Influence of Soil Inoculum Concentrations on Host Range and Disease Reactions Caused by Isolates of *Thielaviopsis basicola* and Comparison of Soil Assay Methods

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ABSTRACT

TABACHNIK, M., J. E. DeVAY, R. H. GARBER, and R. J. WAKEMAN. 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 69:974-977.

Nine isolates of *Thielaviopsis basicola* were tested for host specificity at 10^2, 10^3, and 10^4 endoconidia per gram of soil and for disease reactions caused on cotton, bean, pea, peanut, and soybean. Sunflower, a nonhost, served as control. The isolates of various geographic origin were from cotton, bean, soybean, orange, sesame, and field soils cropped to soybean, tobacco, peanut, or guar. Cotton was the most susceptible at each of the inoculum concentrations, and sunflower apparently was not affected by any of the inoculum concentrations. For all isolates, host specificity was most apparent at an inoculum concentration of 10^4 endoconidia per gram of soil, except for cotton and bean; isolate 415 was nonpathogenic on bean but caused severe black root rot on cotton at 10^4 endoconidia per gram of soil. Although isolates of *T. basicola* were from various hosts, they were not specific for these hosts. In assays for *T. basicola* in soils from nine cotton fields in the San Joaquin Valley in California, each with a history of seedling disease problems, eight fields had 10 or fewer propagules per gram of soil and one field had 70 propagules per gram of soil. Of various selective media compared for sensitivity in detecting low concentrations of propagules of *T. basicola* from naturally infested soils, the carrot disk technique was the most sensitive.

Additional key words: black root rot, soil assays.

Black root rot was first observed on cotton in 1922 in Sacaton, AZ (4). In 1938, the causal organism of black root rot of tobacco in Europe and in some of the eastern and midwestern states of the U.S. was identified as *Thielaviopsis basicola* (Berk. & Br.) Ferraris (4). Experiments were made in 1939 to compare the pathogenicity of strains of *T. basicola* from Arizona (cotton) and Missouri (tobacco) using Pima cotton and Maryland broadleaf tobacco plants as hosts (4). Inoculations of the host plants with both cotton and tobacco strains of *T. basicola* resulted in black root rot (4). *T. basicola* is worldwide in distribution and now has a host range of more than 100 plant species (2).

Keller and Shanks (3) found evidence of host specificity in *T. basicola* in that the strains that attacked tobacco did not attack poinsettia and vice versa. In view of the wide host range of *T. basicola* (2), it is possible that strains overlap in their pathogenicity on some hosts but are specific to others (3). Stover (9) reported the occurrence of physiologic specialization of *T. basicola* in tobacco plants and associated pathogenicity with colony characters; brown wild type strains were more pathogenic than the gray wild type strains. Brown wild type strains were further separated into two physiologic races according to their geographic origin (9).

Lloyd and Lockwood (5) reported that *T. basicola* strains from poinsettia, orange, and pea were moderately to highly virulent on bean and pea but nonpathogenic on tobacco plants. In contrast, strains from tobacco were highly virulent on tobacco but nonpathogenic on bean plants. One tobacco strain was mildly virulent on pea plants (5).

Our objectives were to compare the pathogenicity and possible host specificity of *T. basicola* from various host plants and soils and from different geographic origins in relation to inoculum concentration. Methods for assaying populations of *T. basicola* in field soils also were compared.

MATERIALS AND METHODS

Isolates of *Thielaviopsis basicola*. The following nine isolates of *T. basicola* from various plant hosts or field soils were used and maintained on potato dextrose agar (PDA): 374 from cotton (R. H. Garber, California); 409 from orange (P. H. Tsao, California); 412 from guar field soil (Texas); 414 from tobacco field soil; 415 from sesame, 416 from bean, and 418 from soybean field soil (G. C. Papavizas, Maryland); 420 from peanut field soil (D. L. Lindsey, New Mexico), and 425 from soybean (J. L. Lockwood, Michigan).

Pathogenicity tests. Pathogenicity tests were conducted in the greenhouse at approximately 23 C. Steamed sand and Yolo loam soil were mixed in a 1:1 ratio. Inoculum for infestation of soil was prepared by suspending endoconidia from 10 to 14 day-old cultures of *T. basicola* grown in petri dishes on PDA at 25–27 C, in 10-ml glass-distilled water/dish.

