PYTHIUM ROOT ROT AFFECTING PEAS IN MICHIGAN

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ABSTRACT

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by Cesar A. Escobar

Pythium spp. were isolated from pea plants grown in 20 of 25 soil samples collected in pea fields in the "thumb" area and near Jackson in Michigan. All *Pythium* isolates were able to produce considerable root rot disease severity on pea plants in greenhouse tests.

Three of 5 *Pythium* isolates identified were *P. ultimum*. The other 2 isolates were *P. debaryanum* and *P. monospermum*. All 5 fungus isolates showed a similar degree of pathogenicity on peas in greenhouse tests.

P. ultimum produced a considerable root rot damage on several commercial pea varieties, pea introductions and legumes other than peas. Cereal plants tested as well as tomato, cucumber, *Gomphrena globosa*, *Nicotiana glutinosa*, and *Chenopodium amaranticolor*, although diseased, were less susceptible than the legumes.

Pea plants inoculated with *P. ultimum* 1, 8, 15, 22 and 29 days after seeding, showed a similar degree of susceptibility, a possible slightly increased susceptibility at 22 and again at 29 days. When pea plants were inoculated with *A. euteiches* at the same ages, more severe disease symptoms were observed in those plants inoculated 1 day after seeding soil, but plants became more resistant to the pathogen with each increase in age.

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An additive effect was produced when pea plants were infected with both *P. ultimum* and *F. solani*. The damage produced by these 2 pathogens was almost equivalent to that produced by *A. euteiches*, which is considered as the most damaging root rot fungus on peas.

An even greater enhancement of root rot symptoms occurred when plants previously infected with either BYMV or PMV were inoculated with *P. ultimum*.

Disease severity produced by *P. ultimum* on Miragreen peas increased as soil temperatures increased from 12° to 28° C. The optimum temperature for growth of the mycelium of *Pythium* sp. in culture medium was 24° C. Therefore *Pythium* root rot of peas is most severe in soil at temperature above the optimum for growth of mycelium of the fungus in culture medium and growth of the host in soil.

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By

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INTRODUCTION

Peas are important in the diet of a large percentage of the population of the United States. They are grown to some extent in almost every part of the country for home use, for canning, for freezing, for seed, and for fresh market (41). However, root rots are the limiting factor in pea production in Michigan as well as in other pea growing states (25). *Aphanomyces euteiches* Drechs., has been reported as the most damaging fungus affecting roots of peas in Michigan followed in importance by *Fusarium solani* f. *pisi* (F. R. Jones) Snyder and Hansen (23, 25). A breeding program was begun by Lockwood and Ballard (25), to obtain pea varieties resistant to these pathogens. Selected lines of peas showing some degree of resistance in greenhouse tests sometimes failed to show resistance in the field. Two factors possibly responsible for lack of resistance in the field could be that different strains of these pathogens occur in the field or that fungi other than *A. euteiches* and *F. solani* are involved in the pea root rot complex.

In 1956, Lockwood (23) also obtained pathogenic isolates of *Rhizoctonia* sp. and *Pythium* sp. from root rot infected peas, but these are generally considered to be unimportant in connection with pea root rot.

In 1963, a survey was made in Michigan pea fields in the "thumb" area and near Jackson in order to determine the prevalence of Pythium sp. Studies were also made with selected Pythium isolates found during the survey. These included the effect of soil temperature on severity of the disease, effect of temperature in growth of mycelium in pure culture, effect on the host of combined infections with Pythium and other root rot fungi or viruses, effect of the age of plants on susceptibility to this pathogen, and host range.

LITERATURE REVIEW

Although the pea (*Pisum sativum*) is subject to numerous types of diseases, root rots are generally considered to be the most important factors limiting pea yields and quality in Michigan and other parts of the United States (19, 20, 22, 23, 26, 31).

Pythium species, mainly *P. ultimum* Trow., have been frequently reported to produce root rots in peas, or to be associated with other pathogens which also cause infection in this host, thereby denoting a potential importance of this pathogen in the pea root rot complex (8, 16, 29, 30, 31, 35, 41).

In surveys made during ten years in commercial pea growing areas in New York, fourteen different fungi were associated with pea root rot. However, the following organisms, listed in order of importance were primarily responsible for disease: *Fusarium solani* (Mart.) Appel and Wr., *Aphanomyces euteiches* Drechs., *Pythium ultimum* Trow., *Rhizoctonia solani* Kühn, and *Ascochyta pinodella* L. K. Jones (16).

In 1925, Jones and Linford reported that *Aphanomyces* root rot was the most severe disease affecting peas in Wisconsin and that root rots produced by *Fusarium* sp. and *Pythium* sp. were less important (19). However, *Pythium* isolates from diseased pea plants were capable of preventing germination of pea seeds and caused a considerable degree of root rot. P. ultimum was responsible for pre-emergence damping-off and root rot of pea in Toluca, Mexico (10).

Lockwood (23) also considered that fungi other than A. euteiches and F. solani were involved in the pea root rot complex in Michigan, and obtained pathogenic isolates of Rhizoctonia sp. and Pythium sp. from root rot-infected peas.

McDonald and Marshall (28) found a high percentage of susceptibility to P. ultimum in most of 450 introductions or varieties of peas, with colored flowers which, as a group, seemed to be more resistant to other root rot diseases.

Sometimes, interactions between two pathogens attacking roots of plants have been found. Kerr (20) reported a marked interaction between Fusarium oxysporum f. pisi (Linford) Snyder and Hansen and Pythium ultimum affecting peas in Australia. Either pathogen caused no serious damage when acting alone, both fungi together produced severe wilt symptoms about 6 weeks after sowing and finally caused death of seedlings. On the other hand, Bateman (5) found that Thielaviopsis sp. and Pythium sp. in combination caused less root rot of poinsettia plants than was expected on basis of the expected additive amount of damage caused by the two pathogens separately. However, the amount of damage produced in combination was greater than that produced by either one alone.

Virus infection may predispose pea plants to increased infection by root rot fungi. Farley and Lockwood (11) reported more severe root rot symptoms when pea plants were infected with any of several viruses and either *A. euteiches* or *F. solani* f. *pisi* than when peas were infected with a fungus alone. Wheat plants infected with barley yellow dwarf virus were also more susceptible to some root rot pathogens (37). An increment in post-emergence damping-off of cucumber by *Rhizoctonia* sp. occurred in seedlings infected with cucumber mosaic virus (4).

Pythium spp. have been reported as producing economic damage of different levels of importance to many different commercial plants. For example, this pathogen was found to be the most common cause of seed decay and seedlings diseases in corn (18). It also produces damping-off of cotton seedlings (7), and was responsible for poor emergence and most damping-off of alfalfa seedlings (33, 34) as well as in red clover (15).

Intensity of root rot diseases is affected by soil fertility, moisture level, and temperature. Although *P. ultimum* has been isolated from soils and susceptible hosts collected from many different places in the world, this pathogen frequently causes no important injury (30). Jones and Linford (19) considered that the most obvious explanation for the failure of this fungus to produce more serious injury in plants is that a comparatively high soil temperature is required for its activity. The optimum temperature for *Pythium* disease of various plants generally ranged between 24° and 29° C. (13, 19, 34, 35, 38). Maximum development of Pythium mycelium in culture medium usually occurs at temperatures between 22° and 31° C. (6, 30). On alfalfa and poinsettia plants, a similar effect of temperature upon fungal growth rate and disease intensity was found (6, 27).

Pythium spp. often become progressively less aggressive as plants develop underground lignified tissues (29). Although this pathogen has been reported as responsible for root rot of older and mature plants, the attack is often restricted to immature parts of the root system (9, 13). An increased degree of intensity of Pythium root rot in hosts such as peas and poinsettia has occurred under wet soil conditions (3, 35, 40).

Another factor which may influence disease severity of this pathogen is that Pythia which produce pea root rot belong to several species differing somewhat in pathogenicity and frequency of occurrence (18, 31, 35). Also, lack of a sufficient inoculum level of fungus in certain areas may allow plants to escape disease (13).

