

## Influence of Soil Temperature, Water, and Texture on *Thielaviopsis basicola* and Black Root Rot of Cotton

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### ABSTRACT

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The influence of soil temperature, water, and texture on natural populations of *Thielaviopsis basicola* was examined. The decrease in population of *T. basicola* in soil averaged 2% per week over all treatments, a rate of reduction much lower than in soil artificially infested with chlamydo-spores produced in vitro. Survival was significantly lower at soil temperatures of 24 or 28 C than at 16 C. The presence of cotton plants substantially increased soil populations of *T. basicola*. Increases in soil

populations of the pathogen were correlated with disease incidence, disease severity, and the number of infection sites. Disease incidence was lower at 28 C or at a matric potential of -30 J/kg than at the other temperatures or matric potentials examined. Disease severity was lower at 28 C than at 24 or 20 C. Soil texture did not influence *T. basicola* populations or black root rot.

*Additional keywords:* *Chalara elegans*, water potential.

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*Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick) is an important pathogen of numerous crops (8). Black root rot of cotton (*Gossypium hirsutum* L.) caused by *T. basicola* was first described in the field in Arizona in 1942 (13). The disease may be an important component of the seedling disease complex of cotton in the Mississippi Delta (12,21; Rothrock, unpublished).

Soil environmental conditions are important in the development of black root rot. Cool soil temperatures, less than approximately 26 C, increase disease severity for cotton (5,16), tobacco (*Nicotiana tabacum*) (11), and poinsettia (*Euphorbia pulcherrima*) (4). High soil water increased disease for tobacco (11) and poinsettia (1). Black root rot is more severe for cotton on wet poorly drained

soils than well-drained soils (13). Finer-textured soils also were reported to increase black root rot severity on soybean (*Glycine max*) (15), tobacco (9), and peanut (*Arachis hypogaea*) (10) compared with disease severity on coarser-textured soils. However, a relationship between differences in soil texture and disease severity for tobacco has not been apparent in field surveys (18) or in controlled studies (11). High soil pH (2,7) and soil chemical factors related to soil pH, including cation exchange capacity, base saturation, and calcium or aluminum ion concentration (17,18), also increase disease severity.

*T. basicola* survives in soil primarily as chlamydo-spores (25). In artificially infested soils, survival of *T. basicola* was lower at water-holding capacities of 45% or greater than those of 15% or less (6,20). Soil temperatures of 26 or 34 C also decreased survival compared to 10 or 18 C (20). Loam and loamy sand soil were reported to have lower rhizosphere populations of the

pathogen on bean (*Phaseolus vulgaris*) than sandy loam soil (19).

The selective medium TB-CEN has allowed the quantitative isolation of low populations of *T. basicola* from soil (22). This medium or variations of this medium have been used in previous population studies of the pathogen (18,23). This study examined the importance of soil temperature, water, and texture on the survival of natural populations of *T. basicola* and the development of black root rot disease on cotton.

## MATERIALS AND METHODS

Soil was obtained from a site at the Delta Branch Station, Clarkedale, Arkansas, that had been cropped to cotton continuously for at least 18 yr. Black root rot incidence on cotton seedlings of Deltapine 41 at this site was 32% in the 1990 growing season. The soil is a silty loam (18% sand, 76% silt, 6% clay; pH 5.6) in the Dubbs-Dundee complex.

Differences in soil texture treatments were established by adding either fumigated (98% methyl bromide + 2% chloropicrin) sand or soil from the Clarkedale site to the nonfumigated soil in a 1:1 ratio (w/w) of fumigated soil to nonfumigated soil, a 0.5:0.5:1 ratio of sand to fumigated soil to nonfumigated soil, or a 1:1 ratio of sand to nonfumigated soil. The sand was composed of primarily coarse (43%) and medium (48%) sands. The resulting mixtures had sand contents of 18, 34, or 59% while maintaining equivalent soil populations of *T. basicola* per gram of soil as determined by the selective medium of Specht and Griffin (22).

