. M., Leyendecker, P. J., and Nakayama, R. M. 1953. Observation on black root rot symptoms on cotton seedlings at different soil temperatures. Plant Dis. Rep. 37:473-476.
4. Chittaranjan, S., and Punja, Z. K. 1993. A semi-selective medium and procedures for isolation and enumeration of *Chalara elegans* from organic soil. Plant Dis. 77:930-932.
5. Chittaranjan, S., and Punja, Z. K. 1994. Factors influencing survival of phialospores of *Chalara elegans* in organic soil. Plant Dis. 78:411-415.
6. Christias, C., and Baker, K. F. 1967. Chitinase as a factor in the germination of chlamydospores of *Thielaviopsis basicola*. Phytopathology 57:1363-1367.
7. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN.
8. Gayed, S. K. 1972. Host range and persistence of *Thielaviopsis basicola* in tobacco soil. Can. J. Plant Sci. 52:869-873.
9. Huisman, O. C. 1982. Interactions of root dynamics to epidemiology of root invading fungi. Annu. Rev. Phytopathol. 20:303-327.
10. Ioannou, N., Schneider, R. W., and Grogan, R. G. 1977. Effect of flooding on the soil gas composition and the production of microsclerotia by *Verticillium dahliae* in the field. Phytopathology 67:651-656.
11. Johnson, J. 1916. Host plants of *Thielavia basicola*. J. Agric. Res. (U.S.) 7:289-300.
12. Johnson, J., and Hartman, R. E. 1919. Influence of soil environment on the root-rot of tobacco. J. Agric. Res. 17:41-86.
13. King, C. J., and Presley, J. T. 1942. A root rot of cotton caused by *Thielaviopsis basicola*. Phytopathology 32:752-761.
14. Linderman, R. G., and Toussoun, T. A. 1967. Behavior of chlamydospores and endoconidia of *Thielaviopsis basicola* in nonsterilized soil. Phytopathology 57:729-731.
15. Lloyd, A. B., and Lockwood, J. L. 1962. Precautions in isolating *Thielaviopsis basicola* with carrot discs. Phytopathology 52:1314-1315.
16. Lloyd, A. B., and Lockwood, J. L. 1963. Effect of soil temperature, host variety, and fungus strain of Thielaviopsis root rot of peas. Phytopathology 53:329-331.
17. Maduewesi, J. N. C., Sneh, B., and Lockwood, J. L. 1976. Improved selective media for estimating populations of *Thielaviopsis basicola* in soil on dilution plates. Phytopathology 66:526-530.
18. Maier, C. R., and Staffeldt, E. E. 1960. Cultural variability of selected isolates of *Rhizoctonia solani* and *Thielaviopsis basicola*, and the variability in their pathogenicity to Acala and Pima cotton, respectively. Plant Dis. Rep. 44:956-961.
19. Maloy, O. C., and Alexander, M. 1958. The most probable number method for estimating populations of plant pathogenic organisms in the soil. Phytopathology 48:126-128.
20. Mathre, D. E., Ravenscroft, A. V., and Garber, R. H. 1966. The role of *Thielaviopsis basicola* as a primary cause of yield reduction of cotton in California. Phytopathology 56:1213-1216.
21. Mauk, P. A., and Hine, R. B. 1988. Infection, colonization of *Gossypium hirsutum* and *G. barbadense*, and development of black root rot caused by *Thielaviopsis basicola*. Phytopathology 78:1662-1667.
22. Meyer, J. R., and Shew, H. D. 1991. Development of black root rot on burley tobacco as influenced by inoculum density of *Thielaviopsis basicola*, host resistance, and soil chemistry. Plant Dis. 75:601-605.
23. Meyer, J., Shew, H. D., and Shoemaker, P. B. 1989. Populations of *Thielaviopsis basicola* and the occurrence of black root rot on burley tobacco in western North Carolina. Plant Dis. 73:239-242.
24. Meyer, J. R., and Shew, H. D. 1991. Soils suppressive to black root rot of burley tobacco, caused by *Thielaviopsis basicola*. Phytopathology 81:946-954.
