ABSTRACT

THIELAVIOPSIS BASICOLA ROOT ROT OF PEAS

by A. Bryce Lloyd

A root rot survey was made of the Michigan pea fields. Fusarium solani f. pisi was present in the 19 fields that were sampled for this fungus. Aphanomyces euteiches was present in 14 of 25 fields sampled. Thielaviopsis basicola was isolated from diseased roots from 4 of 25 fields sampled. This fungus had not been reported as part of the root rot complex of peas. Pathogenicity of 10 isolates of T. basicola from the 4 fields ranged from 1.7 - 5.5, based on a scale of increasing disease severity from 0 - 9. The most pathogenic isolates caused a black-brown dry rot which completely destroyed the root cortex. Uniform and severe disease was obtained in greenhouse tests by mixing mycelia and spores of T. basicola with autoclaved soil, and planting seeds dusted with captan in this infested soil.

In one field at East Lansing, T. basicola appeared to be the principal pathogen, based on disease symptoms, on the presence of large numbers of chlamydospores in the diseased tissues, and on isolations made of the fungus. T. basicola was the only pathogen to be isolated from the roots of 10-day old pea seedlings grown in soil from this field.

The severity of T. basicola root rot of peas was similar in soils adjusted at several points between pH 6.5 and 8.1.

Maximum mycelial growth of 3 isolates of T. basicola occurred in potato-dextrose broth at 20° - 24° C. Almost no growth occurred at 32° C. Increasing soil temperatures from 16° to 28° C. resulted in increasing disease severity, with maximum disease indices at 28° C. All the 3 isolates tested produced severe root rot at this temperature. T. basicola root rot of peas is most severe in soils at temperatures above the optimum for both mycelial growth of the fungus in culture, and growth of the host in soil (15° - 18° C.). Warm soil temperatures which are unfavorable for growth of peas are favorable for infection by the fungus. By contrast, T. basicola root rot of warm temperature plants (tobacco, poinsettia, cotton, orange) is most severe in soil at low temperatures. The optimum temperature for root rot disease may extend into the optimum range for mycelial growth of the fungus in culture, but not into the optimum range for vegetative growth of the host.

Three isolates of T. basicola from pea were pathogenic on beans but not tobacco. Citrus and poinsettia isolates were pathogenic on peas, but isolates from tobacco were not.

A. Bryce Lloyd

Twenty-nine commercial varieties and 8 pea introductions all developed root rot when grown in soil infested with T. basicola. As a group, the pea introductions had lower disease indices than the commercial pea varieties.

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By

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INTRODUCTION

In 1955 and 1956, Lockwood et al. (23) made systematic surveys of pea diseases in areas of Michigan where this crop is grown for the canning and freezing industry. They concluded that root rots were the most important group of parasitic diseases of peas. In Michigan and other pea growing states, the important root rot pathogens appear to be Aphanomyces euteiches Drechs. and Fusarium solani f. pisi (F.R. Jones) Snyder and Hansen. Lockwood and Ballard (20, 22), in 1956, began a program of evaluating pea varieties and foreign introductions to obtain varieties of pea resistant to these two fungi. Selected lines of peas showing some degree of resistance in greenhouse tests were planted in a root rot field in East Lansing for further evaluation. Isolations made from these plants grown in the field in 1959 sometimes yielded Thielaviopsis basicola (Berk. & Br.) Ferr. (20). The fungus was shown to be pathogenic on peas.

As T. basicola was not generally considered part of the root rot complex of peas, a survey was made of Michigan pea fields in 1960 to determine the prevalence of this fungus. At the same time information was collected on the incidence of A. euteiches and F. solani f. pisi. Isolates of T. basicola from the disease survey were used to study the effect of soil pH and temperature on disease severity. Information was also obtained on the relative pathogenicity of various isolates,

host specificity, and the possibility of resistance in commercial peas and pea introductions.

LITERATURE REVIEW

T. basicola was first described by Berkeley and Broome (5) in 1850. The fungus was observed on the base of pea stems, and these authors considered it "either destructive of the plant on which it grows, or is developed on it in consequence of previous disease". Zopf (41) in 1876 described the ascomycete Thielavia basicola which he considered the sexual stage of Thielaviopsis basicola. Although other writers were unable to detect perithecia or ascospores, the fungus continued to be identified as Thielavia basicola Zopf. In 1925, McCormick (26) re-examined Zopf's work and showed that the ascomycete Thielavia basicola was not the perfect stage of Thielaviopsis basicola, although the two fungi were commonly associated on the same hosts. More recent work has confirmed McCormick's taxonomic separation of the two fungi (24).

Thielaviopsis basicola has been isolated from soils and susceptible hosts collected from many geographical regions. Yarwood (40) was able to isolate the fungus from soils collected from 7 of 12 localities in California, and reported that in none of these localities was T. basicola observed as a pathogen of the field crops. The fungus is a well known pathogen of tobacco, cotton and other plants, and is reported to have a host range of over 100 species, including peas (14). Until recently, all reported susceptible

hosts of T. basicola were Angiosperms, but recently Vaartaja et al. (38) isolated the fungus from seedlings of several Gymnosperms.

Tobacco, and in recent years poinsettia plants, have been used to study the environmental factors influencing disease severity caused by T. basicola. In 1908 Briggs (8) reported that there was considerably less black root rot of tobacco in acidified soil than in alkaline soil. Anderson, Osmun, and Doran (2) showed that in soil above approximately pH 5.9, T. basicola frequently caused severe root rot. The use of soil below approximately pH 5.6 prevented the disease. Similar results have been reported by several writers, using tobacco (1, 10, 29) and poinsettia (3, 17) as host plants. Johnson and Hartman (15) reported that the optimum soil temperatures for tobacco black root rot were between 17° - 23° C. Similar results have been obtained with tobacco (16, 35, 39), poinsettia (3, 4), cotton (6, 12) and sweet orange (37). The optimum temperature for growth in culture of T. basicola isolates from these hosts has been reported as between 21° - 30° C. (4, 12, 15, 16, 18, 25, 37). The degree of tobacco black root rot has been related to the amount of soil moisture. Soil with a water content near field capacity is favorable to severe root rot injury. Less than field capacity has little effect on the amount of root rot (15, 17, 32).

Infection and root rot severity have also been related to the degree of soil infestation, virulence of the fungus and host specificity. Johnson and Hartman (15) and Anderson et al. (2) considered the number of infection sites on the roots to be related to the concentration of the inoculum in the soil. Severe root rot injury was the result of many infection sites. Papavizas and Davey (30) stated that at least a 100 endoconidia are required per gram of oven dried soil to obtain even a light infection.

Isolates of T. basicola have been shown to vary in pathogenicity when tested on different hosts. The variability may be partly due to physical factors, such as soil temperature during pathogenesis. There are however, several reports of pathogenicity tests made over a range of temperature and soil pH which show evidence of host specificity. Isolates of T. basicola from poinsettia caused root rot of that host but not tobacco, whereas isolates from tobacco did not injure poinsettia (17, 32). Bean, cyclamen, and Primula isolates caused root rot of bean and lupin but not tobacco (31).