MATERIALS AND METHODS

Isolation. Miragreen pea plants were grown in soil samples collected in a survey made in different fields of peas in Michigan. Pathogens infecting the roots of these plants were isolated by the agar plate method. The tap and lateral roots and the lower stem were washed with running water for 45 minutes, then cut into pieces approximately 2 cm. long and surface-sterilized by immersion in 0.5% sodium hypochlorite (Clorox) for 5 minutes. The disinfected pieces were then washed with sterile distilled water. Small portions of tissue from the margin of a disease lesion were excised, dried on sterile filter paper and then placed in Petri dishes on a medium containing 20 g agar and 10 mg Streptomycin per liter (17, 36). The inoculated plates were incubated for three days at 24° C. Mostly, three different kinds of mycelial growth were observed on the surface of the plates. Selected pieces of mycelium from different fungal colonies were transferred to test tubes containing potato-dextrose broth (PDA) (an infusion from 200 g potatoes, 20 g dextrose and 20 g agar per liter).

Preparation of inoculum. To prepare inoculum for pathogenicity tests and another experiments, isolates of *Pythium* were grown for 5 days at 24°C in 500 Erlenmeyer flasks containing 100 ml of potato-dextrose broth. The mycelia were washed 3 times with distilled water and fragmented in a Waring Blender for 15 seconds. Distilled water was added to each mycelial suspension to make 80 ml volume which was used for inoculation of 4 pots.

Selection of substrate for growing peas. A soil mixture composed of 3 parts compost soil and 1 part each of muck and sand was used in all experiments. Soil was autoclaved and placed in pots or glazed crocks. Ten or 12 Miragreen pea seeds were surface-sterilized with a 10% Clorox solution for 15 minutes, then sown in each pot or crock. Seedlings were inoculated 8 days later. This soil mixture was a suitable substrate for production of a uniform root rot on pea plants. Another soil mixture, 1 part each of sand and peat, was tried in same way but results were less satisfactory. In a seedling test for evaluating resistance of peas to *Aphanomyces* and *Fusarium* root rots, Lockwood and Ballard (25) found white sand to be a suitable substrate for this purpose. This growing medium was tried for *Pythium* root rot but disease development was not satisfactory.

Inoculation method. An injection method was used for inoculating plants in autoclaved soil (11, 27). Twenty ml of inoculum were added to each pot by applying 4 ml portions in 5 different positions with a hypodermic syringe inserted to a depth of 1 in. below the soil surface. Another trial for soil infestation consisted in mixing the same amount of inoculum with the soil before planting pea seeds. This method did not produce as uniform infection of the pea roots as did the injection method.

Evaluation of disease severity. Evaluation of disease severity was done by estimating increasing severity using a scale from 0-9 which was developed by Lockwood and Ballard (24, 25). It consists of grading separately the tops of the plants, epicotyls and roots. Slight, moderate and severe disease symptoms were rated as 1, 2 and 3, respectively. The separate ratings from each part of the plant were totalled. The ratings were made for the groups of plants in each pot and an average was obtained for all replications of each treatment. This was designated as the disease index. Each treatment was replicated with 3 or 4 pots and analysis of variance was applied to the individual pot ratings. The multiple range test developed by Duncan was used for ranking the treatment means (21).

RESULTS

Pea root rot survey. A survey was made in 1963 of 25 different fields of peas in the eastern part of Michigan in the area of the "thumb" and near Jackson in the south-central Michigan. Pea root rot in those fields ranged from slight to severe. Before peas were harvested, soil samples were collected from several locations in each field where pea plants showed disease symptoms. Collected soil samples were transported in polyethylene bags and then placed in 6 in. clay pots in the greenhouse. Ten surface-sterilized Miragreen pea seeds were sown in each pot. After 3 weeks most of the plants showed root rot disease symptoms. Isolations made from the diseased roots showed that *Fusarium solani* was present in peas grown in each of the 25 soil samples, *Aphanomyces euteiches* from 6 and *Pythium* sp. from 20 different soil samples.

Pathogenicity of the *Pythium* isolates. Pathogenicity of 20 different *Pythium* isolates from fields sampled in the survey was tested. The isolates tested all produced definite symptoms of root rot. The most severe symptoms of disease observed in Miragreen pea plants were rated as 7 and the least as 3.5 (Table 1). In general, the more severe disease symptoms were confined to the epicotyls and tap and lateral roots of the plants. Symptoms of the disease at an

Table 1. Disease indices of Miragreen peas infected by different *Pythium* isolates.

Pythium isolate	Disease index*	Uninoculated plants
1	6.0	0
2	7.0	0
3	5.0	0
4	4.5	0
5	5.0	0
6	5.0	0
7	3.5	0
8	6.5	0
9	6.0	0
10	5.0	0
11	3.0	0
12	4.0	0
13	5.5	0
14	6.0	0
15	6.0	0
16	6.5	0
17	4.0	0
18	5.0	0
19	6.0	0
20	5.5	0

*Disease index was based on a scale of increasing severity from 0-9. Each figure is a mean index of 3 pots each with 10 plants.

early stage were characterized by a somewhat water-soaked and softened diseased tissue extending above and below the seed attachment. Parts of affected roots showed numerous superficially necrotic spots. In advanced stages of the disease the shrunken tissue presented a black-brown necrosis of the cortex of the tap and lateral roots and lower stem below the soil. The leaves of some plants developed slight symptoms of wilting; the highest rating for foliage damage of plants was 1.5 of a maximum of 3.0.

Identification of species of *Pythium* isolates. An attempt was made to identify some of those *Pythium* isolates more pathogenic to peas. For this purpose, isolates 1, 8, 9, 15 and 16 were selected. Most of these isolates failed to produce both sexual and asexual organs when growing on PDA medium. A synthetic medium was used for obtaining sexual organs. The formula for this medium was supplied by Dr. A. F. Schmitthenner of the Ohio Agricultural Experiment Station. This medium contains sucrose 1.2 g, L-asparagine 0.13 g, K_2HPO_4 0.15 g, $MgSO_4$ 0.05 g, EDTA (Na_2) 0.029 g, ascorbic acid 0.01 g, agar 20 g, distilled water 1000 ml and 5 ml of a trace mineral containing $FeCl_3$ 0.048 g, $CaCl_2$ 0.278 g, $MnCl_2$ 1.438 g, $ZnCl_2$ 0.836 g per liter of distilled water. Another synthetic medium, MP 5, containing 4 g maltose, 1 g peptone and 20 g agar per liter was used for production of sporangia. Small blocks of this medium holding mycelium growth were suspended in sterile distilled water and half a boiled hemp seed was added to promote the production of those asexual organs. Descriptions compiled by Middleton (30) and Gilman (14) were used for identification of the Pythium species. Isolates number 1, 8 and 9 are Pythium ultimum Trow., 15 is Pythium debaryanum Hesse and 16 is Pythium monospermum Pringsheim.

Susceptibility of some commercial peas, foreign pea introductions and other plants to Pythium ultimum. Disinfected seeds of 10 commercial pea varieties, 4 pea introductions and 15 plants other than peas were sown in autoclaved soil mixture (Table 2). Pea seedlings were inoculated with P. ultimum 8 days after sowing. All of the commercial pea varieties and pea introductions developed root rot in the infested soil. The intensity of disease in those plants ranged from 4 to 5.5. Possibly, the pea introductions were slightly more resistant than commercial varieties. Other legumes showed a similar level of disease symptoms. Cereals were more resistant to Pythium root rot, the disease intensity ranging between 2 and 3.5. Nicotiana glutinosa was as susceptible as legume plants but Chenopodium amaranticolor, Gomphrena globosa, Cucumber (National Pickling) and Tomato (Bonny Best) were as resistant as cereal plants. In general, symptoms of disease were located mostly in the lower stem and on the tap and lateral roots of the plants. However, the foliage appeared healthy except in some pea varieties which showed symptoms of wilting and slight stunting.

Table 2. Disease indices of commercial peas, foreign pea introductions and other plants infected with _Pythium ultimum_ in a greenhouse test.

