Thielaviopsis basicola: A Component of the Pea Root Rot Complex in New York State

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ABSTRACT

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Thielaviopsis basicola, previously unreported as a pathogen of peas in New York, was present in tissue of pea plants from 12 of 14 fields sampled near Mount Morris and Auburn in 1976 and 1977. *Fusarium solani, Aphanomyces euteiches, Pythium* spp., and *Rhizoctonia solani* also were found in diseased pea tissue in 14, 7, 14, and 2 fields, respectively. Average soil populations of *T. basicola* in the Mount Morris area ranged from 1,800 to 4,000 propagules per gram of oven-dry soil. The number of plants infected and root-rot severity increased consistently in five locations evaluated three times in a 4-wk period during the growing season. All isolates of *T. basicola* tested were pathogenic, but differed in virulence. In soils from Mount Morris that contained *T. basicola,* addition of benomyl or a benomyl-Dexon combination effectively controlled root rot, but Dexon alone was ineffective. In contrast, in soil from Auburn that lacked *T. basicola*, Dexon alone or a Dexon-benomyl combination controlled root rot, but benomyl alone was ineffective.The addition of *T. basicola* to Dexon-treated Mount Morris soil resulted in pea plants of less weight than the control. The addition of this pathogen to Dexon-treated or nontreated Auburn soil resulted in severe black root rot. Peas planted in a sandy loam infested with *T. basicola* and *F. solani* f. sp. *pisi* developed a black cortical rot similar to that found in plants grown in the Mount Morris soil. It was concluded that in some New York fields, *T. basicola* has become an important component of the fungal complex that causes pea root rot.

Additional key words: Pisum sativum.

A comprehensive survey (19) conducted nearly 40 yr ago established that *Fusarium solani* v. *martii* f. 2 (= *F. solani* [Mart.] Sacc. f. sp. *pisi* [Jones] Snyd. & Hans.), *Aphanomyces euteiches* Drechs., and *Pythium ultimum* Trow were the most widespread and important pathogens in the complex of fungi that cause root rot of pea (*Pisum sativum* L.) in New York state. A preliminary survey in 1975 indicated that *Thielaviopsis basicola* (Berk. & Br.) Ferr., which previously had not been found to be a pea pathogen, was prevalent in diseased pea tissue and also in soils from pea fields. Occasionally this organism has been reported to be a pathogen of pea roots in other states (1,5–8).

The objectives of the present investigation were: to survey the major pea growing areas of central and western New York to determine the extent to which *T. basicola* is presently involved in the pea root-rot complex; to assess the pathogenicity of various isolates of *T. basicola*; and to determine the significance of *T. basicola* in the fungal complex that causes root rot of pea in New York.

MATERIALS AND METHODS

All growth chamber experiments were conducted with 14-hr light periods at 21,600 lux (2,970 watts cool-white fluorescent, 360 watts incandescent) at 20 C and 10-hr dark periods at 16 C. The relative humidity was approximately 70%. Plants of the cultivar Alaska were used in all experiments. Experiments were replicated twice and the data were analyzed by the Waller-Duncan Bayesian K-ratio (LSD) rule (24). Ratings for disease severity on epicotyls and roots were based on a scale of 0 to 6 in which 0 = no symptoms and 6 = dead plant.

Survey for soilborne pea pathogens. In 1976 nine pea fields in central and western New York were sampled at biweekly intervals, beginning after planting and continuing until harvest. Five additional fields were sampled in 1977. Composite soil samples were collected from each field from 10 randomly selected subsamples. Twenty plants per field also were collected, of which 15 were examined microscopically for various root pathogens, and the remaining five plants were used for isolation of pathogens. Soil and plant samples were stored in plastic bags for a maximum of 1 wk at 5 C.

The selective medium (VDYA-PCNB) of Papavizas (13,16) was used for the isolation of *T. basicola.* This medium without nystatin was most reliable for the isolation of *Fusarium* spp. (14). The selective medium ($P_{10}VP$) of Tsao and Ocana (23) was used for the isolation of *Pythium* spp. Attempts to isolate *A. euteiches* were made with the corn-kernel method as reported by Papavizas and Ayers (15). No attempt was made to isolate *Rhizoctonia solani* Kuehn; however, its presence was determined by microscopic observations of typical hyphae in reddish-brown stem lesions.