Soil water drying curves were determined for each soil texture with a pressure-plate apparatus. The matric component of the soil water potential (soil matric potential), expressed as joules per kilogram (1 J/kg = 1 kPa), was established by adding water to bring the soil to the corresponding weight. Matric potentials were maintained by weighing pots daily and adding water to the soil surface to replace the water lost. Since the -50 J/kg matric-potential treatment was below field capacity and could not be maintained, the -50 J/kg treatment was approximated by injecting water horizontally at multiple points with a syringe to better represent this matric potential than by adding water to the soil surface. Water-holding capacity also was determined for the soil treatments, and percent water-holding capacity was calculated for each soil texture-matric potential combination for comparison with previous reports. To examine the influence of nutrient differences in soil amended with fumigated soil or sand on pathogen survival, sand-amended pots watered with only soil extract were compared to sand or fumigated soil-amended pots whose matric potential was adjusted with water. Soil extract for the treatments was obtained by the addition of fumigated soil to an equal weight of water, agitation, and filtration through Whatman filter paper. The experiment examining the survival of *T. basicola* was conducted in 6.5-cm-diameter styrofoam cups with lids perforated to reduce evaporation but allow air exchange. Pots 10 cm in diameter, lined with plastic bags, were used for the plant study. The tops of the plastic bags were folded over to reduce evaporation prior to plant emergence. Controlled environmental chambers with a 12-h photoperiod at a light intensity of  $178 \mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  were maintained to within 0.5 C of the desired temperature. A soil oxygen diffusion rate meter (Jensen Instruments, Tacoma, WA) measured oxygen diffusion rate (ODR) for all pots in the 24 C treatment for the different soil water and texture treatments.

Soil populations of *T. basicola* were quantified with the selective medium TB-CEN (22). Penicillin G (60 mg/L) was added to the medium to improve inhibition of bacteria. No differences in soil population counts were found between glass and disposable dishes (data not shown); therefore, polystyrene petri dishes were used instead of the recommended glass petri dishes (22). Fifteen grams of soil and enough 0.1% agar to bring the volume to 100 ml was placed in Erlenmeyer flasks and shaken on a wrist-action shaker for 20 min. One milliliter of soil suspension was placed in each of six dishes. Cooled molten medium was added, and the dishes were swirled to disperse the soil suspension. Soils adjusted to different textures were assayed for *T. basicola* popu-

lations at the initiation of the experiment. For the pathogen survival study each pot was assayed at 2, 4, and 8 wk. For the experiment that examined the influence of cotton plants on soil pathogen populations, nine metalaxyl-treated seeds (0.31 g a.i./kg of seed) of cultivar Stoneville 506 were planted per pot, and 3 g of soil were assayed 3 wk after planting. Population counts were made after 14 days of incubation at ambient temperature, and counts were expressed as propagules per gram of oven-dried soil. Disease severity was assessed on the cotton plants 3 wk after planting on a 1-5 weighted scale, with 1 = 0%, 2 = 1-10%, 3 = 11-25%, 4 = 26-50%, and 5 = 51-100% root discoloration similar to the weighted scale of Sumner and Patak (24). Fresh weight of plants was determined after the plants were washed in running water for 15 min. Seedlings were then surface-disinfested in 0.5% NaOCl for 1.5 min, and the roots were placed on TB-CEN medium.

Experiments were randomized complete block designs with three replications and a factorial treatment structure. The factors were soil temperature, matric potential, and soil texture. Statistical analyses were by the general linear model procedure using SAS (SAS Institute Inc., Cary, NC). Since most of the quantitative factors had only three levels, trend contrasts were used rather than regression-type modeling. Each significant main effect or interaction was decomposed into linear and nonlinear (quadratic) single degree of freedom trend contrasts. Following a significant