Host	Disease index*
Pea Introductions	
166159	4.0
169604	4.5
180693	4.5
175232	4.0
Commercial Pea Varieties	
Early Perfection	4.0
Miragreen	5.0
Alaska 94003	5.5
Freezer 33	5.5
Early Perfection 63579	4.5
Alderman	5.0
Bliss Everbearing	5.0
Pacific Freezer	5.0
Perfected Wales	4.5
Dark Skin Perfection	5.5
Other Plants	
Corn (Michigan 400)	3.5
Sweet Corn (Golden Cross Bantam)	1.5
Oats (Gopher)	2.5
Barley (Kindred)	3.0
Wheat (Little Club)	2.0
Beans (Bush Wax Pod King)	4.5
Soybeans (Keye)	4.5
Beans (Prince)	4.0
Cowpea (Black)	2.0
Beans (Pinto)	4.0
Tomato (Bonny Best)	2.5
Cucumber (National Pickling)	3.0
Gomphrena globosa	2.5
Nicotiana glutinosa	4.0
Chenopodium amaranticolor	1.5

*Disease index was based on a scale of increasing severity from 0-9. Each figure is a mean of 3 pots each with 10 plants. Uninoculated plants of each kind remained healthy.

<u>Effect of soil temperature on disease severity</u>. Autoclaved soil mixture was placed in glazed crocks and 12 surface-sterilized Miragreen pea seeds were sown in each crock. After 8 days, soil was infested with mycelia of isolates 8, 15 and 16, respectively, <u>P</u>. <u>ultimum</u>, <u>P</u>. <u>debaryanum</u> and <u>P</u>. <u>monospermum</u>. The crocks were kept in tanks containing water at constant temperatures of 12°, 16°, 20°, 24° and 28° C for 2 weeks. Increases in severity of the disease occurred with each increase in temperature from 12° to 28° C. (Table 3; Figs. 1, 2). The highest disease severity was obtained on those plants grown at a soil temperature of 28° C, and <u>P</u>. <u>ultimum</u> produced the highest disease index, 8.5, at this temperature. Each of the 3 <u>Pythium</u> species behaved in a similar way regarding the increment of disease as a response to higher temperatures in the soil. The mean disease indices for all 3 species of <u>Pythium</u> in soil at 12°, 16°, 20°, 24° and 28° C were 1.0, 2.3, 3.6, 5.6 and 7.5, respectively. Differences between these values were statistically significant at the 1% level. Although all 3 isolates behaved similarly, the disease index for <u>Pythium</u> isolate 16 was higher than those of isolates 8 and 15 at 20° C; the index for isolates 15 and 16 were higher than that of isolate 8 at 24° C, and values for isolates 8, 15 and 16 at 28° C all differed at the 5% level of significance. A similar experiment was run twice more using <u>P</u>. <u>ultimum</u> only. Crocks holding uninoculated plants were kept as controls for each tempera-

Table 3. Effect of soil temperature on pathogenicity of *P. ultimum*, isolate 8; *P. debaryanum*, isolate 15; and *P. monospermum*, isolate 16, on Miragreen peas.

Temperature, ^{o}C	Disease index for indicated isolate*			
	P. ultimum (8)	*P. debaryanum* (15)	*P. monospermum* (16)	Mean
12^{o}	1.0	1.0	1.0	1.0
16^{o}	2.5	2.0	2.5	2.3
20^{o}	3.5	3.0	4.5	3.6
24^{o}	5.0	6.0	6.0	5.6
28^{o}	8.5	6.5	7.5	7.5

*Disease index was evaluated using a scale of increasing severity from 0-9. Each figure is a mean of 4 crocks, each with 12 plants. Differences between the mean values were statistically significant at the 1% level.

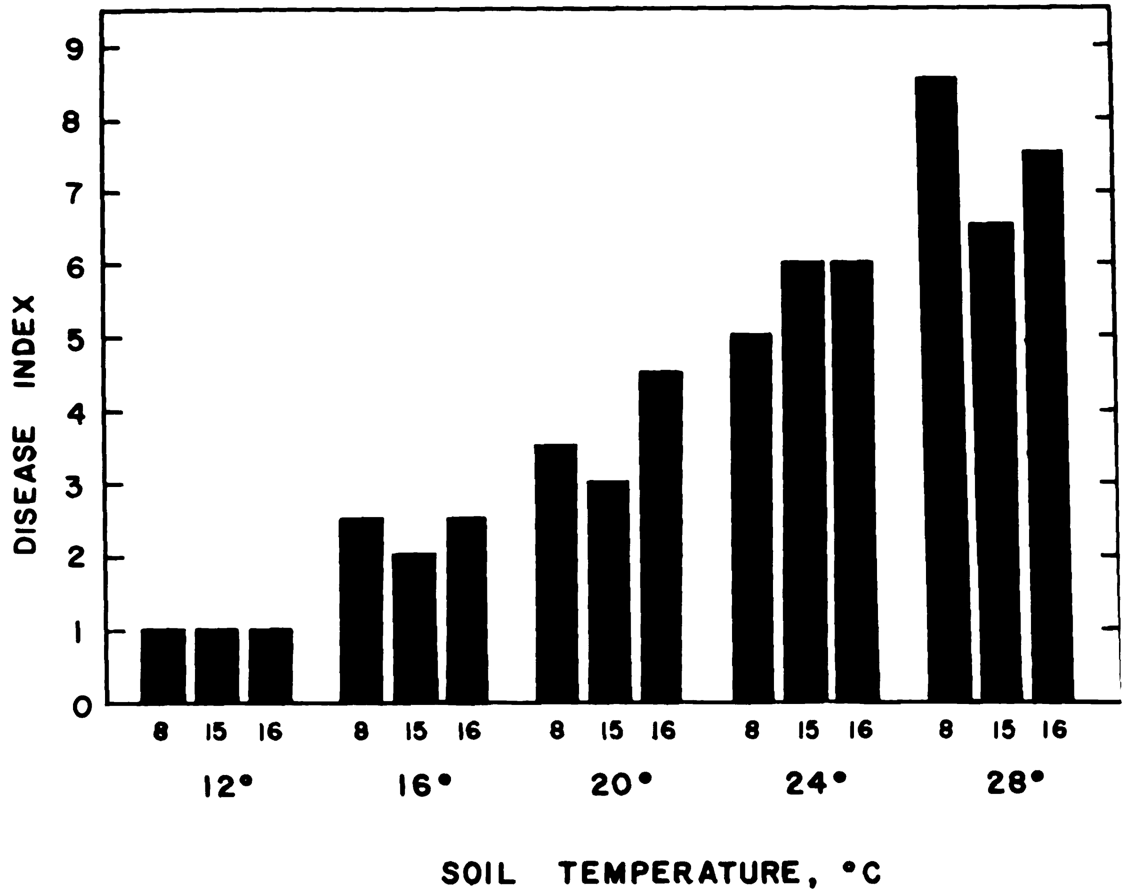

Fig. 1. Effect of soil temperature on pathogenicity of P. ultimum, isolate 8; P. debaryanum, isolate 15; and P. monospermum, isolate 16, on Miragreen peas. Differences between the mean values for 3 Pythium isolates at each different temperature were statistically significant at the 1% level.

Fig. 2. Effect of soil temperature on pathogenicity of Pythium ultimum on Miragreen peas. Left to right: disease symptoms at 12°, 16°, 20°, 24° and 28° C.

ture. Statistically significant increases in disease severity with each increase in temperature were again found. Control plants presented slight root damage rated as 0.5 and 1.0 at temperatures of 24° and 28° C, respectively, but not at lower soil temperatures. Fresh weights of uninoculated plants, at temperatures of 12°, 16°, 20°, 24° and 28° C were 44 g, 38 g, 33 g, 27 g and 26 g per pot, respectively. Thus, pea plants grew best at cooler temperatures. Nevertheless, plants appeared healthy at all temperatures used. Therefore, the increased disease indices which occurred as temperature increased were in fact due to increased root rot symptoms and not to a direct effect of unfavorable temperature on the host. This was confirmed by fresh weights of diseased plants which were 32 g, 28 g, 23 g, 17 g, and 14 g over the same increasing temperature range. It can be seen that the greatest percentage losses in fresh weights occurred at the higher temperatures.