Dilutions of 10^{-2}, 10^{-3}, and 10^{-4} g of soil per milliliter of water were prepared and 1 ml of each dilution was added per petri dish of medium. All dilutions were added to the surface of hard agar. Root and epicotyl samples (0.5 g) were surface-sterilized in a 1% NaOCl (v/v) solution for 1 min, followed by three successive rinses in distilled water. Then the samples were homogenized in a Waring Blendor for 1 min and diluted to give 10^{-3}, 10^{-4}, and 10^{-5} g fresh weight per milliliter. All plant and soil dilution series were replicated three times.

All dilution plates were incubated on laboratory benches at 20–23 C for 7–10 days, with the exception of plates of $P_{10}VP$ which required only 24–48 hr of incubation. Plate counts were made and the pathogens were identified at the end of the allotted time periods. Cultures of *Pythium* spp. were stored at 5 C on slants of Difco cornmeal agar (CMA) and cultures of *T. basicola* and *Fusarium* spp. were maintained similarly on Difco potato-dextrose agar (PDA).

Pathogenicity of *Thielaviopsis basicola*. For pathogenicity studies, 14 isolates of *T. basicola* from nine fields were randomly selected from soil and plant dilution plates. Conidial suspensions

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from 2-wk-old PDA cultures were adjusted to approximately the same spore concentrations with a haemocytometer. Each isolate was atomized on and mixed into a moist greenhouse soil mix (1:1 v/v, pasteurized sand - pasteurized soil) at a rate of 2,500 conidia/g of oven-dry soil. In preliminary experiments, concentrations of >1,000 conidia per gram of soil consistently induced a severe black cortical rot. The test tube method of Maduewesi and Lockwood (10) was used, except we used 2.5 × 10-cm polypropylene centrifuge tubes. Each centrifuge tube was filled with soil infested with an isolate of *T. basicola* and planted with a single nontreated pea seed. Each treatment was replicated 10 times. The centrifuge tubes were randomized in plastic dishes (13.5 × 13.5 cm), with 16 tubes per dish. The soil was kept moist by adding sufficient water to each tube daily to bring the weight of the dish to its weight at the time of planting. Twenty-four days after emergence, the plants were removed from the tubes, washed thoroughly, and rated for root-rot severity. Plant dry weight also was obtained after the plants had been dried for 48 hr at 100 C. Re-isolation of *T. basicola* from soil and infected plant parts was completed by using the selective medium of Papavizas (13,16).

TABLE 1. Relationship between the soil population of *Thielaviopsis basicola,* number of plants infected, and root-rot severity in five fields sampled three times in 1976

Field sampled	Date sampled	Propagules/g oven-dry soil (avg no.)	Plants infected[y] (no.)	Root-rot severity[z]
A	5 June	1,000	4	1.0
	19 June	1,800	9	3.6
	5 July	2,300	10	4.4
B	5 June	1,000	5	0.8
	19 June	2,100	7	3.4
	5 July	2,000	8	4.0
C	5 June	1,000	8	0.8
	19 June	3,250	11	3.2
	5 July	3,500	10	4.2
D	5 June	1,000	8	1.8
	19 June	3,400	8	3.0
	5 July	4,000	10	3.6
E	5 June	1,000	4	2.0
	19 June	2,300	8	3.2
	5 July	2,700	8	3.8

[y]Based on 15 plants sampled.
[z]Ratings were based on a scale of 0 to 6, with 0 = no symptoms and 6 = dead plant.

Significance of *Thielaviopsis basicola* in the root-rot complex. To further determine the significance of *T. basicola* in the pea root-rot complex, a composite soil sample from three fields in the Mount Morris area and a sample from one field from the Auburn area were collected and treated with the following two fungicides: Dexon (70W) [sodium p-(dimethylamino) benzenediazosulfanate] at a rate of 14 mg active ingredient/kg soil, and benomyl (50W) (methyl 1-butylcarbamoyl-benzimidazolecarbamate) at a rate of 120 mg active ingredient/kg soil, or a combination of both. Dexon is active against pythiacious fungi (4) and benomyl is active against *T. basicola* (18) and *F. solani* (2). These fungicides and conidial suspensions of *T. basicola* and *F. solani* f. sp. *pisi*, from 2-wk-old cultures (at a rate of 3,000 conidia per gram of oven-dry soil) were atomized on and mixed into the moist field soils. Additionally, the two pathogens were added separately to a sandy loam, at a rate of 3,000 conidia per gram of oven-dry soil, and together at 1,500 conidia of each fungus per gram of soil. Pea seeds treated with captan were planted in 10-cm-diameter plastic pots filled with the treated soils. Four seeds were planted in each pot and each