25. Nag Raj, T. R., and Kendrick, B. 1975. A Monograph of Chalara and Allied Genera. Wilfred Laurier University Press, Ontario, Canada.
26. Papavizas, G. C. 1964. New medium for the isolation of *Thielaviopsis basicola* on dilution plates from soil and rhizosphere. Phytopathology 54:1475-1481.
27. Papavizas, G. C. 1967. Survival of root-infecting fungi in soil. I. A quantitative propagule assay method of observation. Phytopathology 57:1242-1246.
28. Papavizas, G. C., and Davey, C. B. 1961. Isolation of *Thielaviopsis basicola* from the bean rhizosphere. Phytopathology 51:92-96.
29. Papavizas, G. C., and Lewis, J. A. 1971. Survival of endoconidia and chlamydospores of *Thielaviopsis basicola* as affected by soil environmental factors. Phytopathology 61:108-113.
30. Patrick, Z. A., Toussoun, T. A., and Thorpe, H. J. 1965. Germination of chlamydospores of *Thielaviopsis basicola*. Phytopathology 55:466-467.
31. Pullman, G. S., and DeVay, J. E. 1981. Effect of soil flooding and paddy rice culture on the survival of *Verticillium dahliae* and incidence of Verticillium wilt in cotton. Phytopathology 71:1285-1289.
32. Reddy, M. S., and Patrick, Z. A. 1989. Effect of host, nonhost, and fallow soil on populations of *Thielaviopsis basicola* and severity of black root rot. Can. J. Plant. Pathol. 11:68-74.
33. Rothrock, C. S. 1992. Influence of soil temperature, water, and texture on *Thielaviopsis basicola* and black root rot of cotton. Phytopathology 82:1202-1206.
34. Specht, L. P., and Griffin, G. J. 1985. A selective medium for enumerating low populations of *Thielaviopsis basicola* in tobacco field soils. Can. J. Plant Pathol. 7:438-441.
35. Specht, L. P., and Griffin, G. J. 1988. Relation of inoculum density of *Thielaviopsis basicola* to the severity of black root rot and growth of tobacco in naturally infested soil. Can. J. Plant Pathol. 10:15-22.
36. Specht, L. P., Griffin, G. J., Reilly, J. J., and Komm, D. A. 1987. Inoculum densities of *Thielaviopsis basicola* in tobacco fields and the role of black root rot in tobacco stunting in Virginia. Plant Dis. 71:876-879.
37. Stover, R. H. 1950. The black root rot disease of tobacco. Can. J. Res. 28:445-470.
38. Tabachnik, M., DeVay, J. E., Garber, R. H.,

and Wakeman, R. J. 1979. Influence of soil inoculum concentrations on host range and disease reactions caused by isolates of *Thielaviopsis basicola* and comparison of soil assay methods. Phytopathology 69:974-976.
39. Tsao, P. H. 1962. A quantitative technique for estimating the degree of soil infestation by *Thielaviopsis basicola*. Phytopathology 52:366.
40. Tsao, P. H. 1964. Effect of certain fungal isolation agar media on *Thielaviopsis basicola* and on its recovery in soil dilution plates. Phytopathology 54:548-555.
41. Tsao, P. H., and Bricker, J. L. 1966. Chlamydospores of *Thielaviopsis basicola* as surviving propagules in natural soils. Phytopathology 56:1012-1014.
42. Tsao, P. H., and Canetta, A. C. 1964. Comparative study of quantitative methods used for estimating the population of *Thielaviopsis basicola* in soil. Phytopathology 54:633-635.
43. Yarwood, C. E. 1946. Isolation of *Thielaviopsis basicola* from soil by means of carrot disks. Mycologia 38:346-348.
44. Yarwood, C. E. 1974. Habitats of *Thielaviopsis* in California. Plant Dis. Rep. 58: 54-56.
45. Yarwood, C. E. 1981. Occurrence of *Chalara elegans*. Mycologia 73:524-530.