Effect of the temperature on growth of mycelium in culture medium. The effect of the same 5 different temperatures on the mycelial growth of *P. ultimum*, isolates 8 and 9; and *P. debaryanum*, isolate 15; was determined. Erlenmeyer flasks, each containing 50 ml of potato-dextrose broth were seeded with an 8 mm diameter PDA disk containing a uniform mycelial growth of the fungus. Three flasks containing each isolate were incubated at 12°, 16°, 20°, 24° and 28° C for 5 days. The mycelial mat from each flask was rinsed through a Buchner funnel at low air pressure with 40 ml of distilled water. Mycelial mats were placed in pre-weighed aluminum cups and dried for 24 hr. at 100° C. Thus, total mycelium growth of each fungus isolate was measured by weight of oven-dried mycelium. The optimum temperature for growth of mycelium of the 3 Pythium isolates was 24° C (Table 4; Fig. 3). At temperatures higher or lower than 24° C mycelial growth was significantly less. A poor development of the fungi was produced at 12° C, but there was a significant increment of growth at 16° C. No significant difference was found among the mycelial weights at temperatures of 16°, 20° and 28° C. Differences between isolates at different temperatures were not significant. The optimum temperature for mycelial growth in artificial medium was below the soil temperature which produced the highest intensity of the disease (Fig. 4). Disease severity was always increased in soil from 12° to 28°C, and although mycelial growth increased at temperatures from 12° to 24°C, it decreased at 28°C.

Effect of age of pea plants on susceptibility to *Pythium ultimum* and *Aphanomyces euteiches*. Sixty clay pots filled with autoclaved soil mixture were planted at different time intervals with 10 surface-sterilized Miragreen pea seeds. Soil in all pots was infested at the same time with either *P. ultimum* or *A. euteiches* when pea plants were 1, 8, 15, 22 and 29 days old.

Table 4. Effect of temperature on growth of mycelium of three *Pythium* isolates in potato-dextrose broth.

Temperature, ^{o}C	Mean dry wt. of mycelium, mg*			
	P. ultimum (8)	*P. ultimum* (9)	*P. debaryanum* (15)	Mean
12^{o}	38	43	38	40^{a}
16^{o}	191	200	196	196^{b}
20^{o}	213	222	217	217^{b}
24^{o}	255	248	270	258^{c}
28^{o}	218	239	210	222^{b}

*Each figure is a mean of 3 dried mycelial mats. Means followed by different letters differed statistically at the 5% level of significance. Differences between isolates at the same temperatures were not significant.

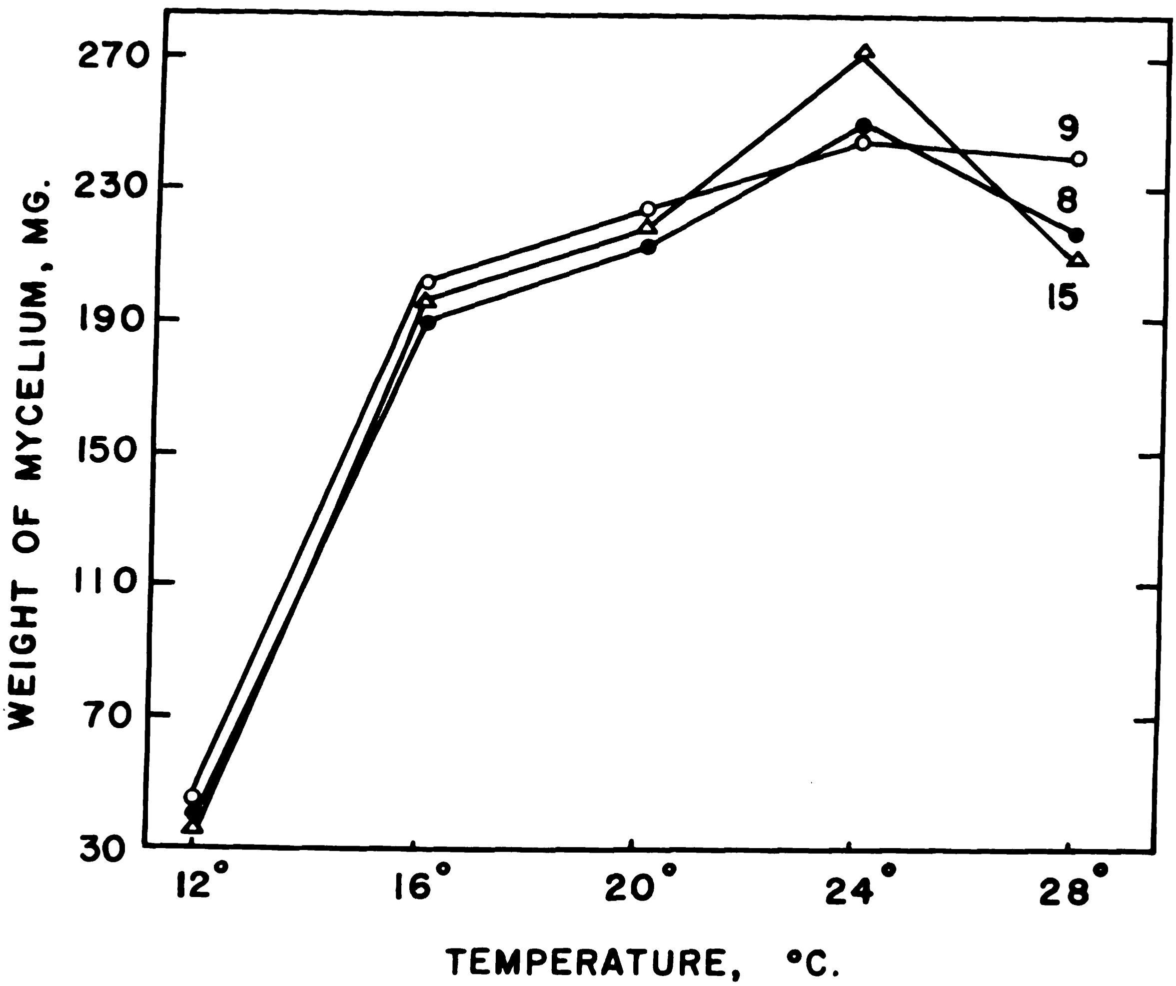

Fig. 3. Effect of temperature on growth of mycelium of P. ultimum, isolates 8 and 9; and P. debaryanum, isolate 15, in potato-dextrose broth. Mean values of the 3 Pythium isolates, at 16°, 20° and 28° C did not differ at the 5% level of significance.

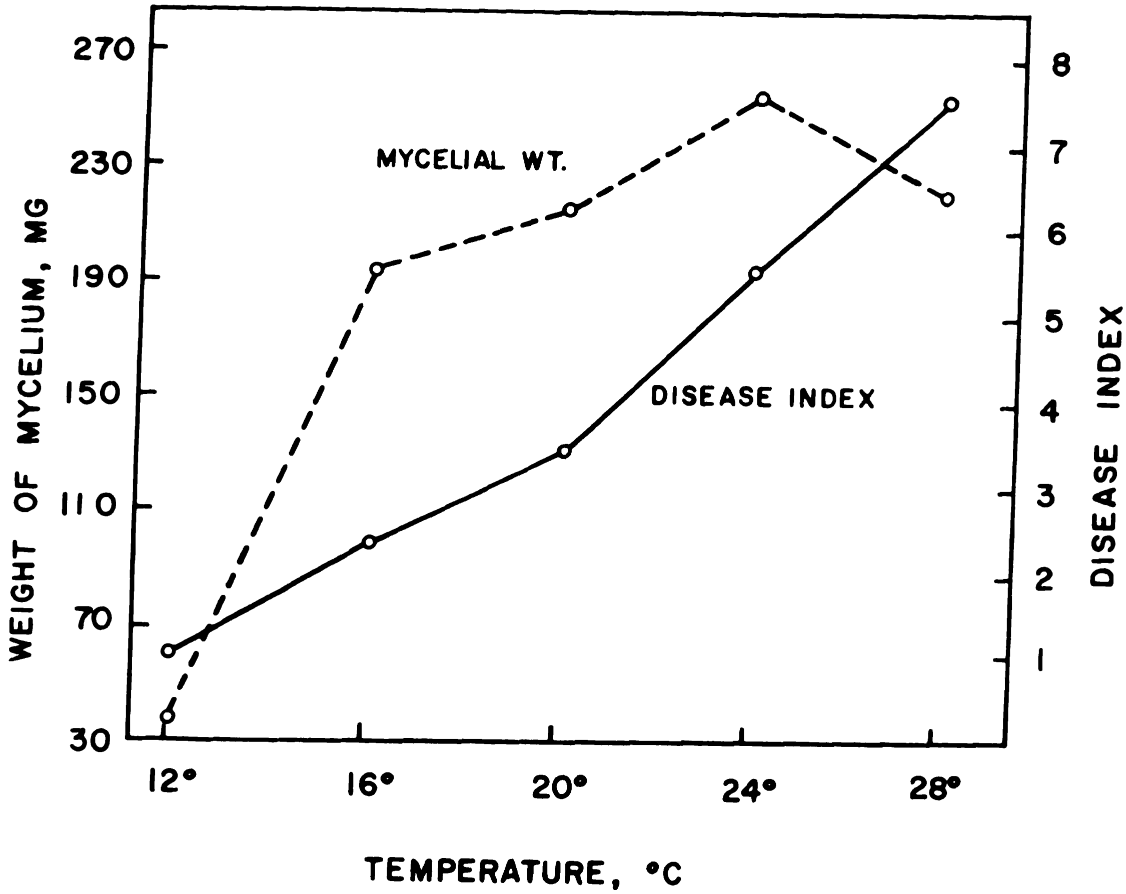

Fig. 4. Effect of soil temperature on Pythium disease severity compared with the effect of temperature on growth of mycelium.