Johnson (14) grew 200 species of plants in soil collected from a tobacco field known to be naturally infested with T. basicola. Microscopic examination of the root tissues showed the presence of chlamydospores of T. basicola in the diseased roots of approximately 100 species of plants. He considered this as evidence that no specialization of the

fungus appears to exist. In naturally infested soil the population of T. basicola is composed of individuals which differ in virulence (34, 35, 36). Stover considered field isolates of T. basicola to be of two types, which he loosely classified as brown and gray. The brown type culture was more virulent and able to survive for longer periods as a saprophyte in the soil (34, 35).

The pea has been reported as a host of T. basicola (9, 14, 28). Linford (19), in 1928, made a survey of pea fields in the United States and reported T. basicola chlamydospores on the blackened surface of the roots of a few plants from a field in Idaho. Lockwood (20) isolated T. basicola from peas grown in East Lansing and from peas grown in the greenhouse in root rot soil from three other Michigan locations. All isolates produced root rot of peas in greenhouse tests.

MATERIALS AND METHODS

F. solani f. pisi was isolated from diseased pea roots by the agar plate method. Tap roots were washed in running water, cut into transverse pieces, and surface-sterilized by immersion in 0.5% sodium hypochlorite for 5 minutes. The cut pieces were then washed in sterile water, dried and placed on water agar (per litre: 20 gm agar dissolved in distilled water) and corn meal agar (per litre: 20 gm corn meal, 20 gm agar). Streptomycin at 100 µg per ml and chloramphenicol at 10 µg per ml were added to the agar to inhibit growth of bacteria. T. basicola was isolated from diseased roots by the carrot disk method (40). Macerated pieces of root tissue were placed on carrot disks on moist filter paper in a petri dish. Single microconidial isolations were made from colonies of Fusarium growing on the agar substrates, and single endoconidial isolations from T. basicola colonies growing on the carrot disks. In addition to isolations, diseased root tissues were examined microscopically for the presence of chlamydospores of T. basicola and for oospores of A. euteiches.

All cultures were maintained on potato-dextrose agar slants (per litre: infusion from 200 gm potatoes, 20 gm dextrose, 20 gm agar). Additional isolates of T. basicola were obtained from citrus plants (supplied by Dr. P.H. Tsao, California), tobacco (Dr. L. Henson, Kentucky), and isolates

from poinsettia which were also pathogenic on beans (Dr. A.W. Dimock, New York). To prepare inoculum for pathogenicity tests, isolates of T. basicola were grown for 7 days at 24° C. in 250-ml Erlenmeyer flasks containing 50 ml of potato-dextrose broth. Mycelial mats were washed in distilled water, ground in a Waring Blendor, diluted with water and the homogenate used to infest autoclaved soil. Endoconidial suspensions from T. basicola cultures grown on potato-dextrose agar were used to infest sand. The concentration of the endoconidia was standardized with a haemacytometer. Potato-dextrose broth in 250-ml Erlenmeyer flasks was used for liquid cultures. Each flask was seeded with a standardized endoconidial suspension. For determination of mycelial dry weights, the content from each flask was separately centrifuged, washed in distilled water and dried at 75° C. overnight in preweighed aluminum pans.

Several different methods were used for infesting autoclaved soil with T. basicola (Table 1). The method which resulted in severe and uniform disease is as follows: Inoculum was mixed with autoclaved soil, using a volume of homogenate equivalent to one mycelial mat per 1.5 litres of soil. The infested soil was put in 4 inch clay pots. Pea seeds (Miragreen) were lightly dusted with captan, and 12 seeds planted in each of 4 pots. The pots were kept for 5 weeks in a greenhouse at approximately 22° C. Dusting the seeds with captan prevented seed decay when they were planted

Table 1. Effect of different methods of infesting autoclaved soil on the severity of root rot on Miragreen peas, caused by T. basicola isolate 25.

Treatment[a]	Average disease index[b]	Escapes[c]
1	9.0[d]	0
2	8.8[d]	0
3	6.3	0
4	3.5	0
5	5.1[d]	0
6	5.5	0
7	3.0	6
8	3.4	1
9	2.2	17
control	0.0	-

[a]Treatments as follows:
1. Seeds planted in soil infested with a mixture of mycelia and spores from broth cultures.
2. Seeds soaked in water before planting in infested soil.
3. Seeds lightly dusted with captan before planting in infested soil.
4. Seeds planted in autoclaved soil overlying infested soil.
5. Seeds soaked in water and planted as in treatment 4.
6. Inoculum mixed with surface soil after seedling emergence.
7. Inoculum placed in holes in soil after seedling emergence.
8. Surface soil infested with an endoconidial suspension after seedling emergence.
9. Soil infested after seedling emergence by placing a suspension of agar, mycelia, and spores from PDA cultures in holes in the soil.

[b]Disease index was based on a scale of increasing severity from 0-9. Each figure is a mean index of 4 pots, each with 12 plants.

[c]Plants with no disease symptoms.

[d]Low seedling emergence.

in the infested soil. There did not appear to be any residual effect from the fungicide as the test plants had uniformly severe root rot symptoms. This method (Table 1, treatment 3) was used in all other work that required infested soil. Metal pans (size: 16 in. x 17 in. x 4 in., or 13 in. x 26 in. x 4 in.) were sometimes used as soil containers instead of pots.

Several other methods of infesting soil with T. basicola gave unsatisfactory results (Table 1). Non-dusted seeds planted in infested soil (treatment 1), seeds soaked in water before planting in infested soil (treatment 2), and seeds soaked in water and planted in autoclaved soil overlying infested soil (treatment 5), all resulted in low seedling emergence. Seeds planted in autoclaved soil overlying infested soil (treatment 4), inoculum placed in holes in soil after seedling emergence (treatment 7), surface soil infested with an endoconidial suspension after seedling emergence (treatment 8), and a homogenate of potato-dextrose agar cultures put in holes in soil after seedling emergence (treatment 9) all gave variable results. Inoculum mixed with the surface soil after seedling emergence (treatment 6) produced uniform disease symptoms. This method was not used however, as mixing the inoculum with the soil damaged the plant roots.

Sand instead of soil was also used as a substrate for pathogenicity tests, following the method described by Lockwood and Ballard (22) for evaluating Aphanomyces euteiches and Fusarium solani f. pisi root rot of peas. Rows of surface sterilized pea seeds (Miragreen) were sown in washed silica sand in metal pans. After seedling emergence (approx. 6 days), each row was infested with 10 ml of a suspension containing a determined number of endoconidia per ml. The pans were kept in a greenhouse at 20° C. for 5 weeks. The severity of root rot on the plants was low, even when the sand was infested with a suspension containing 2,500,000 endoconidia per ml.

The Fusarium isolates were tested for pathogenicity by placing a suspension of agar, mycelia, and spores made from cultures of the fungi growing on PDA, into holes in the soil after seedling emergence.