Inoculum was adjusted to 10^2, 10^3, and 10^4 endoconidia per gram of soil, using a cell-counting chamber and known amounts of air-dry soil. The soil and the inocula were mixed with a rotary mixer. The infested soil usually was dispensed into metal flats ($55 \times 40 \times 8$ cm) or plastic trays ($36 \times 12 \times 6$ cm) and kept moist in the greenhouse for 10–14 days before planting. Seeds of the following plant species were used: Cotton (*Gossypium hirsutum* L. 'Acala SJ-2,' California Planting Cotton Seed Distributors, Bakersfield); bean (*Phaseolus vulgaris* L. 'Bountiful,' Burpee Seeds, Riverside, CA); pea (*Pisum sativum* L. 'Early Bird,' Burpee Seeds); peanut (*Arachis hypogaea* L. 'Spanish,' Burpee Seeds); sunflower (*Helianthus annuus* L. 'Mammoth,' Burpee Seeds); and soybean (*Glycine max* (L.) Merr. 'Amsoy 71,' D. G. Gilchrist, University of

California, Davis).

Seeds were planted in the infested soil at 20 per row in six rows per metal flat or 10 per row in two rows per plastic tray. About 10 days after emergence, seedlings were uprooted and indexed for severity of black root rot: 0 = healthy; 1 = up to 25% of taproot blackened; 2 = 26–50% taproot blackened; 3 = 51–75% taproot and few lateral roots blackened; 4 = 76–100% taproot blackened and few or no lateral roots remaining.

In another pathogenicity test that involved a wide range of inoculum concentrations of isolate 374 on cotton, soil was prepared as above but infested with 1, 5, 10, 25, 50, 75, 100, 10^3, 5×10^3, 10^4, 5×10^4, 10^5, and 5×10^5 endoconidia per gram of soil. Cotton seeds were planted, and seedlings were grown for 6 wk in the greenhouse and compared for growth and disease development.

Collection of soil samples for assays of *T. basicola*. Soil assays of eight cotton fields in the San Joaquin Valley of California were made to determine the concentration of propagules of *T. basicola*. This was done to compare the populations of *T. basicola* in these fields with those found by Mathre et al (7) in the same area in 1964 and also to compare these populations with the concentrations of endoconidia necessary to cause various levels of severity of black root rot on seedlings of crop plants in greenhouse tests using steamed soil.

Two soil samples were collected by bulking eight soil cores taken 30 m apart from the upper 30 cm of soil from eight rows per field per sample.

Soil assay methods. To compare the sensitivity of selective media for estimating populations of *T. basicola,* two soil samples were collected from a cotton field near Bakersfield, CA; it was heavily infested with *T. basicola* and had a high incidence of black root rot. The media were the TBM-C medium (6), a sugar beet disk medium, and a carrot disk medium (12).

Assay methods used with the TBM-C medium (6) were: (i) 1 g of soil (moisture at approximately 70-80% of field capacity) was suspended in 0.5% water agar to give 10^{-1}, 10^{-2}, and 10^{-3} (w/v) dilutions, and then 1 ml of each dilution was spread evenly on the agar surface in each of three petri dishes; and (ii) 100 mg of pulverized, air-dry soil were impacted on the agar medium with a modified Anderson sampler (1) that deposited the soil at 400 individual locations on the agar per dish.

To prepare the sugar beet disk medium, sugar beet roots were washed and surface-sterilized by immersing them in 2.5% sodium hypochlorite solution for 20 min; then they were rinsed in three washes of sterile water. Slices or disks of root (3–4 mm thick) were cut to a diameter of 100 mm to fit the petri dishes. After having been placed in the dishes on a sterile Whatman No. 1 filter paper disk, 100 mg of pulverized soil was impacted on the disks with the modified Anderson sampler (1). The disks then were treated with 2 ml of 50 ppm streptomycin solution.

For the carrot disk medium, carrot roots were washed and surface-sterilized as described for sugar beet roots. Disks (3–4 mm thick) then were placed in petri plates on a sterile disk of Whatman No. 1 filter paper. One series of tests involved five disks per plate per soil sample with three replications, and in a second series, the carrot disks were fitted to cover the bottom of the entire plate. For plates containing five disks, a soil spoon containing 21 mg of soil was used to apply the soil evenly on the upper cut surface of each disk. Then, 5 ml of 50-ppm streptomycin solution was added per plate. A disk of filter paper was placed in the top of each plate and sprayed with the streptomycin solution. The soil on the carrot disks also was sprayed with the streptomycin solution and kept moist during incubation. For the other series of plates with carrot disks covering the entire plate, 100 mg of soil was impacted on carrot disks in each plate with the modified Anderson sampler (1).

