The Effect of Temperature on the Pathogenicity of Pythium aphanidermatum, P. debaryanum, and P. ultimum on Soybean

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ABSTRACT

Pythium aphanidermatum, P. debaryanum, and *P. ultimum* were all pathogenic on soybeans. Three methods of inoculation were used: (i) placing a small piece of mycelium in an incision in the hypocotyl; (ii) pouring macerated mycelium over seed in the soil; and (iii) placing seeds on agar overgrown with the fungi. All three techniques led to infection except when inoculum of *P. aphanidermatum* was poured over seeds in soil. Temperature has a differential effect on severity of disease incited by these fungi. *Pythium aphanidermatum* infected soybeans between 24 and 36 C, while *P. debaryanum* and *P. ultimum* were more virulent at 15 to 20 C or after preconditioning at 4, 8, or 12 C more than 4 days. Phytopathology 61:933-935.

Additional key words: soybean seed, root rot.

Three species of *Pythium* have been reported to be pathogenic on soybean (*Glycine max* [L.] Merr.). *Pythium debaryanum* Hesse was first reported on soybean in 1926 by Lehman & Wolf (3) in North Carolina; *P. ultimum* Trow was added to the list in 1952 when Hildebrand & Koch (2) identified it as the causal agent of a root and stem rot of soybean in Ontario; *P. aphanidermatum* (Edson) Fitz. was first reported as a virulent pathogen of soybean by Morgan & Hartwig in 1964 (5).

Pythium debaryanum and *P. ultimum* have generally been associated with soybean seed rot and seedling root rot under cool, wet conditions. The effect of temperature on the growth rate of various species of the genus has been studied by many workers. *Pythium ultimum* and *P. debaryanum* have the same temperature range for mycelial growth. The minimum is about 1 C, the maximum 37 C, and the optimum 28 C (1, 4, 6, 7, 8). With *P. aphanidermatum* the minimum is 10 C; the optimum, 34 C; and the maximum, 45 C (4, 10). Temperature may influence the distribution and the amount of infection caused by each species.

Many soybean cultivars are resistant to *Phytophthora* root rot. This has made damage from *Pythium* more obvious, and a need for resistant cultivars more apparent.

The purpose of this study was to determine (i) if *P. aphanidermatum, P. debaryanum,* and *P. ultimum* are equally pathogenic on soybeans; (ii) if the species differ in their temperature requirements for infection; and (iii) methods of inoculation that could be used in evaluating resistance.

MATERIALS AND METHODS.—The cultures of *Pythium* used were: *P. aphanidermatum* ATCC No. 16994; *P. debaryanum* ATCC No. 9998, and *P. ultimum* isolated from soybeans in 1963 in Indiana. Their identity was verified by using keys prepared by Middleton (4) and Waterhouse (9).

A single lot of the soybean cultivar Harosoy 63, resistant to *Phytophthora* root rot caused by *Phytophthora megasperma* (Drechs.) var. *sojae* A. A. Hildeb., was used throughout the study.

To determine the pathogenicity of the three species, hypocotyl inoculations were made. Inoculum was prepared by growing the fungus on oatmeal agar for 5 days. The mat that formed was cut into pieces which were individually placed into an incision in the hypocotyl of 8- to 9-day-old plants. The inoculation site was covered with petroleum jelly to prevent the wounded stem tissue and mycelium from drying out. Control plants were wounded without insertion of mycelium. Pots were placed in temperature chambers at 15, 20, 22.5, 24, 28, 30, 32, and 36 C with a photoperiod of 16 hr. The number of plants killed was recorded 8-9 days after inoculation.

To determine the effect of the three species on soybean germination, surface-disinfected seed were placed on potato-dextrose agar (PDA) seeded with cultures of the fungus and on control plates. Disinfection was accomplished by immersing seed in a 1:5 Clorox (5.25% sodium hypochlorite)-water solution for 1 min. Five seeded agar plates of each organism and five nonseeded control plates were placed in temperature chambers ranging from 16 to 36 C at 4 degree intervals. A second group of seeded and nonseeded plates was kept at a 4-C conditioning temperature. Every 2 days for a period of 10 days, five plates of each fungus and five control plates were transferred to the temperature chambers. Germinated seeds were counted when bean roots in the control plates were 5-7 cm long at 24 C and above and 2-3 cm at 20 C and below. Similar experiments were conducted in which the conditioning temperatures were either 8 or 12 instead of 4 C.

The effect of the three fungi on germination of soybeans in a nonsterile soil mixture was also studied. Inoculum was prepared by macerating 5-day-old fungus mycelium from 10 petri plates in 900 ml water. Inoculation was accomplished by pouring 50 ml of the mycelial suspension over 10 seeds on 8 cm of soil in 4-inch pots and covering them with 2-3 cm of sand. Tap water was poured over seeds in control pots. Fifteen pots of each isolate and five control pots were placed in temperature chambers at 15, 22.5, and 30 C with a

TABLE 1. The effect of temperature on survival of soybeans following hypocotyl inoculation with three species of *Pythium*

Temp (C)	% Plants killed[a]		
	P. aphanidermatum	*P. debaryanum*	*P. ultimum*
15	65	98	100
20	98	91	73
22.5	90	58	84
24	88	32	35
28	100	14	36
30	96	15	32
32	99	3	15
36	99	7	4

[a] One hundred to 150 plants inoculated at each temperature with each species. Similar numbers of noninoculated control plants at each temperature showed no disease.