Disease evaluation was based on a scale of increasing severity from 0 - 9. Disease in the tops, epicotyls and roots was separately rated for each group of plants in a pot or row, using a scale similar to that of Lockwood and Ballard (22). Severe disease symptoms were rated as 3, intermediate symptoms as 2, and slight observable symptoms as 1. The separate ratings for the tops, epicotyls and roots were totalled, and the average obtained for all replications of each treatment.

Autoclaved soil was adjusted to pH 8.5 with calcium carbonate and calcium oxide, then to lower pH levels with 0.1N sulfuric acid. The sand pH was adjusted by watering with a dilute phosphate buffer. The required buffer pH was obtained by altering the concentration of potassium salts of the monohydrogen and dihydrogen phosphates.

RESULTS

Pea root rot survey. The survey was made in 5 important pea growing areas in Michigan: Caro, Saginaw, Croswell (all in the 'thumb'), Montague (western Michigan), and Jackson (south-central Michigan), and also at East Lansing. Fields were visited 2 - 3 weeks before harvest, and samples were taken only from fields where root rot was observed. Sixteen plants with visible root rot symptoms were taken from each field sampled. Tap roots from 8 of the plants were washed, cut into 9 transverse pieces and surface-sterilized. Three of the sections from each root were placed on water agar, 3 on corn meal agar, and 3 macerated pieces placed on carrot disks. The remaining 8 plants from each field were examined microscopically for chlamydospores of T. basicola and oospores of A. euteiches.

F. solani f. pisi was readily isolated from surface-sterilized root tissues plated on water agar. T. basicola was rarely isolated by the agar plate method and microscopic examination of diseased root tissue showed chlamydospores of the fungus in plants from only one area (East Lansing). The number of root infections caused by this fungus was therefore based on isolation by the carrot disk technique. No colonies of A. euteiches were observed growing on the agar substrates. The number of A. euteiches infections recorded was based on microscopic identification of the

characteristic thick-walled oospores of the fungus embedded in the root tissues.

Table 2 lists the number of diseased plants infected with F. solani f. pisi, A. euteiches, and T. basicola based on a total of 8 plants collected from each field sampled. Pathogenic isolates of F. solani f. pisi were obtained from plants in all of the 19 fields that were sampled for this fungus. A. euteiches was observed in plants from 14 of the 25 fields sampled. T. basicola isolates were obtained only from diseased plants collected in 3 fields at Jackson and 1 field at East Lansing.

Pathogenicity of Isolates. Pathogenicity tests were made using 10 separate isolates of T. basicola obtained from plants collected in the Jackson and East Lansing areas. At the same time 35 of the F. solani f. pisi isolates were also tested for pathogenicity.

The T. basicola isolates tested were all pathogenic on peas and had average disease indices from 1.7 - 5.5 (Table 3). In these tests the maximum rating for tops of plants was 1, showing that disease in epicotyls and roots caused by some isolates was near the maximum. All of the 35 isolates of F. solani f. pisi were pathogenic, and the roots and lower stems of the infected pea plants had disease symptoms similar in degree and appearance to those caused by T. basicola.

Table 2. Frequency of infection of roots of pea plants with 3 pathogenic fungi from Michigan pea fields.

Area	No. of fields sampled	No. plants infected with each fungus[a]		
		F. solani	*A. euteiches*	*T. basicola*
Caro	3	16	8	0
Montague	4	-	18	0
Saginaw	2	-	8	0
Croswell	6	28	6	0
Jackson	8	34	36	12
East Lansing	2	11	8	8
Total	25	89	84	20

[a]Eight plants were used for isolation, and 8 were used for microscopic observations from each field. Data for *F. solani* f. *pisi* and *T. basicola* are based on isolations; those for *A. euteiches* are based on microscopic identification of oospores in diseased roots.

Table 3. Results of greenhouse pathogenicity tests of isolates of T. basicola on Miragreen peas.

Area	Isolate	Average disease index[a]
Jackson	21	5.0
	26	3.0
	27	2.5
	29	4.7
	33	1.7
East Lansing	23	2.0
	25	5.5
	93	4.7
	94	4.3
	95	4.7

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[a]Disease index was based on a scale of increasing severity from 0 - 9. Each figure is a mean index of 3 pots, each with 12 plants.

Pea seedlings infected with T. basicola show discoloration of the root tissue below the cotyledons and at several infection sites along the tap and lateral roots (Fig. 1A). These diseased regions coalesce, resulting in the characteristic black-brown necrosis of the cortex of the tap and lateral roots, and of the lower stems below the soil level (Fig. 1B, 1C). Severe infection resulted in almost complete decay of the root system, wilting of the leaves, and stunting of the plants. Sometimes the stem below the soil line was girdled leaving the stele as the only connection between the top and roots. The external symptoms strongly resemble those caused by F. solani f. pisi which is also a cortical invader. T. basicola could easily be isolated from these plants by the carrot disk technique (Fig. 2). The characteristic dark chlamydospores were abundant in the decayed tissues of severely diseased plants (Fig. 3).

In one field at East Lansing, T. basicola was isolated from all the sampled plants. Microscopic examination showed large numbers of chlamydospores embedded in the diseased tissues. Dusted pea seeds were again planted in this field in July, 1960. On several different days, seedlings were taken from the field, surface-sterilized and tap roots plated on water agar, corn meal agar, and carrot disks.