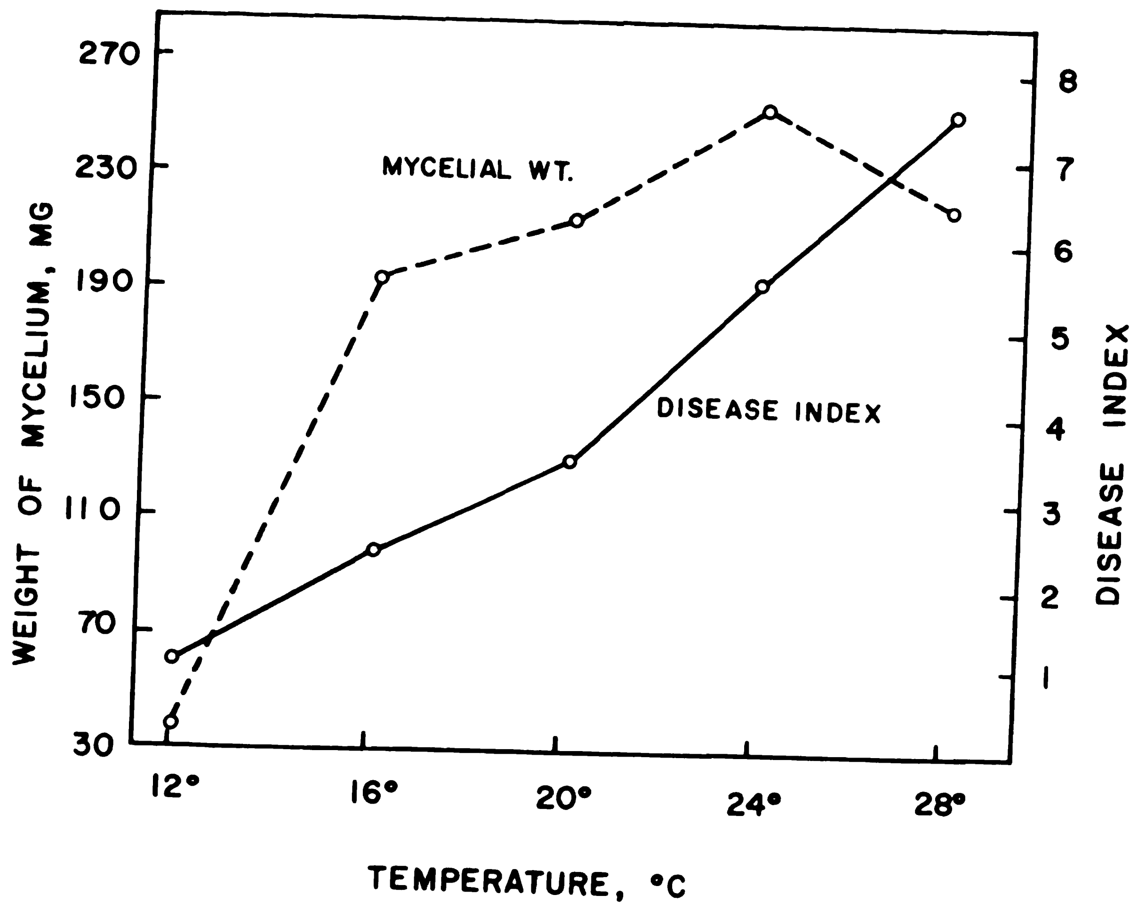

Fig. 4. Effect of soil temperature on *Pythium* disease severity compared with the effect of temperature on growth of mycelium.

Uninoculated plants were kept as controls for each age. Seventeen days after inoculation, plants were examined and the intensity of the disease was evaluated. Disease index of Aphanomyces root rot was 8.8 in those plants inoculated 1 day after seeding soil, but the disease intensity decreased in older plants (Table 5; Figs. 5, 6, 7, 8, 9, 10). The lowest disease index, 4.5, occurred in plants inoculated at 29 days of age. On the other hand, pea plants grown in soil infested with P. ultimum showed increases in disease severity with each increase in age of the plants from 1 to 29 days. Disease severity was rated as 3.8, 4.1, 4.6, 5.6 and 6.2 in soil infested when plants were 1, 8, 15, 22 and 29 days old, respectively. Statistically significant differences (5% level) were obtained between disease indices 4.6, 5.6 and 6.2. Control plants presented some root rot decay which was assessed as 0.3, 0.8, 1.1 and 1.2 in plants at 8, 15, 22 and 29 days of age. Similar results were obtained in another similar experiment. However, in this experiment the disease index in plants inoculated when 29 days old was not significantly different from that at 22 days.

Effect of combinations of Aphanomyces euteiches, Fusarium solani and Pythium ultimum on severity of pea root rot. Standard inoculum suspensions of mycelia of A. euteiches, F. solani and P. ultimum were used to infest soil containing 8 day-old pea plants. One-half the usual quantity of inoculum was used for some treatments

Table 5. Effect of age of pea plants on susceptibility to *Pythium ultimum* and *Aphanomyces euteiches*.

Age of plants at inoculation, days	Disease index*		
	P. ultimum	*A. euteiches*	Control
1	3.8[a]	8.8	0
8	4.1[a]	8.2	0.3
15	4.6[b]	7.0	0.8
22	5.6[c]	6.1	1.1
29	6.2[d]	4.5	1.2

*Disease index was evaluated using a scale of increasing disease severity from 0-9. Each figure is a mean index of 4 pots, each with 10 plants. Values followed by different letters differed statistically at the 5% level of significance.