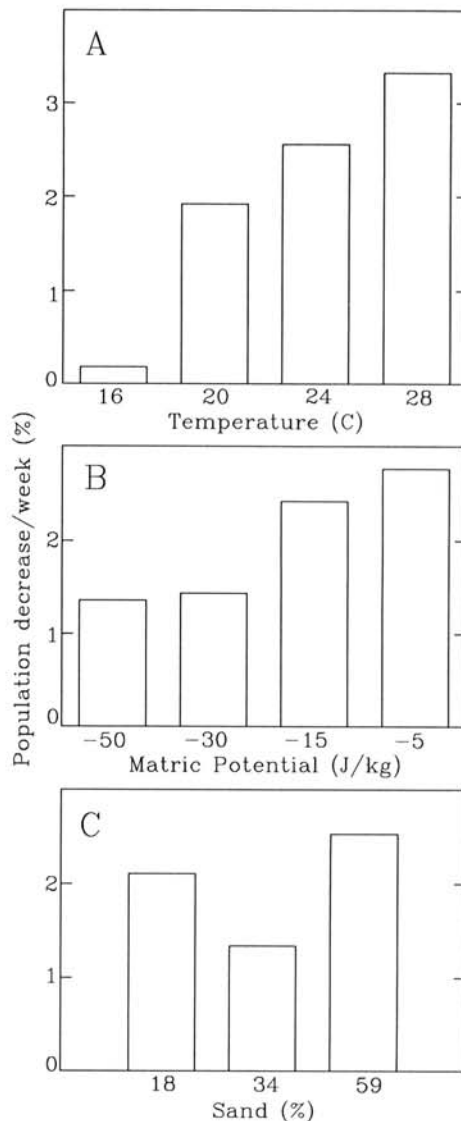


Fig. 1. Survival of *Thielaviopsis basicola* in soil as influenced by A, soil temperature (LSD = 1.8,  $P = 0.01$ ), B, matric potential (not significant,  $P = 0.01$ ), and C, soil texture (not significant,  $P = 0.01$ ).

TABLE 1. General linear model sum of squares for main effects and interactions for *Thielaviopsis basicola*, black root rot, and cotton growth

Source	df	Infections	Disease incidence	Disease severity	Soil population	Seedling weight
Temperature (Tm)	2	2,993.2*** <sup>y</sup>	8,365.7***	42.570***	11.329***	6.416***
Linear (lin)	1	2,831.1***	6,783.6***	38.873***	10.963***	6.345***
Nonlinear (nonlin)	1	162.1	1,582.1	3.697***	0.366**	0.072
Matric potential (Mp)	2	2,843.6***	10,813.6***	0.193	2.425***	0.903**
Lin	1	2,400.5***	10,003.6***	... <sup>z</sup>	2.397***	0.799**
Nonlin	1	476.1	901.9	...	0.036	0.113
Texture (Tx)	2	239.2	879.7	1.705	0.036	3.137***
Lin	1	...	...	...	...	3.081***
Nonlin	1	...	...	...	...	0.057
Tm × Mp	4	1,715.8*	2,368.3	2.051	0.829***	0.216
Lin × lin	1	1.4	...	...	0.295**	...
Lin × nonlin	1	1,114.9**	...	...	0.297**	...
Nonlin × lin	1	233.1	...	...	0.212*	...
Nonlin × nonlin	1	348.2	...	...	0.021	...
Tm × Tx	4	439.7	1,972.1	1.401	0.065	0.118
Mp × Tx	4	235.6	179.5	1.572	0.305*	0.285
Lin × lin	1	...	...	...	0.250*	...
Lin × nonlin	1	...	...	...	0.017	...
Nonlin × lin	1	...	...	...	0.034	...
Nonlin × nonlin	1	...	...	...	0.005	...
Tm × Mp × Tx	8	1,069.9	6,557.2	1.354	0.338	0.534

<sup>y</sup> Main effects or interactions are significant at  $P = 0.01$  (\*), 0.001 (\*\*), or 0.0001 (\*\*\*).

<sup>z</sup> These main effects or interactions were not decomposed, because the overall test was not significant.

TABLE 2. Interactive effect of matric potential and soil temperature on infections by *Thielaviopsis basicola*<sup>y</sup>

Soil temperature (C)	Matric potential (J/kg)		
	-10	-20	-30
20	24.9 c <sup>z</sup>	37.8 a	14.8 ef
24	31.8 b	20.9 d	12.2 fg
28	17.2 de	10.4 g	6.3 h

<sup>y</sup> Infections per pot.

<sup>z</sup> Soil temperature by matric potential means not followed by the same letter are significantly different at  $P = 0.01$ , LSD.

*F* test, a means separation test, LSD, was done. *T. basicola* populations were transformed to log<sub>10</sub> for analysis. Interaction means were examined where appropriate. Survival of the pathogen in soil was analyzed as slopes from regression analyses based upon populations at 2, 4, and 8 wk. All experiments were repeated at least once, with consistent results for the main effects for infection, disease, plant growth, and pathogen population in fallow soil or soils planted to cotton, except for the influence of soil texture on plant growth. Significant interactions between matric potential and temperature or texture were not found in all experiments.