T. basicola was isolated by the carrot disk method from most plants older than 7 days (Table 4). On corn meal

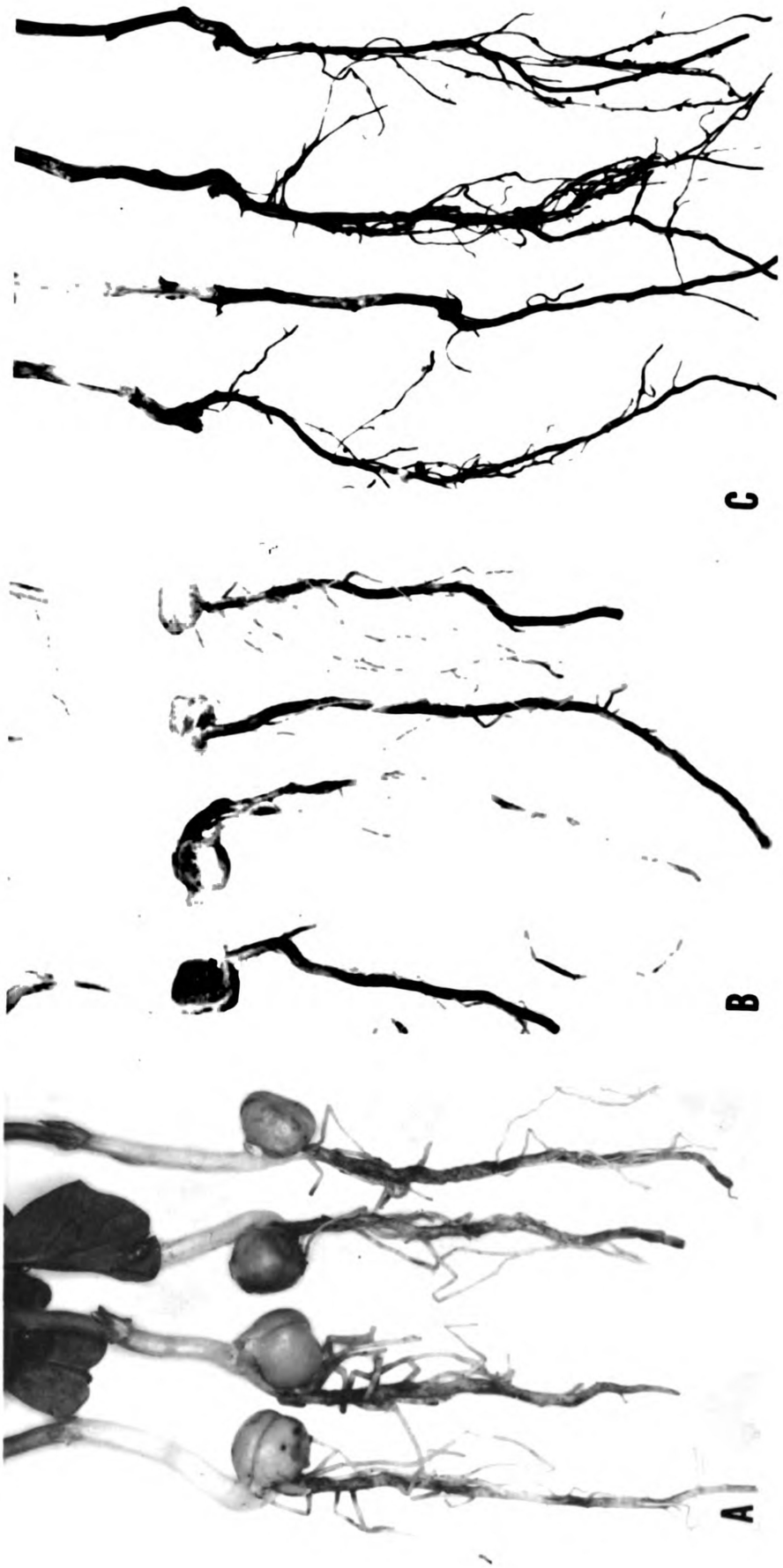

Fig. 1. Thielaviopsis basicola root rot on Miragreen peas. A: seedlings 10 days old. B: seedlings 14 days old. C: plants 30 days old.

Fig. 2. Nine-day old colonies of Thielaviopsis basicola isolated from diseased pea roots by the use of carrot disks.

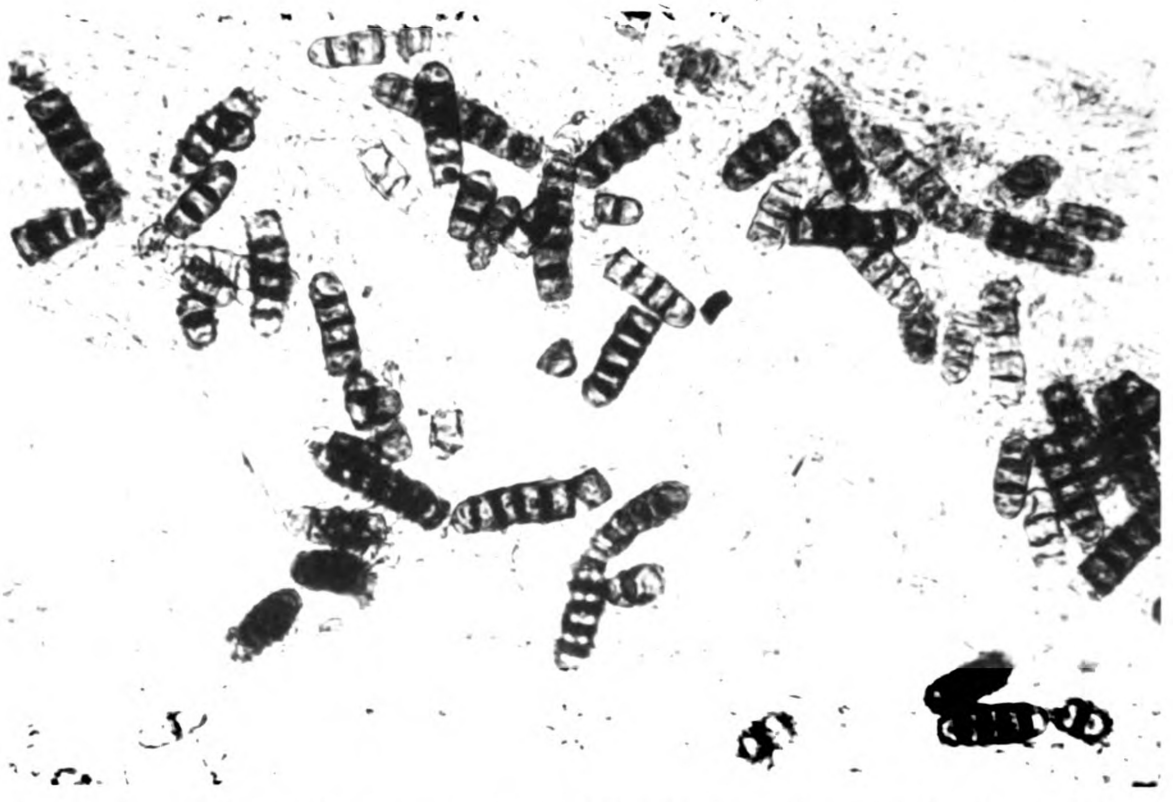

Fig. 3. Chlamydospores of Thielaviopsis basicola in infected cortical tissues of pea roots.

Table 4. Isolation of 2 pathogenic fungi from pea plants grown in the East Lansing pea field.

Days after planting	No. of plants yielding the indicated fungus[a]	
	F. solani	T. basicola
7	0	0
12	6	8
15	5	7
17	7	7
18	7	7
22	5	8

[a]Eight plants were used for isolation at each sampling period. Data for F. solani f. pisi was based on agar plate isolations, those for T. basicola was based on carrot disk isolations.

and water agar, this fungus was only isolated at 12 and 15 days, although the older plants were infected. Saprophytic fungi and the faster growing F. solani f. pisi colonies probably masked the presence of T. basicola on the agar substrates. At 12 days, F. solani f. pisi and T. basicola were isolated from the roots of most plants. In greenhouse tests using infested field soil, it was shown that 10 days after planting, T. basicola was the only fungus isolated from most of the seedlings. Both T. basicola and F. solani f. pisi are important root rot pathogens in this field soil, and from these results it seems that T. basicola is the principal pathogen.

Effect of soil pH on disease severity. Soil at adjusted pH levels of 5.5, 6.5, 7.5 and 8.5 was placed in metal pans and infested with T. basicola isolates 25, 27 or 29. Twenty dusted pea seeds were planted in each of the infested soils. The experiment was duplicated for each isolate at each pH level, making a total of 24 infested soils. Difficulty was experienced in controlling the extreme pH values, the buffering of the soil tending to reverse changes in the altered soil pH. At the end of the experiment, the adjusted pH levels of 5.5 had altered to 6.5 and pH 8.5 to 8.1.