The plates for all soil assays were incubated for 7–10 days at 24 C. The soil was then rinsed from the media, and the number of colonies of *T. basicola* per plate and/or the number of carrot disks (five per plate) colonized by *T. basicola* were determined by microscopic examination.

For each method of assay, duplicate analyses of each soil sample with three replications per medium were made.

RESULTS

Comparisons of selective media for *T. basicola*. Carrot disks were the most sensitive selective medium for estimating soilborne populations of *T. basicola* (Table 1). Although an excellent medium when tested with steamed soil infested with *T. basicola,* sugar beet disks were of limited use because other fungi grew when field soil was used. Although the Anderson sampler method gave the highest propagule counts and made it possible to detect 3–4,000 propagules per gram of soil, the soil spoon method with carrot disks, capable of detecting 3–50 propagules per gram of soil, was easier to use. Hence, the soil spoon method with carrot disks was chosen for comparisons of field soils in other experiments. All soil samples were assumed to contain an even distribution of propagules of *T. basicola.*

Assays of field soils for *T. basicola*. We used carrot disk assays for *T. basicola* with soil samples from eight cotton fields with a history of seedling disease in the San Joaquin Valley. The concentration of propagules of *T. basicola* measured in seven cotton fields was 10 or fewer per gram of soil, but in one field that had a high incidence of black root rot of cotton seedlings, the concentration was more than 50 propagules per gram of soil.

Concentrations of *T. basicola* in soil in relation to black root rot development in cotton. At 100 or fewer endoconidia per gram of soil, there were no apparent differences in growth and development of cotton plants. At 5×10^5 endoconidia per gram of soil, however, cotton plants growing in infested soil were still in the cotyledon stage, whereas control plants had developed to the third internode stage when the roots were indexed for disease severity. The disease index varied in relation to inoculum concentration: one

TABLE 1. Comparison of selective media and soil-distribution methods for assaying two samples of field soil for *Thielaviopsis basicola*

Medium	Soil distribution method	Propagules per gram of soil in sample[a] A	B
TBM-C[b]	Soil dilution in 0.5% water agar	0	0
TBM-C	Soil impaction, modified Anderson Sampler	6	0
Sugar beet disk	Soil impaction, modified Anderson Sampler	4	0
Carrot disk	Soil impaction, modified Anderson Sampler	36	44
Carrot disk	Soil spoon	29	31

[a] Figures are averages of three replications per medium with duplicate analyses of two soil samples (A and B) from a cotton field with a high incidence of black root rot.

[b] A carrot-based agar medium containing several antibiotics (6).

TABLE 2. Relative susceptibility of crop plants to nine isolates of *Thielaviopsis basicola* at different inoculum concentrations

Crop	Relative susceptibility[z] (endoconidia per gram of soil) 10^2	10^3	10^4
Cotton	2.1 a	2.5 a	2.4 a
Peanut	1.0 b	0.5 b	1.2 b
Bean	0.2 c	0.3 c	1.3 b
Soybean	0 c	0.1 cd	0.8 c
Pea	0 c	0.1 cd	0.3 d
Sunflower (nonhost)	0 c	0 d	0 e

[z] Relative susceptibility is the average of disease indices for each of nine isolates of *T. basicola* on each crop plant. Rating scale: 0 = healthy; 1 = up to 25% of taproot blackened; 2 = 26–50% of taproot blackened; 3 = 51–75% of taproot blackened and few lateral roots; and 4 = 76–100% of taproot blackened and few or no lateral roots remaining. Figures in vertical columns followed by different letters are significantly different at the $P = 0.01$ level, Duncan's multiple range test.

TABLE 3. Disease reactions of cotton (*Gossypium hirsutum*, 'Acala SJ-2' and bean (*Phaseolus vulgaris*, 'Bountiful') seedlings to three isolates of *Thielaviopsis basicola* at four concentrations of inoculum

Inoculum concentration (endoconidia per gram of soil)	Plant and disease indices[z] per isolate of *T. basicola*							
	Bean				Cotton			
	374	415	418	Av.	374	415	418	Av.
0	0 a	0 a	0 a	0	0 a	0 a	0 a	0
10^2	0.3 b	0 a	0.2 b	0.2	0.8 b	0.8 b	1.2 b	0.9
10^3	1.4 c	0 a	1.7 c	1.0	2.7 c	0.7 b	2.5 c	2.0
10^4	2.6 d	0 a	3.4 d	2.0	3.1 d	3.0 c	3.6 d	3.2