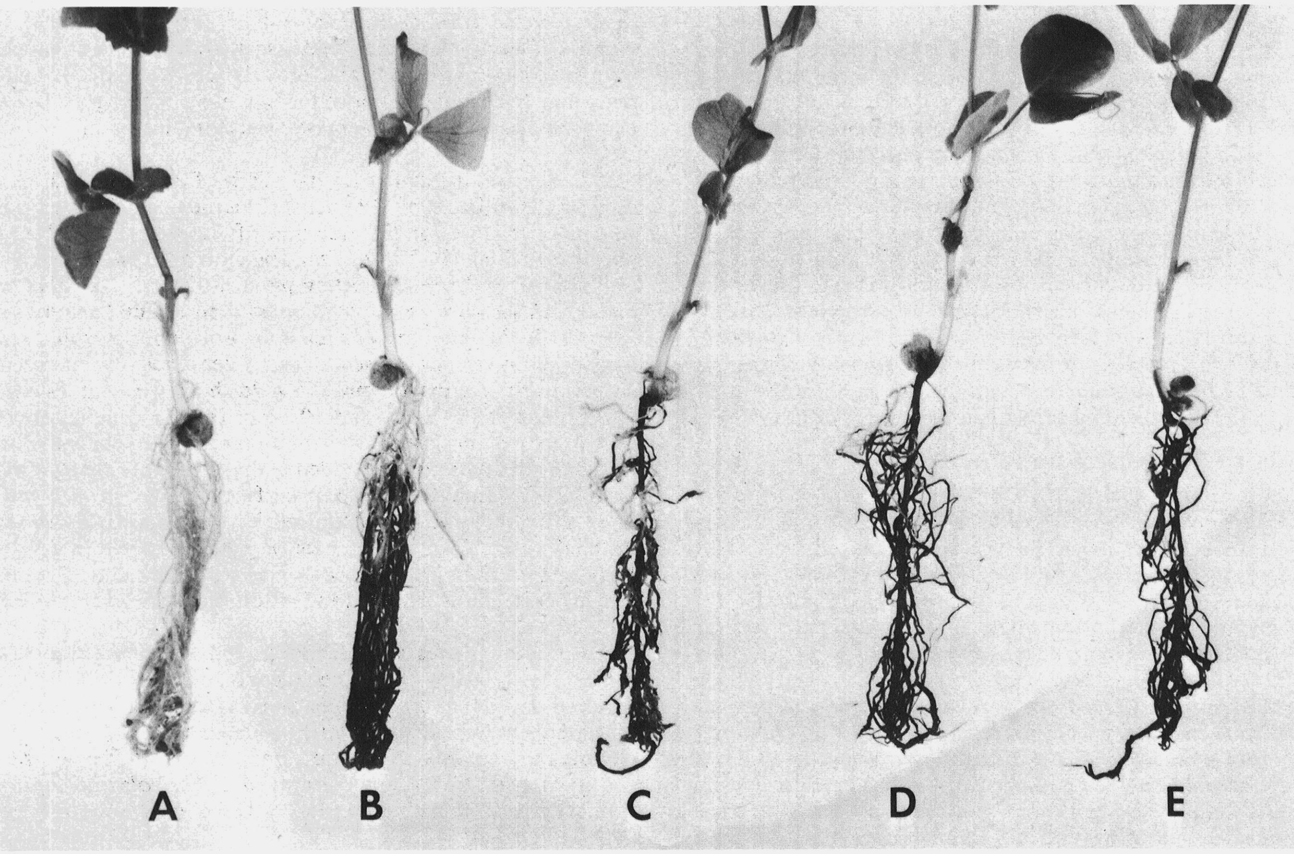

Fig. 1. Root-rot symptoms incited by isolates of *Thielaviopsis basicola* on 28-day-old pea plants in a pasteurized greenhouse soil mix: **A,** Control; and **B-E,** pea roots infected with four different isolates of *T. basicola.*

treatment was replicated four times. All pots were watered daily to keep the soil moist. Twenty-four days after emergence, the plants were removed from the pots, washed thoroughly, and rated for root-rot severity. Fresh weight of roots was determined after the surface of the root segments had been blotted dry. Reisolation of the root pathogens and microscopic examination of the infected root tissue were done as described above.