The severity of the disease was similar in all soils between pH 6.5 and 8.1 (Table 5). As a result of the

Table 5. Effect of soil pH level on pathogenicity of T. basicola isolates on Miragreen peas.

Initial pH	Final pH	Average disease index for indicated isolate[a]		
		25	27	29
5.5	6.5	2.8	2.8	5.0
6.5	6.5	2.8	2.8	5.0
7.5	7.6	2.8	2.8	5.0
8.5	8.1	2.8	2.8	4.5

[a] Disease index was based on a scale of increasing severity from 0 - 9. Each figure is a mean index of 2 replications, each with 20 plants.

change in soil pH, no information was obtained on reduction in disease severity in very acid soils as reported by other writers (1, 2, 3, 8, 10, 17, 29).

Sand instead of soil was used as a substrate in an experiment on the effect of pH on disease severity. The sand in metal pans was adjusted to pH 6.8, 8.8 or 9.2 by watering when necessary with a phosphate buffer. T. basicola isolate 25 was used for preparation of the inoculum. After seedling emergence, each row of peas was infested with 10 ml of a suspension containing 100,000, 500,000 or 2,500,000 endoconidia per ml and kept for 5 weeks at an air temperature of 20° C. The average disease index for each treatment was between 1.0 and 2.0. These low indices made the sand test unsatisfactory for evaluating the effect of pH on pea root rot caused by isolates of T. basicola.

Effect of temperature on disease severity. Autoclaved soil was put in metal pans and infested with T. basicola isolates 25, 27 or 29. Twenty dusted pea seeds were planted in each of two rows in all the pans. The seed plantings were repeated using sand instead of soil as a substrate. Each row of seedlings in the sand was inoculated with 10 ml of a suspension containing 500,000 endoconidia per ml of T. basicola isolates 25, 27 or 29. The pans were kept in soil temperature tanks at 16°, 20°, 24°, or 28° C. for 4 weeks.

Results of these tests are shown in Table 6 and Figs. 4, 5 and 6. The highest disease indices in both soil and sand were recorded at 28° C. In soil at 28° C., T. basicola isolate 27 gave a disease index of 5.5, and isolates 25 and 29 gave maximum disease indices of 9.0. In sand at 28° C., isolate 27 gave a disease index of 1.5, isolate 25 a disease index of 2.0, and isolate 29 an index of 4.8. All 3 isolates in both soil and sand showed increases in disease severity with each increase in temperature from 16° to 28° C. The mean disease indices for the 3 isolates in soil at 16°, 20°, 24°, and 28° C. were, respectively, 2.5, 3.8, 6.2 and 7.8. In sand at these temperatures the mean disease indices were, respectively, 0.2, 1.2, 1.7 and 2.7.

Effect of culture temperature on growth of mycelium. Suspensions containing 100,000 endoconidia per ml were prepared from cultures of T. basicola isolates 25, 27 and 29. Fifty-four flasks, each containing 50 ml of potato-dextrose broth were seeded with 10 ml of an endoconidial suspension. The flasks were kept at 24° C. for 15 hours to induce spore germination. Three flasks of each isolate were then incubated at 12°, 16°, 20°, 24°, 28°, and 32° C. for 7 days.

Table 7 and Fig. 7 show the results of this work. Maximum mycelial growth occurred at 20° and 24° C. for isolates 25 and 29, and at 24° C. for isolate 27. There was a marked

Table 6. Effect of soil and sand temperature on pathogenicity of T. basicola isolates 25, 27 and 29 on Miragreen peas.

Temperature	Average disease index for indicated isolate[a]							
	Soil				Sand			
	25	27	29	Mean	25	27	29	Mean
16°	3.0	0.5	4.0	2.5	0.0	0.0	0.5	0.2
20°	4.0	1.5	6.0	3.8	0.8	0.5	2.5	1.2
24°	6.5	3.0	9.0	6.2	1.0	1.0	3.0	1.7
28°	9.0	5.5	9.0	7.8	2.0	1.5	4.8	2.7
LSD 5%	0.4	0.4	0.4	0.3	0.8	0.8	0.8	0.5

[a]Disease index was based on a scale of increasing disease severity from 0 - 9. Each figure is a mean index of 2 rows, each with 20 plants.

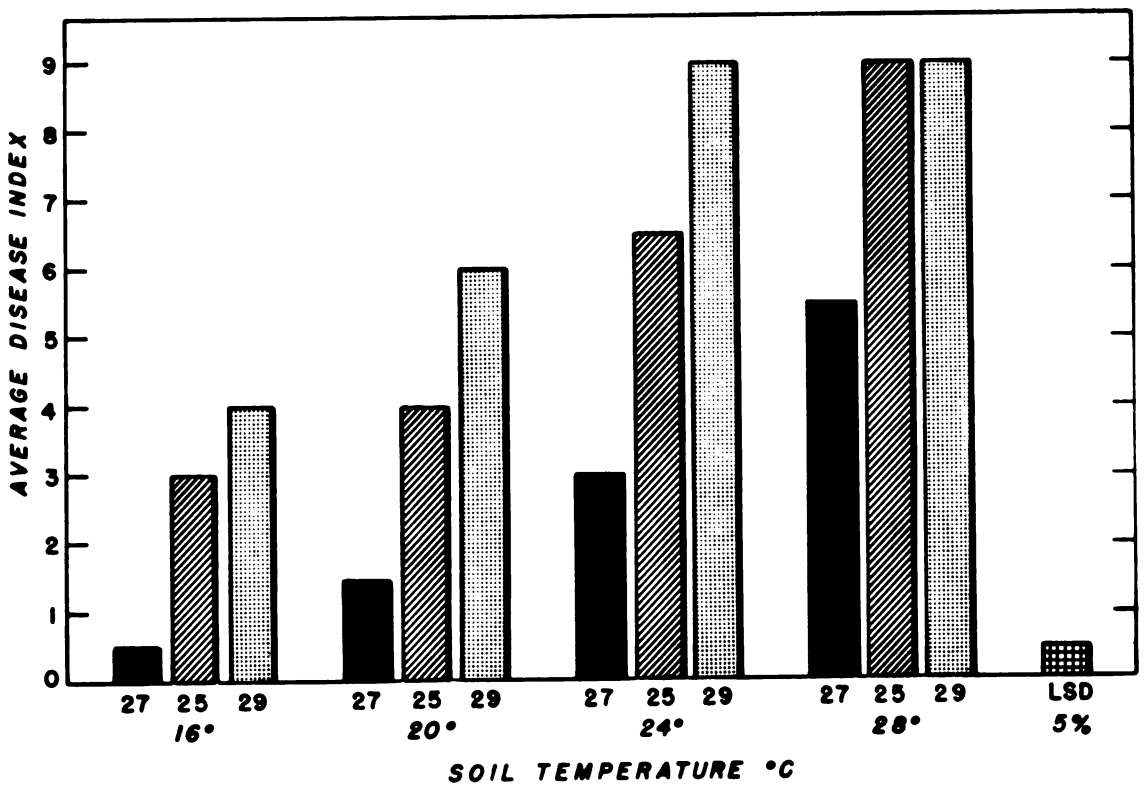

Fig. 4. Effect of soil temperature on pathogenicity of Thielaviopsis basicola isolates 25, 27 and 29 on Miragreen peas.