## RESULTS

**Influence of soil factors on soil populations of *Thielaviopsis basicola*.** Initial soil populations for the soil mixtures were 43, 44, or 44 propagules per gram of soil for soils with 18, 34, or 59% sand, respectively. Soil populations increased slightly after 2 wk compared to the soil population prior to the initiation of the experiment. Soil populations of *T. basicola* decreased at a rate of 2% per week over all treatments after the 2-wk sample (Fig. 1). Soil temperatures of 24 and 28 C had significantly lower survival of the pathogen than 16 C (Fig. 1A). No significant reduction in population of the pathogen occurred at 16 C between 2 and 8 wk of incubation (Fig 1A). There was a significant linear trend for soil temperature ( $P = 0.001$ ). No differences were found for *T. basicola* survival among the soil water or texture treatments (Fig 1B and C), and no interactions were found among the main effects. Nutrient differences between amendment of nonfumigated soil with fumigated soil or fumigated sand did not influence the

TABLE 3. Influence of soil temperature, texture, and matric potential on black root rot and cotton growth

Main effect	Disease incidence (%)	Disease severity <sup>y</sup>	Seedling weight (g)
Temperature (C)			
20	81.7 a <sup>z</sup>	4.5 a	0.72 c
24	80.1 a	4.2 b	1.00 b
28	59.3 b	2.9 c	1.40 a
Matric potential (J/kg)			
-10	85.1 a	3.9 a	1.14 a
-20	78.5 a	3.8 a	1.09 a
-30	57.6 b	3.8 a	0.89 b
Texture (% sand)			
18	77.4 a	3.7 a	1.26 a
34	74.3 a	3.8 a	1.08 b
59	69.4 a	4.1 a	0.78 c

<sup>y</sup> Disease severity was assessed on the cotton plants 3 wk after planting on a 1–5 scale with 1 = 0%, 2 = 1–10%, 3 = 11–25%, 4 = 26–50%, and 5 = 51–100% root discoloration.

<sup>z</sup> Means within a column and main effect not followed by the same letter are significantly different at  $P = 0.01$ , LSD.

survival of *T. basicola*. The 59% sand treatment watered with soil extract had a mean population of 58 or 54 propagules per gram of soil compared to 55 or 55 propagules per gram of soil for the same treatment without soil extract at a matric potential of -15 J/kg at 24 or 20 C, respectively, over all sampling times.

**Influence of soil factors on black root rot and populations of *Thielaviopsis basicola* in soils planted to cotton.** There was a significant two-way interaction between matric potential and temperature for the number of infections per pot (Table 1). Contrast trends indicated a significant linear response for temperature and a significant curvature for matric potential in the temperature–matric potential interaction. More infections of cotton seedlings occurred at 24 and 28 C at higher matric potentials than lower matric potentials (Table 2). The greatest number of infections was found at 20 C at -20 J/kg. The number of infections was lowest at 28 C for all matric potentials. No differences in number of plants per pot were found between treatments (data not shown). Disease incidence was significantly lower at 28 C than 24 or 20 C or at a matric potential of -30 J/kg than that of -20 or -10 J/kg (Table 3). Trend contrasts indicated a linear

relationship for both matric potential and temperature for disease incidence (Table 1). Disease severity, measured as the amount of root discoloration, was lower at 28 C than at either 24 or 20 C (Table 3). There was some curvature for disease severity as the temperature changed (Table 1). Growth of cotton plants was greatest at 28 C or 18% sand, compared to lower temperatures or higher sand contents (Table 3). Matric potentials of -10 J/kg or -20 J/kg resulted in higher plant weights than -30 J/kg. For plant weight there were linear relationships for temperature, texture, and matric potential (Table 1).

Populations of *T. basicola* increased substantially in soils planted to cotton. Initial soil populations were 85 propagules per gram of soil. There was a significant two-way interaction between matric potential and temperature or matric potential and texture (Table 1). Contrast trends indicated a significant curvature for temperature and matric potential in the temperature-matric potential interaction and a significant linear response for texture and matric potential in the significant interaction between these main effects. At 20 and 24 C, populations of *T. basicola* in soils containing cotton plants were significantly lower at -30 J/kg than at -10 J/kg (Table 4). For each matric potential, soil populations following cotton were lower at 28 C than at 20 C. Soil texture did not affect soil populations during the period of incubation (Table 4). The 18% sand treatment at a -10 J/kg matric potential had an ODR reading of 30  $\mu\text{g}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The range of ODR readings for the other treatments at 24 C was 77-126  $\mu\text{g}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

Soil populations of *T. basicola* in soil planted to cotton were positively correlated with number of infections ( $r = 0.69$ ), disease incidence ( $r = 0.55$ ), and disease severity ( $r = 0.75$ ) ( $P = 0.0001$ ). Plant weight was negatively correlated with disease severity ( $r = -0.72$ ) and pathogen soil populations ( $r = -0.46$ ) ( $P = 0.0001$ ).