RESULTS

Survey for soilborne pea pathogens. *Thielaviopsis basicola, F. solani,* and *Pythium* spp. all were isolated readily from surface-sterilized root tissues from 12, 14, and 14 fields, respectively. *Aphanomyces euteiches* only rarely was isolated by the corn-kernel method described by Papavizas and Ayers (15), but it was detected microscopically as thick walled oospores embedded in root tissue of plants from 7 of the 14 fields. Hyphae of *Rhizoctonia solani* were detected in root tissue of plants from two fields. No attempt was made to distinguish the different *Pythium* spp. on P_{10}VP. In 1976, five fields were chosen to be used in a study of the seasonal progress of root rot incited by *T. basicola.* Average soil populations of *T. basicola* ranged from 1,800-4,000 propagules per gram when symptoms of black root rot were prevalent. At all five sites, number of fungal propagules, number of infected plants, and root-rot severity ratings increased in three readings taken during a 4-wk interval (Table 1).

Pathogenicity of *Thielaviopsis basicola*. Of 14 isolates of *T. basicola* all were pathogenic on peas and induced average root-disease, indices ranging from 3.2 to 5.0. The average disease rating of epicotyls was 0.3. Dry weight per plant ranged from 0.34 to 0.50 g with a reduction in plant weight ranging from 12–40% of the control.

Peas with black root rot were characterized by a black necrosis of the cortex of the tap and lateral roots. Lesions also developed in the tap root below the cotyledons (Fig. 1). Chlamydospores of *T. basicola* were abundant in the decayed tissue of diseased plants.

Significance of *Thielaviopsis basicola* in the root-rot complex. In the Mount Morris soil, benomyl or a Dexon-benomyl combination significantly increased root fresh weight and decreased eipcotyl and root-rot-severity ratings in comparison with nontreated soil. There were no significant differences in root weight and disease severity ratings of plants grown in the Dexon-treated or nontreated soil. Additionally, when *T. basicola* was added to the Dexon-treated soil, the root weight was less than that in the nontreated and the Dexon-treated soil. Root weight was not changed when *F. solani* f. sp. *pisi* was added to the Dexon-treated soil (Table 2). *Thielaviopsis basicola, F. solani,* and *Pythium* spp. were readily isolated from infected root tissue from plants grown in nontreated field soil. Plants grown in this soil developed typical black root-rot symptoms. *Aphanomyces euteiches* was not observed upon microscopic examination of plants grown in the nontreated or benomyl-treated soil from the Mount Morris fields.

In the Auburn soil, a Dexon-benomyl combination or Dexon alone significantly increased root fresh weight and decreased disease severity ratings over the field control. A benomyl application alone did not increase the root weight over the field control. The addition of *T. basicola* to the field control or Dexon treated soil resulted in less root weight than similar soils without addition of the pathogen (Table 2). Microscopic observations and isolations from plants grown in the nontreated soil revealed that *A. euteiches, Pythium* spp., and *F. solani* were associated with common root-rot symptoms. *Thielaviopsis basicola* was not detected in infected root tissue or soil in the Auburn field control.

The addition of *T. basicola* alone or in combination with *F. solani* f. sp. *pisi* to a sandy loam significantly decreased plant dry weight from the control and the *F. solani* treatment (Table 3). Additionally, the combination of these two pathogens produced a black cortical rot similar to that found in pea plants grown in the Mount Morris field soil.

DISCUSSION

Reinking (19) considered *A. euteiches, F. solani* f. sp. *pisi*, and *P. ultimum* to be the principal pathogens responsible for root rot of peas in New York State. However, frequent recovery of *T. basicola* from diseased root tissues of peas in 12 of 14 fields surveyed in 1976 and 1977, and the results of pathogenicity tests suggest that this fungus is of considerable importance as a component of the complex of fungi associated with pea root rot in New York. Additionally, black root rot was reduced by benomyl or a Dexon-benomyl combination but not by Dexon, indicating that this symptom is incited by benomyl-sensitive organisms such as *T. basicola.* In Mount Morris soils, *F. solani* f. sp. *pisi* (2) and *T. basicola* (18) were the major benomyl-sensitive pathogens. Addition of *T. basicola* to a sandy loam with a low root-rot potential (20) resulted in plants of less weight than the control and with black roots, whereas the addition of *F. solani* f. sp. *pisi* resulted in epicotyl darkening but did not affect plant weight. The addition of both organisms resulted in characteristic black root-rot symptoms similar to those induced by *T. basicola* alone. Similarly, the addition of *T. basicola* to Dexon-treated Mount Morris soil or Dexon-treated or nontreated Auburn soil resulted in black root rot and plants of less weight than the control. All the evidence suggests that *T. basicola* is of major importance in New York pea fields and that it incites black root rot.