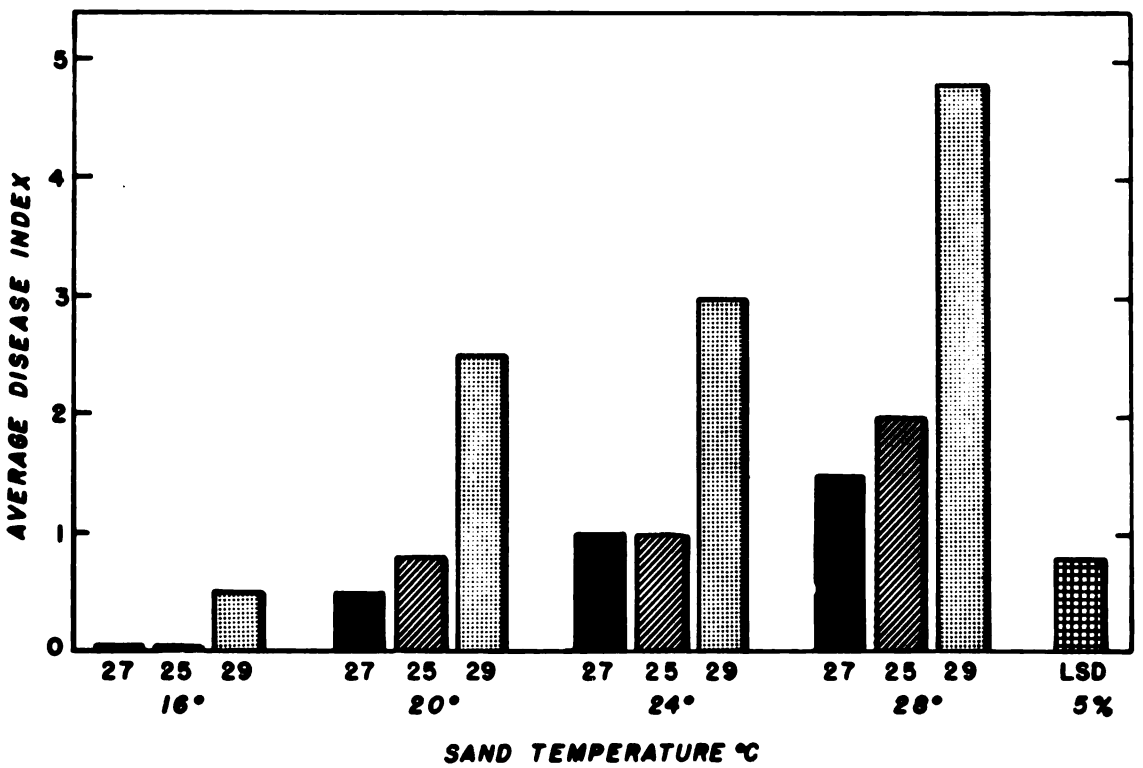

Fig. 5. Effect of sand temperature on pathogenicity of Thielaviopsis basicola isolates 25, 27 and 29 on Miragreen peas.

Fig. 6. Thielaviopsis basicola root rot on Miragreen peas grown in infested soil at the indicated temperatures.

Table 7. Effect of culture temperature on growth of T. basicola isolates 25, 27 and 29 in potato-dextrose broth.

Temperature	Mean dry weight of mycelium, mg[a]			
	25	27	29	Mean
12°	95	72	59	75
16°	147	97	187	144
20°	157	125	235	172
24°	166	172	242	193
28°	115	151	175	147
32°	8	4	3	5
LSD 5%	15	15	15	9

[a]Each figure is mean of 3 dried mycelial mats.

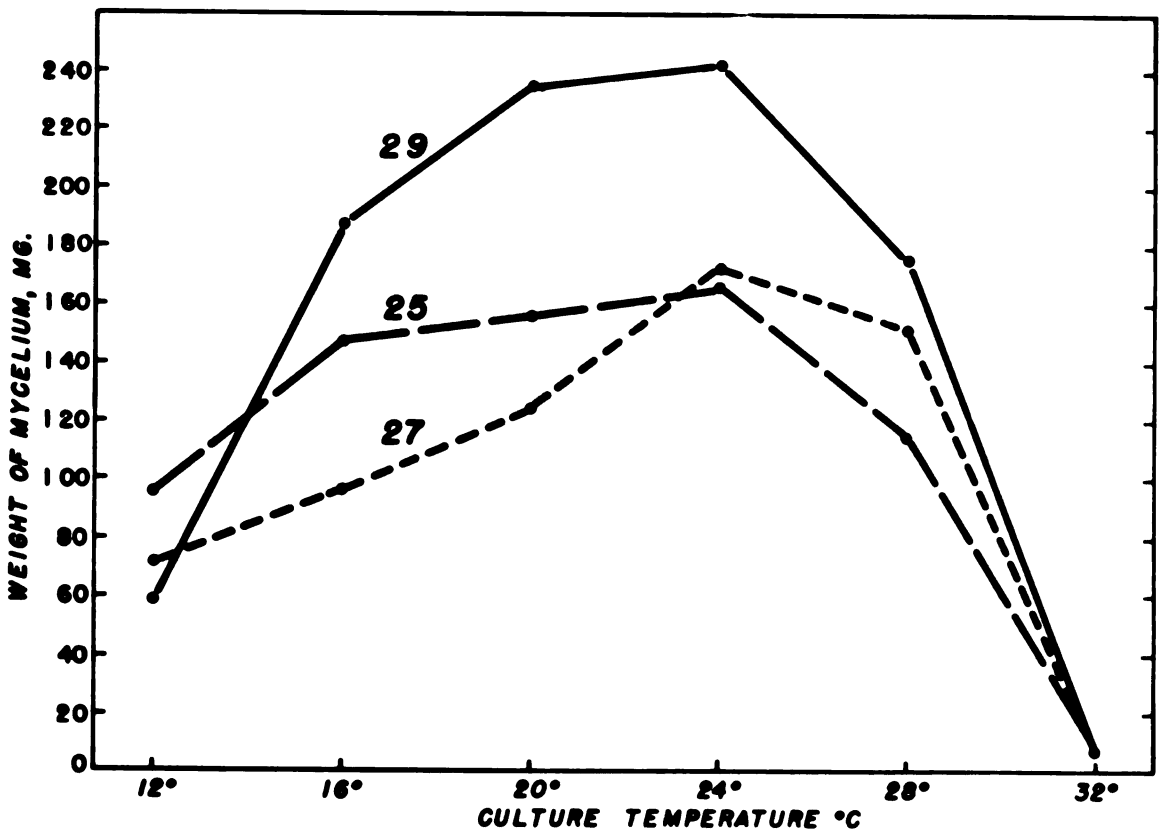

Fig. 7. Effect of culture temperature on growth of Thielaviopsis basicola isolates 25, 27 and 29 in potato-dextrose broth.

reduction in growth of all isolates at 12° and 32° C. Almost no growth occurred at 32° C. Growth of mycelium at 24° C. was significantly higher than growth at 16° and 28° C. for all isolates. A second test showed similar growth differences.