[z] Average disease index 10 days after emergence of plants. Rating scale: 0 = healthy; 1 = up to 25% of taproot blackened; 2 = 26–50% of taproot blackened; 3 = 51–75% of taproot blackened and few lateral roots; and 4 = 76–100% of taproot blackened and few or no lateral roots remaining. Disease indices in vertical column followed by different letters are significantly different at the $P = 0.01$ level, Duncan's multiple range test.

endoconidium per gram of soil caused a disease index of 0–1; five to ten endoconidia per gram of soil, 1 or 2; 25 endoconidia per gram of soil, 2 or 3; and 50 to 100 endoconidia per gram of soil caused a disease index up to 4.

Pathogenicity tests and host specificity. The relative susceptibility of the crop plants to disease caused by the nine isolates of *T. basicola* was markedly different (Table 2). Cotton developed the most and peanut the next greatest amount of disease at each of the inoculum concentrations used. Beans, peas, and soybeans showed an increase in severity of black root rot with increasing inoculum concentrations. Sunflower, a nonhost, apparently was not affected by *T. basicola* at any concentration of inoculum.

When the virulence of each of the nine isolates of *T. basicola* on the different crop plants was compared using disease indices, a low degree of host specificity among the isolates was apparent. The most marked differences were on roots of cotton and bean plants; isolates 414, 415, and 420 caused little or no disease on bean roots at any of the inoculum concentrations, whereas on cotton, disease severity greatly increased with increasing concentrations of inoculum. Isolates 374, 412, 418, and 425 caused increased disease on both bean and cotton roots with increasing concentrations of inoculum. However, disease indices on cotton were always greater than those on bean at each inoculum concentration. Isolate 416 was intermediate in virulence on cotton and pea roots and weakly virulent or nonpathogenic on the roots of the other crop plants. Among the isolates, 409 was the weakest pathogen, causing little or no disease on any of the crop plants.

These results were essentially verified in a second experiment (Table 3) but the most marked differences in interactions between host plants and isolates of *T. basicola* were obtained in the first experiment.

Although host specificity was found for some isolates of *T. basicola,* isolates from various hosts were not necessarily specific for these hosts. For example, isolate 416 from bean was pathogenic on pea and cotton but caused little or no disease on bean. Also, pea was susceptible only to isolate 416 from bean, and cotton was the most susceptible host to all the isolates.

DISCUSSION

Among 49 cotton field soils in the San Joaquin Valley assayed for *T. basicola* in 1964 by Mathre et al (7), 38 had no detectable propagules of *T. basicola,* seven had populations of 1–100, two had populations of 100–1,000, and two had populations greater than 1,000 propagules per gram of soil. In our study, nine fields were chosen for soil assays because of their histories of seedling disease problems. The concentrations of propagules of *T. basicola* in these soils varied less than those found by Mathre et al (7) because only one field soil had more than 50 propagules per gram of soil. Maduewesi et al (6) reported that natural infestations of *T. basicola* in Michigan soils ranged from 10 to 100 propagules per gram of soil.

Most of the field soils we tested had concentrations of propagules of *T. basicola* lower than 10 per gram of soil. Because black root rot may develop in soils with only 5–10 propagules per gram of soil, a selective medium and soil distribution method was needed that could detect low concentrations of propagules. An even distribution of soil on freshly cut surfaces of carrot root disks was the most sensitive of the various methods for detecting low concentrations of naturally occurring propagules of *T. basicola.* Carrot root disks also were used by Mathre et al (7) and by Tsao and Canetta (10) who also found that carrot medium was more sensitive and selective than agar media for detecting low populations of *T. basicola* in soils.

In field soils, the naturally occurring propagules of *T. basicola* are mainly chlamydospores (11) or "secondary chlamydospores" (8). However, the role of endoconidia as infectious propagules in natural soils is largely unknown. Two of the authors (J. E. DeVay and R. H. Garber) have found that the infestation of steamed soil with endoconidia is an effective procedure for the evaluation of seed treatments for controlling black root rot. Moreover, the infectiveness of the propagules remains high for several months. In the present study, the use of endoconidia was convenient and effective for quantifying the inoculum concentrations of *T. basicola* in steamed soil and comparing the virulence of the fungal isolates or the relative susceptibility of crop plants to black root rot.