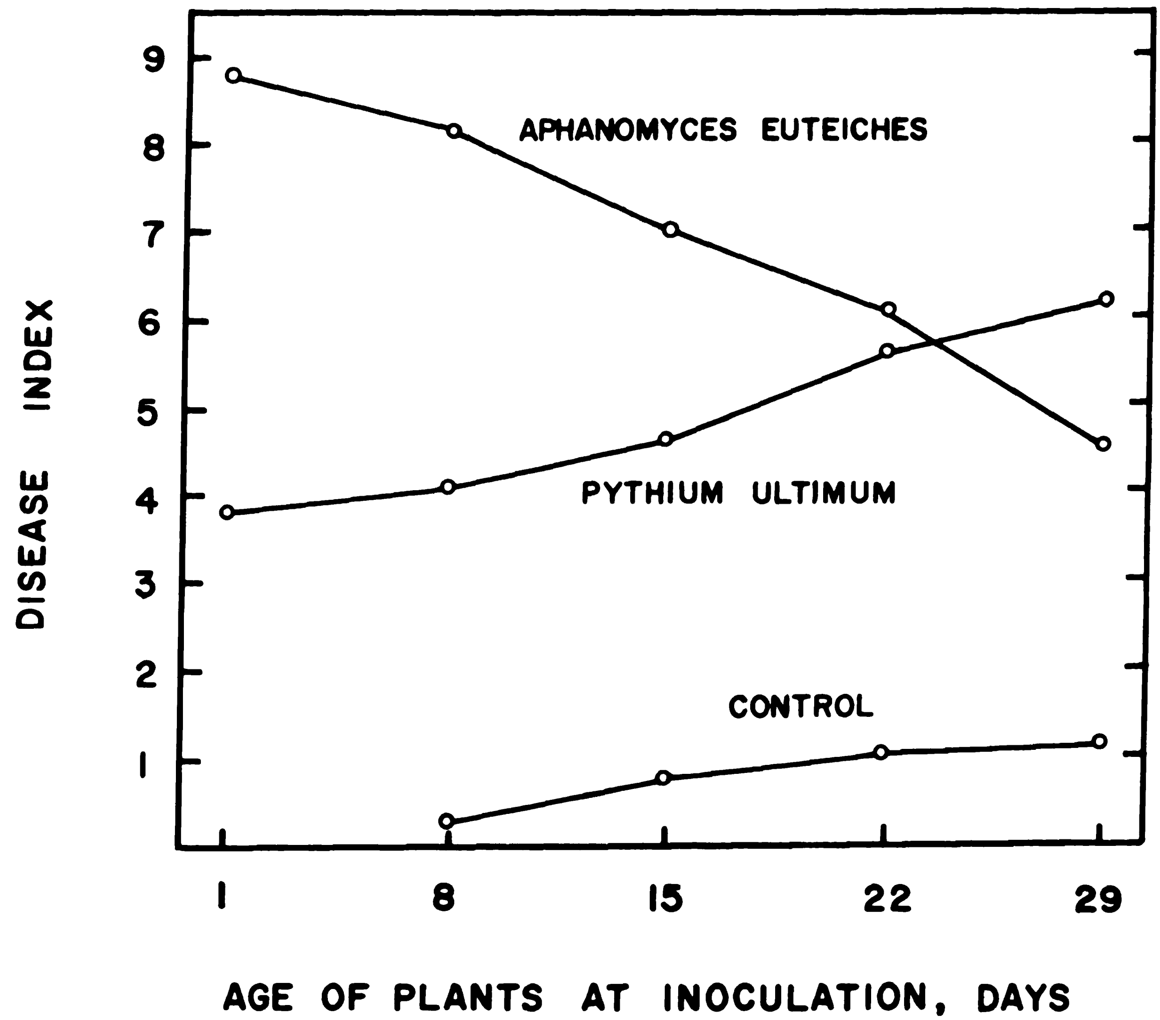

Fig. 5. Effect of age of pea plants on susceptibility to Pythium ultimum and Aphanomyces euteiches. Index values for P. ultimum at 8, 15, 22 and 29 days were statistically different at the 5% level of significance. Values for 1 and 8 days were not statistically different.

Fig. 6. Effect of age of Miragreen peas on susceptibility to *Pythium ultimum* and *Aphanomyces euteiches*. Symptoms when plants were inoculated one day after sowing. (left) *P. ultimum*, (center) control and (right) *A. euteiches*.

Fig. 7. Effect of age of Miragreen peas on susceptibility to *Pythium ultimum* and *Aphanomyces euteiches*. Symptoms when plants were inoculated 8 days after sowing. (left) *P. ultimum*, (center) control and (right) *A. euteiches*.

Fig. 8. Effect of age of Miragreen peas on susceptibility to *Pythium ultimum* and *Aphanomyces euteiches*. Symptoms when plants were inoculated 15 days after sowing. (left) *P. ultimum*, (center) control and (right) *A. euteiches*.

Fig. 9. Effect of age of Miragreen peas on susceptibility to *Pythium ultimum* and *Aphanomyces euteiches*. Symptoms when plants were inoculated 22 days after sowing. (left) *P. ultimum*, (center) control and (right) *A. euteiches*.

Fig. 10. Effect of age of Miragreen peas on susceptibility to Pythium ultimum and Aphanomyces euteiches. Symptoms when plants were inoculated 29 days after sowing. (left) P. ultimum, (center) control and (right) A. euteiches.

for better comparison with single fungi. Pots were inoculated with F. solani alone, A. euteiches alone, P. ultimum alone, P. ultimum plus F. solani (1/2 quantity each), P. ultimum plus F. solani (usual quantities), P. ultimum plus A. euteiches (1/2 quantity each) and P. ultimum plus A. euteiches (usual quantities). Disease was estimated 2 weeks after inoculation (Table 6, Fig. 11). Respective disease indices for F. solani, P. ultimum and A. euteiches were 4, 4.5 and 8. Disease severities ranging between 7.5 and 8.7 were obtained in those treatments in which any 2 pathogens acted together. Significant differences were not found between these values, thus, similar results were produced whether half or full quantities of inoculum were used. Of particular interest was the additive effect of combined inoculation with P. ultimum and F. solani. These pathogens together caused a disease severity almost equivalent to the sum of disease indices produced by the same pathogens acting separately. Other pathogens combinations did not produce this effect. Another similar experiment gave the same results.

Increased root rot severity of Miragreen peas infected with a virus and Pythium ultimum. The 2 first leaves of 8 day-old pea plants were inoculated with a virus by dusting them with carborundum, then rubbing twice with a cotton swab which had been dipped in sap of pea plants containing either bean yellow mosaic virus (BYMV) or pea mosaic virus (PMV). Soil was infested with P. ultimum 3 days

Table 6. Effect of combinations of *Aphanomyces euteiches*, *Fusarium solani* and *Pythium ultimum* on pea root rot severity.

Treatment	Mycelial mats per 4 pots	Disease index*
Pythium	1	4.5[a]
Fusarium	1	4.0[a]
Aphanomyces	1	8.0[b]
Pythium + *Fusarium*	1/2 + 1/2	7.5[b]
Pythium + *Fusarium*	1 + 1	8.0[b]
Pythium + *Aphanomyces*	1/2 + 1/2	8.0[b]
Pythium + *Aphanomyces*	1 + 1	8.7[b]
Control	-	0.0[c]

*Disease index was evaluated using a scale of increasing disease severity from 0-9. Each figure is a mean index of 4 pots, each with 10 plants. Means followed by different letters differed statistically at the 5% level of significance.