Increasing soil temperatures from 16° to 28° C. resulted in increasing disease severity, with maximum disease indices at 28° C. Optimum culture temperature for mycelial growth occurred at 20° and 24° C. At temperatures above 24° C., the disease severity increased and the mycelial growth decreased (Fig. 8). T. basicola root rot of peas was most severe at soil temperatures above the optimum for growth of mycelium in culture.

Host specificity of T. basicola. Nicotiana glutinosa tobacco (33) and red kidney bean plants (27) are reported as susceptible hosts of T. basicola. Both species were used to test the pathogenicity of T. basicola isolates from pea.

Mycelial homogenates were prepared from cultures of T. basicola isolates 25, 27 and 29. A row of 10 dusted bean seeds and a row of tobacco seedlings were planted in infested soil in metal pans. The pans were put in soil temperature tanks (21) at regulated water temperatures of 20° and 28° C., and kept for 5 weeks. All treatments were duplicated. Both temperatures were used as T. basicola

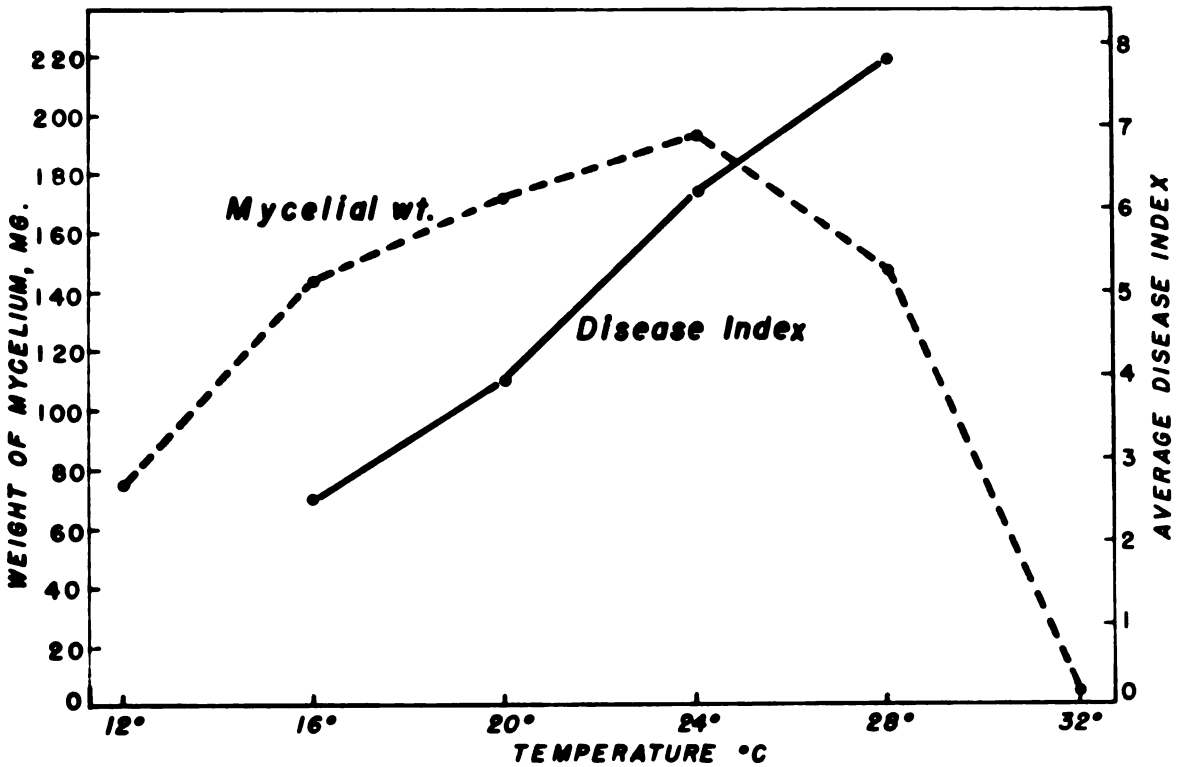

Fig. 8. Effect of soil temperature on disease severity compared with the effect of culture temperature on growth of mycelium. The disease indices and mycelial weights are means of Thielaviopsis basicola isolates 25, 27 and 29.

root rot is reported as severe in soil at approximately 20° C. for most plants (Table 9), yet for peas the maximum disease severity was at a soil temperature of 28° C.

No root rot symptoms occurred on the tobacco plants at either 20° or 28° C. All T. basicola isolates were pathogenic on bean plants at both temperatures. The average disease index was 1.0 for isolate 25, 4.0 for isolate 27, and 3.0 for isolate 29. Disease indices for each isolate were the same at 20° and 28° C. Bean plants grown in soil infested with T. basicola isolates 27 or 29 showed severe girdling of the collars at the junction of the root and shoot. There was no wilting of the leaves on bean plants with severe root rot symptoms. Beans form adventitious roots from the lower stem region, and in moist soil, sufficient uptake of water by these roots prevented wilting and death of the plants.

Isolates of T. basicola from citrus, poinsettia (also pathogenic on bean), and tobacco were used for pathogenicity tests on peas. The pots were kept in a greenhouse at 20° C. for 4 weeks, and 28° C. for one week. The two T. basicola isolates from poinsettia caused severe root rot of peas, both with average disease indices of 8.0. The 3 isolates from citrus plants were all pathogenic, giving average disease indices of 4.0, 2.5, and 1.0. No root rot symptoms occurred on pea plants grown in soil infested with

any of the 3 isolates from tobacco. This indicates that there is some host specificity of the fungus.

Resistance of commercial peas and pea introductions. Dusted seeds of 29 commercial pea varieties and 8 pea introductions were each sown in 2 rows in separate metal pans containing soil infested with T. basicola isolate 94. The pans were kept in a greenhouse at 28° C. The soil was watered to near saturation shortly after seedling emergence. This high moisture apparently favored the development of the disease. Some plants showed wilting symptoms only 3 weeks after planting the seed, and about 2 weeks before plants were usually removed from the soil for disease evaluation.

Commercial varieties and pea introductions all developed root rot in the infested soil (Table 8). The highest disease index was 9.0 and the lowest 4.5. As a group, the pea introductions had lower disease indices than the commercial varieties of peas. The lowest disease rating for a commercial variety was 6.0. The pea introductions with relatively low disease indices (4.5 - 6.0) often had severe root rot with necrosis of the cortex of the tap and lateral roots and lower stem below the soil level. The shoots of the plants appeared healthy and showed no wilting of the leaves or apparent stunting of the plants. The healthy top, in spite of a severely diseased root system, was not observed in the commercial varieties of peas.