The present study, with known concentrations of inoculum, confirmed the occurrence of a low level of host specificity for *T. basicola.* Except on bean and cotton, an inoculum concentration of 10^4 endoconidia per gram of soil usually allowed discrimination between isolates by the disease index on roots. However, bean and cotton plants best showed differences in the specificity of various isolates.

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Effects of Controlled Night Temperatures on Incidence of Verticillium Wilt in Field-Grown Cotton

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ABSTRACT

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Cotton plants grown in field growth chambers developed foliar symptoms of Verticillium wilt after they were subjected to controlled night temperatures ranging from 10 to 27 C. High incidence of disease occurred at 10 and 15 C and lower levels at 20 and 25 C, but symptoms developed in only one of several susceptible cultivars at 37 C. Significant disease symptoms developed on cotton plants grown under 10 and 15 C temperature regimes, even though the maximum day temperatures exceeded 30 C, the level above which the development of Verticillium wilt is inhibited under field conditions. Cultivars with different levels of resistance to Verticillium wilt were more easily separated into disease classes about the middle of September than either earlier or later in the season. Interaction was significant between cultivars and temperatures. Application of methyl parathion to cotton plants did not affect disease development, but the interaction of insecticide and temperature treatments was significant.

Additional key words: Verticillium dahliae, Gossypium hirsutum.

The Verticillium wilt disease of upland cotton (*Gossypium hirsutum* L.) is caused by the fungus *Verticillium dahliae* Kleb. The incidence, severity, and distribution of this disease are increased by temperatures of less than 30 C (5,7–9,12,16,21). Expression of genetic plant resistance also can be altered by varying temperatures (1–6,11,12). The development of the disease in cotton plants grown under controlled temperatures in greenhouses and in growth chambers changed rather significantly over a narrow temperature (22–29 C) range (6,10,15).

The most favorable temperature for the growth of the fungus in culture is between 20 and 27 C (1,3,15). The incidence and severity of Verticillium wilt in cotton in the greenhouse decrease when soil temperature exceeds 25 C (15). Cotton cultivars that varied from resistant to highly susceptible in field evaluations were resistant when grown at 32 C or higher and susceptible when grown at 22 C or lower, but they were distributed among classes ranging from susceptible to resistant when grown at 25 C (1–4,6,12,19). Foliar symptoms of Verticillium wilt in both susceptible and resistant cotton cultivars grown at a low temperature decreased when the plants were grown at 27–30 C in the greenhouse for several additional months (3).

In field evaluations, high daily air temperatures prevented the development of foliar symptoms of Verticillium wilt in resistant and susceptible Acala cottons, but during a cool growing season, the two cottons were easily separated into resistant classes by the difference in percentages of foliar disease symptoms (12). Cotton grown on raised seedbeds, in skip-row planting, and under alternate-furrow irrigation schemes where soil temperatures are higher tend to show reduced disease severity.

High temperature on the High Plains of Texas during July usually prevents the expression of external symptoms of Verticillium wilt, but as temperatures decline in August and September, symptoms generally develop rapidly and plants with various resistance levels may be distinguished in the field (18,20). Severe disease occurs sooner if the temperature declines earlier (18).

Our objective was to evaluate the effects of controlled night temperatures in conjunction with ambient day temperatures on the development of foliar symptoms of Verticillium wilt in cotton plants grown in field growth chambers.

MATERIALS AND METHODS

Controlled night temperature regimes were imposed on cotton growing in the field at the Texas A&M University Agricultural Research and Extension Center at Lubbock in 1966, 1971, and 1972. Night temperature control was maintained in field growth chambers equipped with air conditioners or gas-fired furnaces (13,14). The chambers, mounted on wheels and tracks, were rolled onto the plots at sunset and off at sunrise. No attempt was made to control air temperature during the day. Daily ambient maximum and minimum temperatures recorded during the study were averaged on a monthly basis.

In 1966, night temperature regimes of 10 and 27 C were imposed on cultivars Acala 1517 BR 2, Gregg 35, Lankart 57, Stoneville 7A, and CA 491 from first bloom until frost. Cultivars were replicated twice within each temperature treatment with 30 cotton plants per replication.

In 1971, night temperatures of 10, 15, 20, and 25 C were imposed on Gregg 35 and Deltapine 16, beginning at seedling emergence and continuing until frost. Within temperature regimes, each cultivar was replicated three times in a double row pattern with 20 plants per replication. Methyl parathion [*o*, *o*-dimethyl *o*-(*p*- nitrophenyl) phosphorothioate] at 0.19 kg/ha was sprayed on one-half of the