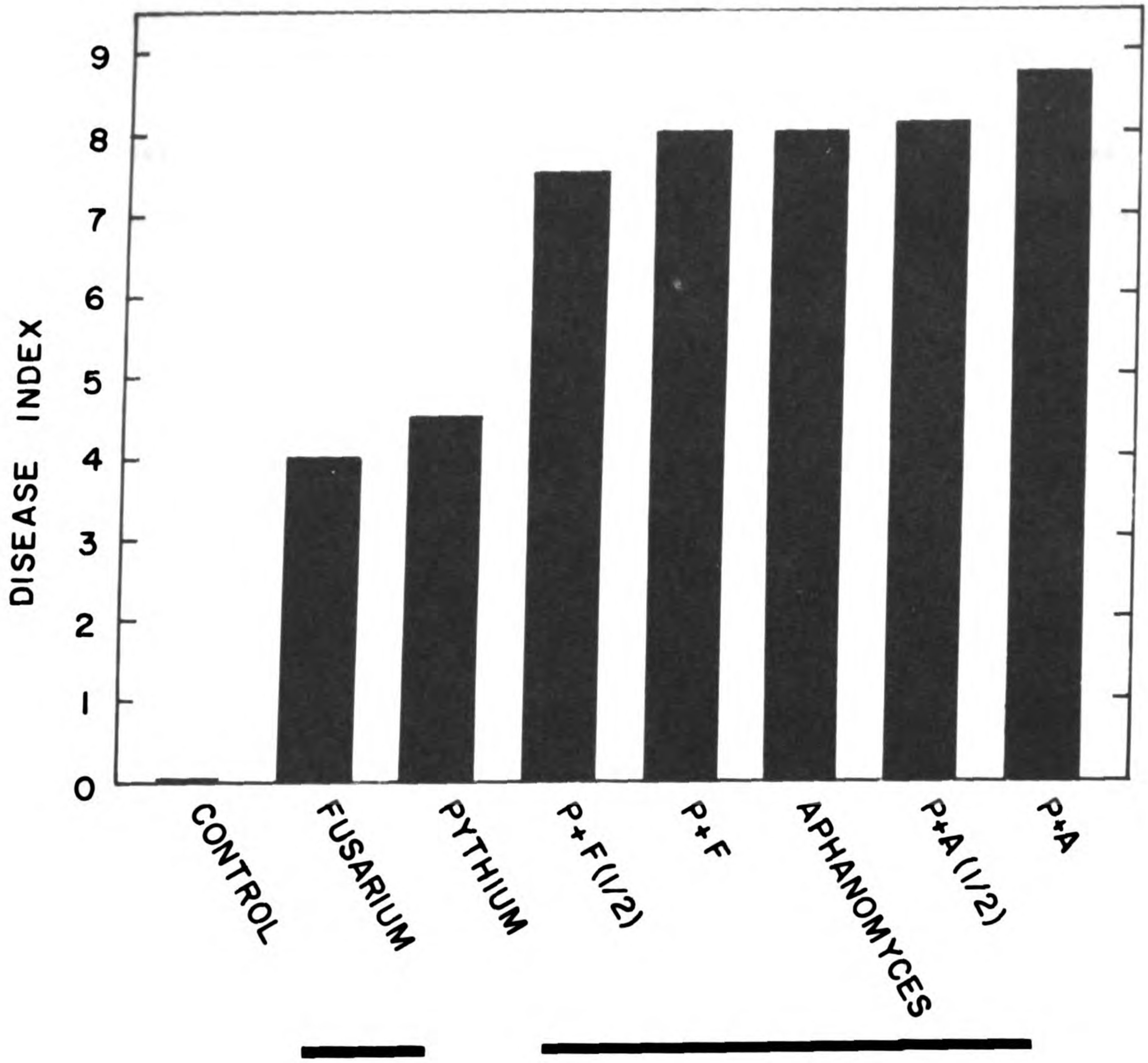

Fig. 11. Effect of combinations of Aphanomyces euteiches, Fusarium solani and Pythium ultimum on pea root rot severity. Treatments underscored by a line did not differ statistically at the 5% level of significance.

after the fungus was applied. The highest disease index, 7.8, was obtained in plants infected with both a virus and the fungus (Table 7; Fig. 12, 13, 14). The effect produced by P. ultimum alone was rated as 4.4, a value significantly lower than that produced in plants infected with either virus or the fungus. Disease levels in plants infected with either BYMV or PMV alone were significantly lower than those in plants infected by the fungus alone. Disease severity in virus-fungus infected plants was considerably higher than the sum of disease indices of plants infected by both the fungus alone and either virus alone. It can be noted, in Figs. 13 and 14, that disease severity in virus-fungus infected plants was increased in all parts of the plants and was characteristic of a severe root infection. Similar results were obtained in another experiment.

Table 7. Increased root rot severity of Miragreen peas infected with a virus and *Pythium ultimum*.

Treatment	Disease index*
Pythium ultimum	4.3[a]
BYMV	0.3[b]
PMV	0.2[b]
P. ultimum + BYMV	7.7[c]
P. ultimum + PMV	7.7[c]
Control	0.0[b]

*Disease index was evaluated using a scale of increasing severity of the disease from 0-9. Each figure is a mean index of 4 pots, each with 10 plants. Values followed by different letters differed statistically at the 1% level of significance.

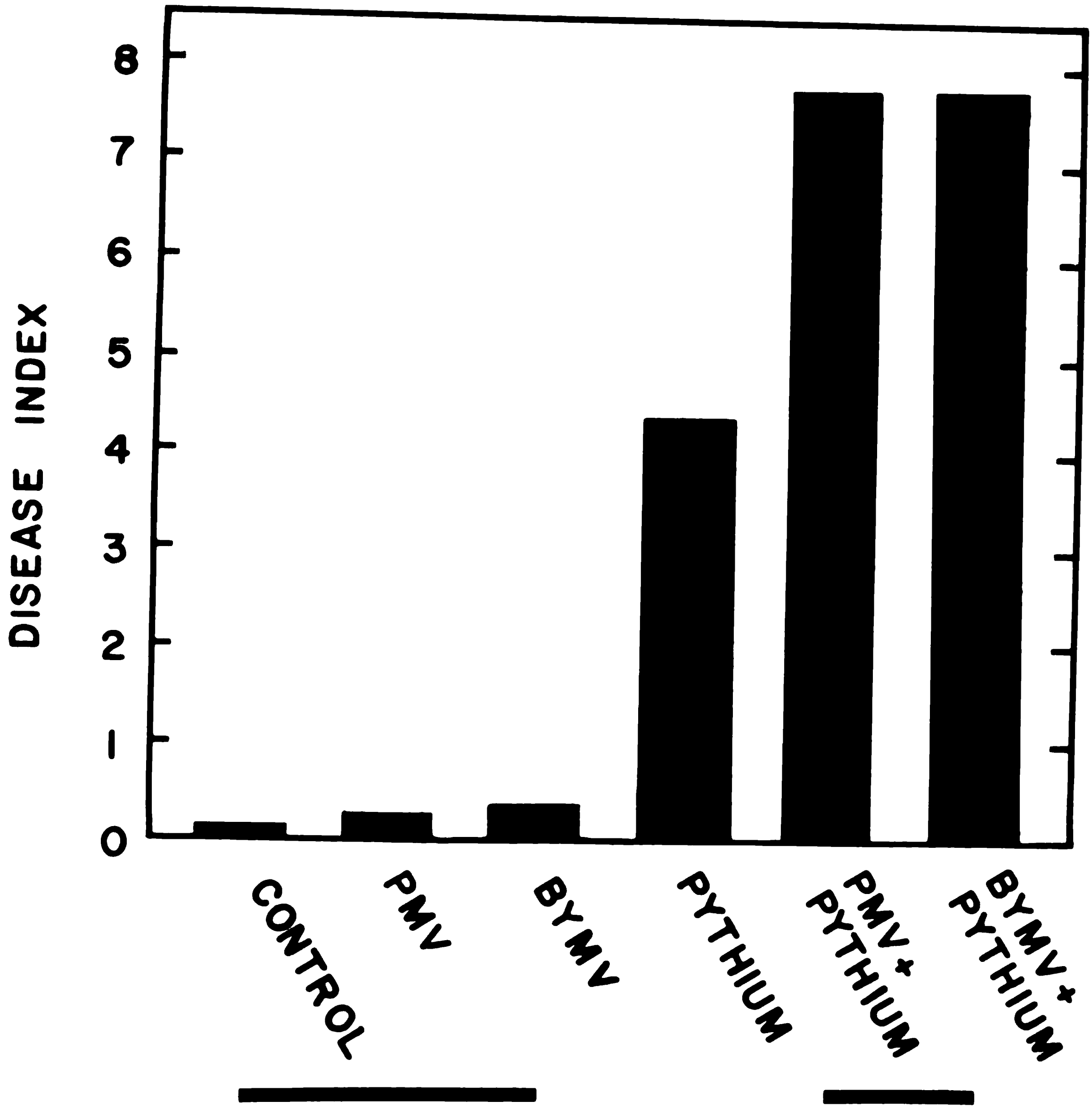

Fig. 12. Increased root rot severity of Miragreen peas infected with a virus and Pythium ultimum. Treatments underscored by a line did not differ statistically at the 1% level of significance.