Aphanomyces euteiches was not detected in areas where it previously had been common (19). This organism may be less prevalent now, particularly in the Mount Morris area. However, it is still important in the Auburn region where in combination with other fungi it produces common root rot (19). This conclusion was borne out by fungicide experiments in which Dexon or a Dexon-benomyl combination controlled root rot in this soil. Of the pathogens found in the Auburn soil, Dexon controls only pythiacious fungi (4). The root-rot symptoms were characteristic of

TABLE 2. Effect of soil application of Dexon and benomyl on severity of pea root rot in a growth chamber study of soils from two pea fields with histories of root rot

	Disease severity rating[x]				Root fresh weight g/plant	
	Epicotyl		Root			
Soil treatment[w]	Mt.Morris[y]	Auburn[y]	Mt.Morris	Auburn	Mt.Morris	Auburn
None (field control)	2.3 A[z]	4.9 A	4.8 A	4.4 A	0.60 B	0.77 B
Benomyl	0.6 B	3.8 B	1.1 B	4.4 A	0.90 A	0.58 BC
Dexon	2.5 A	1.5 D	4.5 A	1.9 B	0.68 B	1.20 A
Dexon + benomyl	0.6 B	1.4 D	0.3 C	0.9 C	0.95 A	1.20 A
Dexon + *Thielaviopsis basicola*	2.5 A	2.6 C	4.9 A	4.2 A	0.45 C	0.76 BC
Dexon + *Fusarium solani*	2.7 A	...	4.8 A	...	0.61 B	...
T. basicola	...	4.2 A	...	4.7 A	...	0.50 C

[w] *Thielaviopsis basicola* and *F. solani* f. sp. *pisi* were incorporated into the soil at a rate of 3,000 propagules/g of oven-dry soil. Dexon and benomyl were incorporated into the soil at rates of 14 mg a.i./kg and 120 mg a.i./kg of oven-dry soil, respectively.
[x] Disease severity ratings were based on a scale of 0 to 6 with 0 = no symptoms and 6 = dead plant.
[y] Mount Morris soil elicited black root-rot symptoms and Auburn soil elicited common root-rot symptoms.
[z] Means in a column followed by the same letter do not differ significantly ($P = 0.05$) according to the Waller-Duncan's K-ratio (LSD) rule.

TABLE 3. Effect of *Thielaviopsis basicola* and *Fusarium solani* f. sp. *pisi* singly and in combination on root-rot development of peas in a sandy loam field soil

Treatment[x]	Disease severity ratings[y] Epicotyl	Root	Dry wt/ plant (g)
Control	0.0 A[z]	0.0 A	0.40 A
F. solani	3.0 C	1.7 B	0.37 A
T. basicola	1.6 B	3.3 C	0.32 B
T. basicola + *F. solani*	3.2 C	3.6 C	0.31 B

[x] *Thielaviopsis basicola* and *F. solani* f. sp. *pisi* were incorporated into the soil at a rate of 3,000 propagules per gram of oven-dry soil singly and 1,500 propagules per gram of each when in combination.
[y] Disease severity ratings were based on a scale of 0 to 6 with 0 = no symptoms and 6 = dead plant.
[z] Means in a column followed by the same letter do not differ significantly ($P=0.05$) according to the Waller-Duncan's Bayesian K-ratio (LSD) rule.

A. euteiches (19).

The results of the pathogenicity tests indicate that infection by *T. basicola* is confined primarily to the tap and lateral roots since average epicotyl ratings were 0.3. Also, *T. basicola* does not appear to interfere with seed germination because the percent germination of nontreated Alaska pea seeds was 92%.

Thielaviopsis basicola has frequently been reported to be a component of the fungal complexes causing root rot of bean, soybean, and other hosts in other states (1,3,9,11,12,17,21,22,25), but this is believed to be the first report of *T. basicola* as a pathogen of pea in New York State. This organism is not frequently associated with pea root rot elsewhere, but it has been reported (1,5,6,7,8). Apparently it is becoming more prevalent in New York, which is corroborated by the work of G. Abawi (*personal communication*) with root rot of snap beans.

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