## DISCUSSION

Survival of *T. basicola* in naturally infested soils was prolonged in the absence of a host by low soil temperature, with no decrease in the population at 16 C during the period of the study. A similar relationship between soil temperature and survival occurred in artificially infested soils (20). However, in artificially infested soils approximately 40% of the propagules survived after 4 wk at 26 C at a water-holding capacity of 45-60%. This was a much greater loss in viability than that found with naturally infested soils in this study, in which 89% of the initial population survived after 4 wk at 24 C over treatments having a similar range of water-holding capacities.

In the present study, no significant differences were found in pathogen survival at the matric potentials used in the absence of cotton plants. Survival rates in this study in the naturally infested soil over a 4-wk period ranged from 89 to 95% for the

TABLE 4. Interactive effect of matric potential and soil temperature or matric potential and soil texture on populations of *Thielaviopsis basicola* in soils containing cotton plants<sup>x</sup>

Soil factor	Matric potential (J/kg)		
	-10	-20	-30
Temperature (C)			
20	1,515 a <sup>y</sup>	1,408 ab	461 bc
24	996 ab	489 abc	245 cd
28	160 cd	109 d	112 d
Texture (% sand)			
18	538 ab <sup>z</sup>	400 ab	271 ab
34	554 ab	386 ab	245 b
59	809 a	487 ab	189 b

<sup>x</sup> Propagules per gram of oven-dried soil.

<sup>y</sup> Soil temperature by matric potential means not followed by the same letter are significantly different at  $P = 0.01$ , LSD.

<sup>z</sup> Soil texture by matric potential means not followed by the same letter are significantly different at  $P = 0.01$ , LSD.

matric potentials used, which corresponded to survival rates of 96, 89, or 90% for treatments ranging from 28 to 38%, 48 to 56%, or 71 to 74% water-holding capacity, respectively. With artificially produced inoculum only 25% survival was found at 22 C (6), and 5% survival was found at 20 C (20) after 4 wk at 60% water-holding capacity. At 45 and 30% water-holding capacity only 15 and 65% of the propagules survived, respectively, in artificially infested soils at 20 C (20). Very little decline in populations was found at low water-holding capacities (6,20).

Soil temperature and soil water were important factors in the root infection and development of black root rot and reproduction of *T. basicola* in the presence of cotton. This supports previous research and observations that low soil temperatures (6,16) and high matric potentials (13) favor black root rot on cotton. The presence of a host appears to be important in the growth and reproduction of *T. basicola*. Bateman (3) also found that soil populations increased dramatically in the rhizosphere of bean as disease developed.

Soil texture did not significantly influence pathogen survival or disease development in these studies. The relationship of black root rot with soil texture may be connected to other factors associated with soil texture, such as soil compaction, water infiltration rates, and soil aeration. Soil matric potential was important in disease incidence and reproduction of the pathogen as discussed earlier. Bulk densities in this experiment averaged 1.2, 1.4, or 1.2  $\text{g}/\text{cm}^3$  for the soil textures containing 18, 34, or 59% sand, respectively. The only ODR that was below the critical rate for cotton, 33  $\mu\text{g}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (14), was recorded with the 18% sand treatment at a -10 J/kg matric potential, which had an ODR reading of 30  $\mu\text{g}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

This study indicates that natural propagules, presumably chlamydo spores (25), are more tolerant of deleterious environmental conditions, including high soil temperature and water, than chlamydo spores produced in vitro and used to infest soil. Clough and Patrick (6) observed differences in envelope thickness of the cell wall surrounding chains of chlamydo spores produced in vitro and in vivo, which may be one factor responsible for the superior survival of naturally produced chlamydo spores. Both high temperatures and high soil matric potentials favor microbial activity, which has been associated with decreased survival of *T. basicola* in soil (6). This research emphasizes the importance of evaluating differences between artificially and naturally produced inoculum when conducting ecological studies. The data from this study indicate that only a small reduction in populations of the pathogen is expected during the winter months in the Mississippi Delta.

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