16-hr photoperiod. A second group of pots was placed at 4 C and transferred to the temperature chambers at 2-day intervals over a period of 10 days. The number of emerged seedlings was counted after the first trifoliolate leaves had appeared on the noninoculated control plants.

RESULTS.—*Hypocotyl inoculation.*—The effect of temperature on survival of soybeans inoculated in the hypocotyl with the three fungi is given in Table 1.

Sixty-five per cent of the plants inoculated with *P. aphanidermatum* were killed at 15 C; at 20-36 C, between 88 and 100% succumbed. *Pythium debaryanum* and *P. ultimum* destroyed nearly 100% of the plants at 15 C. At higher temperatures, the percentage of dead plants was less; at 24 C there was a sharp decline, and at 36 C only 4-7% of the plants died.

Seed germination in the presence of the pathogens.—The effect of temperature and the three species of *Pythium* on the germination of seed on PDA is given in Table 2. All seeds germinated in the presence of the fungi were abnormal. The roots were swollen, misshapen, and discolored, with poor lateral root development. The seeds that did not germinate were usually completely rotted. Essentially the same percentage of germination occurred with each conditioning temperature, and only the data for the seeds conditioned at 4 C are presented. *Pythium aphanidermatum* reduced germination in seeds held above 20 C. This was most apparent in those seeds that were not subjected to a conditioning temperature treatment and in those held 2 days at 4, 8, or 12 C. Little damage was incurred below 20 C regardless of the time subjected to a conditioning temperature. *Pythium debaryanum* and *P. ultimum* greatly reduced germination below 24 C or at any temperature when the conditioning treatment was more than 4 days. In the absence of the pathogen, germination of seeds kept at 4, 8, and 12 C and later placed at higher temperatures was 94, 96, and 95%. This did not include an average of 3% abnormal germination.

Infection of soybeans in soil.—The effect of temperature on the emergence in soil artificially infested with two *Pythium* species and preconditioned at 4 C for 10 days is given in Table 3. The germination of seeds in soil infested with *P. aphanidermatum* was essentially the same as that of seeds in noninfested soil irrespective of temperature or the time of the conditioning treatment and is not included. *Pythium debaryanum* and *P. ultimum* caused decreased emergence when planted seeds were kept at 4 C for more than 2 days before being placed at 15 C. These fungi also reduced emergence markedly when seeds were preconditioned for 6-10 days before being placed at 22.5 or 30 C.

TABLE 2. The effect of temperature and of three species of *Pythium* on the germination of soybean seed on potato-dextrose agar after different intervals at 4 C

Days at 4 C	% Germination[a] at indicated temp					
	16 C	20 C	24 C	28 C	32 C	36 C
P. aphanidermatum						
0	96	69	6	4	4	0
2	94	94	54	66	22	0
4	92	100	88	88	88	62
6	92	96	96	96	90	90
8	100	96	100	98	94	98
10	98	100	98	96	98	92
P. debaryanum						
0	12	56	94	96	98	100
2	14	80	100	96	98	100
4	6	28	56	72	78	90
6	0	2	20	8	22	46
8	0	0	0	0	2	2
10	0	0	0	2	0	0
P. ultimum						
0	52	38	76	86	98	98
2	48	38	84	82	100	98
4	44	32	46	82	88	92
6	8	8	12	24	42	90
8	6	30	34	20	18	22
10	4	2	10	10	20	6

[a] All germination abnormal as described.

TABLE 3. The effect of temperature on the emergence of soybeans in soil infested with two species of *Pythium* after different intervals at 4 C

Days at 4 C	Temp (C)	% Emergence[a]		
		P. debaryanum	*P. ultimum*	*Non-inoculated*
0	15	58	66	54
2		32	46	62
4		20	20	50
6		14	18	70
8		4	0	60
10		5	3	42
0	22.5	88	80	66
2		74	74	64
4		64	56	60
6		28	42	58
8		8	10	74
10		5	3	42
0	30	86	88	90
2		88	82	88
4		68	58	90
6		38	34	80
8		22	16	84
10		22	14	78

[a] Each figure based on 50 seeds. Emergence in soil infested with *P. aphanidermatum* was essentially the same as that of the noninoculated control.

DISCUSSION.—*Pythium aphanidermatum, P. debaryanum,* and *P. ultimum* were highly pathogenic on soybeans. There appeared to be a relationship between the temperatures at which the fungus mycelium grew best and the temperatures at which it was pathogenic on the host. *Pythium aphanidermatum* was most virulent in a temperature range of 24-36 C, and showed little effect below 20 C. *Pythium debaryanum* and *P. ultimum* were most virulent at 15-20 C, or also at 24-36 C if the fungus and the host were given a preliminary cold treatment.

Pythium aphanidermatum was less effective in causing damping-off than the other two species in a nonsterile soil artificially infested with mycelium of the fungus. Control seeds held at low temperature often germinated poorly. This may have been due to upset of physiological processes that normally occur at optimum temperatures for soybean germination and growth. Furthermore, germination was slow at low temperatures, and together these factors may have rendered the seed more subject to attack by the species which grow best at lower temperatures.

Although inoculation of the hypocotyl by insertion of mycelium is highly artificial, it yields results comparable to those obtained by other artificial methods that more closely simulate the phases of pathogenesis.

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