Table 8. Average disease indices of commercial peas and pea introductions infected with T. basicola in greenhouse tests.

Variety or P.I. No.	Average disease index[a]	Origin of seed
Alaska Sweet Pea	8.0	Rogers Bros. Seed Co.
Alaska 28-57 W.R.	7.3	Northrup, King & Co.
Alderman	6.5	Ferry Morse Seed Co.
Alsweet	6.0	
Ameer	9.0	Corneli Seed Co.
Bliss Everbearing	8.0	Ferry Morse Seed Co.
Dark Skin Perfection	9.0	" " " "
Early Perfection	8.5	" " " "
Eureka	8.5	Rogers Bros. Seed Co.
Freezer 37	8.5	Asgrow Seed Co.
Gradus W.R.	7.0	Ferry Morse Seed Co.
Green Seeded Pea	8.5	" " " "
Lincoln	8.5	Northrup, King & Co.
Little Marvel	8.5	Asgrow Seed Co.
Miragreen	8.5	Ferry Morse Seed Co.
Notts Excelsior	9.0	" " " "
Pacific Freezer	8.0	" " " "
Pacific Perfection	8.0	Ferry Morse Seed Co.
Perfected Wales	9.0	Northrup, King & Co.
Premium Gem	8.5	Ferry Morse Seed Co.

Variety or P.I. No.	Average disease index[a]	Origin of seed
Pride	6.0	Northrup, King & Co.
Progress	9.0	Ferry Morse Seed Co.
Resistant Surprise	8.5	Northrup, King & Co.
Thomas Laxton	8.0	Ferry Morse Seed Co.
Victory	8.5	
Willetts Wonder	8.0	Corneli Seed Co.
Wisconsin Perfection	8.5	Rogers Bros. Seed Co.
Wyola	8.5	Asgrow Seed Co.
100 Fold	9.0	Rogers Bros. Seed Co.
140165	4.5	USDA Plant Introduction Sta.
169604	7.0	" " " "
167250	5.0	" " " "
173059	8.5	" " " "
174198	6.0	" " " "
174917	6.5	" " " "
180868	4.5	" " " "
184128	5.0	" " " "

[a]Disease index based on a scale of increasing severity from 0 - 9. Each figure is a mean index of 2 rows, each with 20 plants.

DISCUSSION

The results of this survey and previous work (23) show that F. solani f. pisi and A. euteiches are the most important pathogens in the pea root rot complex in Michigan. T. basicola was isolated from diseased root tissue of peas growing at East Lansing and at Jackson, but not at the 4 other locations. In one field at East Lansing, T. basicola appeared to be the principal pathogen based on disease symptoms, on the presence of large numbers of chlamydospores in the diseased tissues, and on isolations made of the fungus. T. basicola was the only pathogen to be isolated from the roots of 10-day old seedlings grown in this soil. T. basicola is considered to be of potential importance in the pea root rot complex since isolates of this fungus were highly pathogenic in greenhouse tests, producing disease symptoms equal in severity to those caused by isolates of F. solani f. pisi.

The use of soil below approximately pH 5.6 prevents T. basicola root rot of tobacco (1, 2, 10, 29). Similar results have been found using poinsettia as hosts (3, 17). In tests with peas, the severity of root rot was similar in all soils between pH 6.5 and 8.1.

The average disease indices for tests in sand were considerably lower than those in soil. Although the concentration of inoculum in the soil can influence the degree

of root rot (30, 35), in this work it was not considered a limiting factor, as concentrations of inoculum as high as 2,500,000 spores per ml were used to infest the sand. Massee (28) considered the fungus unable to infect the host except in the presence of organic matter, which favored the growth of the fungus in soil. No real evidence was given for this belief, other than his finding that sand and volcanic ash used in seed beds for growing tobacco plants were effective in controlling the disease. Johnson and Hartman (15), however, reported that the severity of T. basicola root rot of tobacco was the same in sand and pure leaf mold. Nutrients were added to the sand during the period of the experiment, and this may have influenced the disease severity in sand. The use of sand as a substrate has been found satisfactory for evaluating root rot of peas caused by A. euteiches and F. solani f. pisi (22).

Peas are cool temperature plants with an optimum temperature for vegetative growth at approximately 15^{o} - 18^{o} C. (7). T. basicola root rot of peas was most severe in soils at a temperature (28^{o} C.) above the optimum for both mycelial growth of the fungus in culture (20^{o} - 24^{o} C.), and growth of the host in soil. Optimum temperature for growth of mycelium in culture, disease severity, and vegetative growth of the host have been determined for tobacco, poinsettia, cotton, and sweet orange (Table 9). These hosts may be grouped as warm temperature plants with an optimum temperature

Table 9. Comparison between optimum temperature for growth of T. basicola in culture, for disease, and for vegetative growth of host (^{o} C.).

Host	Opt. temp. host	Opt. temp. fungus	Opt. temp. disease	Reference
Tobacco	27-31	22-30	17-23	15, 16, 25, 35, 39
Poinsettia	26	21-24	17-20	3, 4
Cotton	30	24-30	21	6, 11, 12, 18
Sweet orange	30	21-27	15-25	37
Pea	15-18	20-24	28	7

for vegetative growth of approximately 26° - 31° C. T. basicola root rot of these plants is most severe in soils at temperatures of approximately 17° - 25° C. This range of temperature is below the optimum for growth of both the pathogen (21° - 30° C.) and hosts (26° - 31° C.). From these results and those with peas, it appears that the optimum temperature range for root rot disease may extend into the optimum range for mycelial growth in culture, but not into the optimum range for vegetative growth of the host. Temperatures unfavorable for growth of the host are favorable for infection by the fungus, whereas temperatures favorable to growth of the plant are unfavorable for infection by the fungus. The optimum temperature for fungal growth is not a factor in pathogenesis. Garrett (13) believes T. basicola to be a primitive type of parasite which infects plants grown under somewhat adverse conditions. Warm temperature plants are susceptible to T. basicola root rot when grown in cold soil. It has been shown with peas that T. basicola root rot is most severe on plants grown in warm soil. This is believed to be the first reported example using a cool temperature crop.

Pea isolates were pathogenic on beans but not tobacco. Citrus and poinsettia isolates were pathogenic on peas, but isolates from tobacco were not. This can be considered evidence that there is some host specificity of the fungus, although each isolate probably has a wide host range.

Pea introductions had lower disease indices than the commercial varieties of peas. This suggests that they might be used in breeding for T. basicola root rot resistance in peas. Frequently the pea introductions had severe root rot, although the shoots appeared healthy and showed no wilting of the leaves or apparent stunting of the plants. Lockwood (20), has reported a similar expression of resistance in the foliage of pea introductions with partial resistance to A. euteiches and F. solani f. pisi root rots.

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