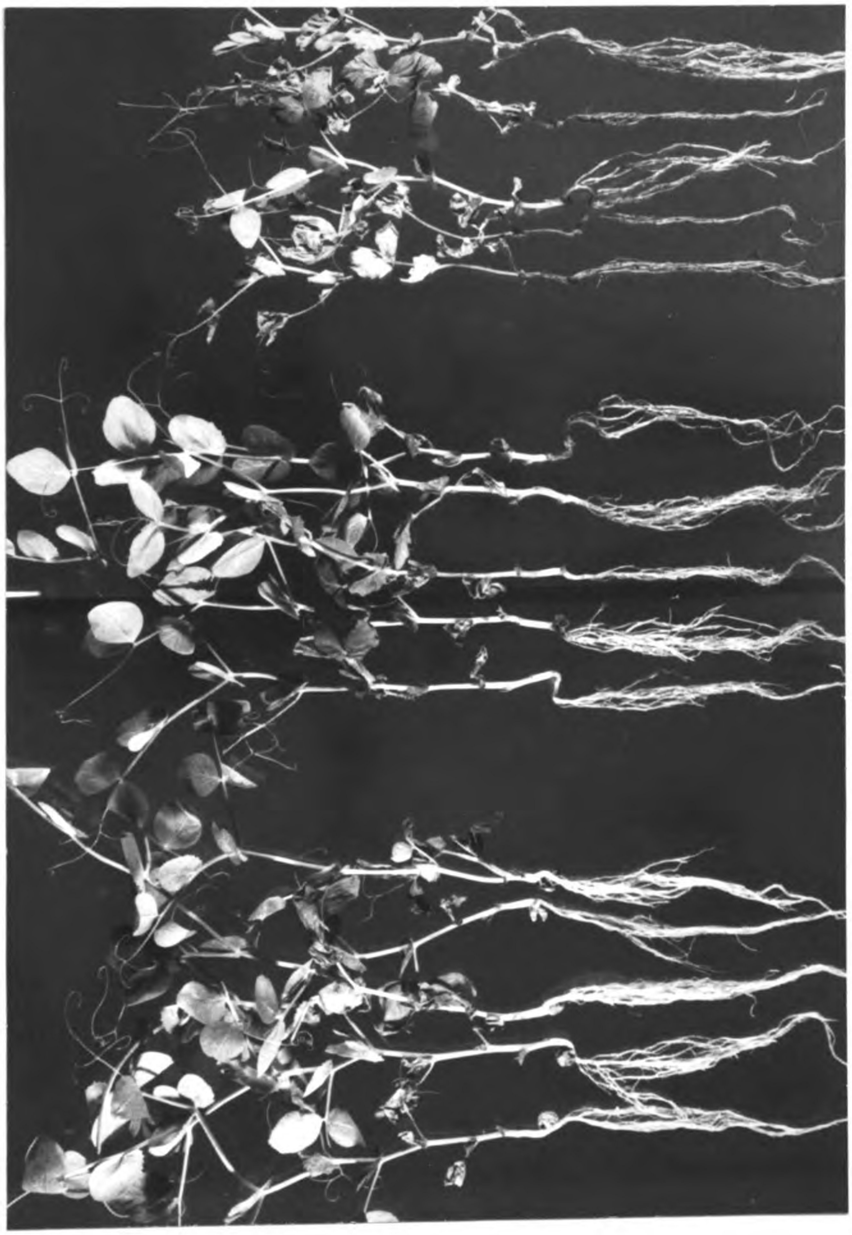

Fig. 13. Miragreen peas infected with: (left) Bean Yellow Mosaic Virus, (center) *Pythium ultimum* and (right) Bean Yellow Mosaic Virus and *P. ultimum*.

Fig. 14. Miragreen peas infected with: (left) Pea Mosaic Virus, (center) *Pythium ultimum* and (right) Pea Mosaic Virus and *P. ultimum*.

DISCUSSION

Pythium sp. may be an important component in the pea root rot complex in Michigan. The results of this survey show that *Pythium* sp. was present in soil samples from 20 out of 25 different pea fields in Michigan in the area of the "thumb" and near Jackson. Moreover, all isolates of this fungus were able to produce considerable root rot disease on pea plants in greenhouse tests. In 1960, Lockwood (23) considered that fungi other than *A. euteiches* and *F. solani* involved in the pea root rot complex in Michigan, and obtained pathogenic isolates of *Pythium* sp. and other pathogens from root rot infected peas.

Three of 5 *Pythium* isolates were identified as *P. ultimum*. According to the literature, when *Pythium* spp. are reported as causing damage on pea plants, *P. ultimum* is frequently mentioned as producing the disease, or associated with other pathogens which also cause infection in this host (16, 35, 41). The other 2 isolates identified were *P. debaryanum* and *P. monospermum*. These were as virulent in greenhouse tests as were the isolates of *P. ultimum*. It would be of interest to identify other isolates of *Pythium* pathogenic to peas in order to establish which species of *Pythium* are most frequently associated with pea root rot.

P. ultimum isolated from infected pea plants produced considerable damage to roots of several commercial pea varieties, pea introductions and legumes other than peas. McDonald and Marshall (28) found a high percentage of susceptibility to this fungus in about 450 pea introductions and varieties. In the present study cereal plants, tomato (Bonny Best), cucumber (National Pickling), Gomphrena globosa, Nicotiana glutinosa and Chenopodium amaranticolor were less susceptible to this fungus. However, isolates of P. ultimum from peas, in this work, were more pathogenic on legumes in general than on other plants tested, suggesting some degree of host specificity with respect to virulence.

Christensen has reported Pythium sp. as responsible for root rots of older plants of wheat, oats and barley, but with damage restricted mainly to immature parts of the root system (9). Although Pythium spp. often become progressively less aggressive as plants develop underground lignified tissues results obtained with pea plants inoculated with P. ultimum 1, 8, 15, 22 and 29 days after seeding, showed a similar degree of susceptibility, a possible slightly increased susceptibility at 22 and again at 29 days.

In peas, damage was not restricted to younger parts of the root system. When pea plants were inoculated with A. euteiches, most severe disease symptoms were observed in those plants inoculated 1 day after seeding, but plants became more resistant to the pathogen with each increase in age.

Kerr (20) considered a synergistic interaction which occurred between *F. oxysporum* f. *pisi* and *P. ultimum* as an important factor limiting the production of peas in Australia. In the present work an additive effect was produced when pea plants were infected with both *P. ultimum* and *F. solani*. Damage produced by these 2 pathogens together was almost equivalent to that produced by *A. euteiches* alone. Since the latter pathogen is considered the most damaging root rot fungus on peas (23), it should be considered that combined infection by *P. ultimum* and *F. solani*, both of which are very frequently found in agricultural lands, may produce a disease severity similar to that produced by *A. euteiches*.

An even greater enhancement of disease occurred in plants infected with a virus and *P. ultimum*. Miragreen peas infected with either BYMV or PMV and *P. ultimum* showed more severe symptoms than peas infected with the fungus alone. Final symptoms shown by the virus-fungus-infected plants of peas were characteristic of those produced by the fungus alone. Farley and Lockwood (11) reported more severe root rot symptoms when pea plants were infected with any of several viruses and either *A. euteiches* or *F. solani* f. *pisi* than when peas were infected with either fungus alone. Several cases of synergism between virus-fungus-infected plants have been reported in legumes other than peas and in cereals (4, 11, 37).

Since virus diseases are second in importance to pea root rot in Michigan (26), the synergistic reaction found in those virus-fungus-infected plants of peas suggest the desirability further of work to better understand the interaction between these 2 pathogens. The practical importance of this interaction is supported by the work of Watson and Guthrie (39) who reported a severe root rot epiphytotic on pea plants associated with widespread virus infection. The root fungi isolated were unable to reproduce the original syndrom when tested either singly or in combination.

The actual mechanism which brings about the increase in susceptibility in virus-infected plants to root rot pathogens is as yet little understood. One possibility is that changes in the rhizosphere microflora due to altered host metabolism may result in more suitable conditions for the fungi infecting roots of peas (32). Sadasivan (32) also found that rhizospheres of the legume Dolichos lablab showed a considerable increment of bacteria and actinomycetes after inoculation with Dolichos enation mosaic virus. He also found an increase in the free amino acid contents of the infected plants. Possibly, this might result in a larger amount of amino acids in exudations from roots of such plants, and provide a more suitable source of nutrition for the pathogens. Moreover, Farley and Lockwood (11), through a transplanting experiment, found evidence which suggested that virus infection in pea plants increased fungus inoculum potential at the root surface. The possibility also exists that the host resistance is decreased as a result of virus infection.

In most cases, diseases caused by *Pythium* are reported to be unfavorable for growth of the host, with little relation to the temperature favoring fungus growth (7). Increased disease severity produced by *P. ultimum* on Miragreen peas increased with soil temperatures from 12° to 28° C. On the other hand, the optimum temperature for growth of the mycelium of *Pythium* sp. in culture medium was at 24° C, a temperature below that producing most severe disease on pea plants, but above the optimum for growth of the host which is reported to be between 15° and 18° C (7). *Thielaviopsis basicola* behaves in a similar way with respect to the relation between soil temperature, growth temperature and root rot severity on peas. *Thielaviopsis* root rot in high temperature plants such as tobacco, orange and poinsettia was most severe at low soil temperatures (6, 27). Analogous results can be cited for *Pythium* sp. and other pathogens of cotton seedlings which grew best at 28° C (2). In general, disease severity was greatest at 21° C whereas the optimum for the crop was 30° C (12).

In view of fact that isolates of *Pythium* sp. pathogenic to pea occur in most pea fields in Michigan, further research on this root rot disease would be profitable. If the *Pythium* disease on peas is an important factor limiting production of this legume, it would be important to search for possible sources for resistance for use in breeding.

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