Effect of Soil Temperature on Virulence of Pythium aphanidermatum and Pythium myriotylum to Rye and Tomato

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ABSTRACT

Optimum temperature for mycelial growth of *Pythium aphanidermatum* and *P. myriotylum* on V-8 juice agar was 35 C; slight growth occurred at 15 and 43 C. When seeds were planted in infested soil, both species reduced stands of rye 100% at 27 C and above, but caused no damage at 15 C. *P. aphanidermatum* was most virulent to tomato at 27-35 C. *P. myriotylum* caused almost complete stand loss in tomato at 35 C and was less virulent than *P. aphanidermatum* at 23-31 C. *P. myriotylum* caused root and crown rot and reduction in wet wt of established rye plants at 23 C, and virulence increased as soil temperature increased to 35 C. *P. aphanidermatum* caused little damage to established rye plants below 31 C. Both species were about equally damaging to established tomato plants above 23 C, and disease was most severe at 35 C. Rye kernels were more readily infected by *P. aphanidermatum* than by *P. myriotylum*. Seventy-three per cent of the kernels were infected after exposure in *P. aphanidermatum*-infested soil for 24 hr at 35 C, as compared to 15% infection with the other species. Tomato seed were relatively resistant to invasion, as indicated by failure to recover either species after exposure in infested soil for 48 hr at 31 C. Phytopathology 60:704-707.

Information is extensive, dealing with effect of soil temperature on virulence of *Pythium aphanidermatum* (Edson) Fitz. to various hosts, but very limited for *Pythium myriotylum* Drechs. Disease caused by *P. aphanidermatum* is most severe at 32-36 C; optimum for growth in culture is 30-37 C (3, 9, 11, 13). Mycelia will not grow above 38-46 or below 5-12 C. Luna & Hine (9) found that the optimum for growth of *P. aphanidermatum* in sterile and nonsterile soil was 28-31 C. *P. myriotylum* grows best at 34-37 C, and will not grow below 10 C or above 43 C (11). Bell found that *P. myriotylum* was pathogenic to peanut seed in pure culture from 18-35 C, but root necrosis of seedlings was most severe at 35 C (1).

Sechler & Luke (12) reported that damping-off of rye (*Secale cereale*) and other small grains was caused primarily by *P. aphanidermatum* when soil temperatures were high (32 C). Recently, McCarter & Littrell (10) proved pathogenicity of *P. myriotylum* to rye and tomato (*Lycopersicon esculentum*). Littrell (*unpublished data*) found that *P. myriotylum* was one of the organisms involved in stand loss in early (September) rye plantings in Georgia. Pythium blight of tomato caused by *P. aphanidermatum* is recognized as one of the major diseases in the production of transplants (5). A need exists to define the influence of soil temperature on diseases of fall-seeded small grains, especially rye, and of tomatoes caused by these fungi. The objectives of this study were to (i) determine the cardinal temperatures for radial growth of the organisms in culture; (ii) study the influence of soil temperature on their capacity to cause pre- and postemergence damage to rye and tomato; and (iii) compare virulence to tomato seed and rye kernels at various soil temperatures. An abstract on a portion of this work has been published (7).

MATERIALS AND METHODS.—The culture of *P. aphanidermatum* (F-17) used in all studies was originally isolated from *Citrus* sp. in Florida (obtained from F. F. Hendrix). *P. myriotylum* (F-8) was isolated from diseased rye plants at Tifton, Georgia. Both isolates were highly virulent to rye and tomato. Stock cultures were maintained at 19 C on V-8 juice agar (V-8A) consisting of 200 ml unstrained V-8 juice, 13 ml 1.0 N KOH, 25 g agar, and 787 ml distilled water. One additional isolate of each species was also studied, but results given are for the isolates described above, as results were comparable.

The cardinal temperatures for in vitro growth of the two *Pythium* spp. were determined by growing them at 4-C intervals from 11-31 C, and at 2-C intervals from 31-43 C. Petri plates containing 30 ml of V-8A were each inoculated with 5-mm mycelial plugs taken from the periphery of actively growing V-8A cultures. Three plates of each isolate were placed at each temperature, and colony diam was measured after 16 hr. The test was run twice.

In all tests, seed were planted in an autoclaved (2 hr at 15 psi) Goldsboro loamy sand mixed with vermiculite (3:1 v/v) and fertilized with a complete mixture (5% N, 10% P, 15% K). Constant soil temperatures from 15 to 35 C at 4-C increments were maintained with water bath temperature tanks. Ambient greenhouse temperatures were 25-29 C during the day, 22-24 C at night. Seventy-five seed of tomato (Campbell 17) or rye (Wrens Abruzzi) were planted in 4-liter glazed crocks filled with infested or noninfested (control) soil. Four replications of each treatment combination were used.

Methods for infesting soil for pre-emergence tests and for inoculating plants in postemergence tests were described earlier (10). In postemergence tests, stands

of tomato were thinned to 20 plants at time of inoculation. The crocks containing rye were not thinned, as they were quite uniform and no crowding occurred. Tomato and rye plants were 8 and 15 cm tall, respectively, when inoculated.

In the pre-emergence tests, counts on seedling emergence and final survival were made 7 and 14 days after seeding, respectively. In postemergence tests, plants were removed from the soil 14 days after inoculation, the roots were washed, and plants were assigned a disease severity rating based on a previously described (10) 0-6 scale on which 0 = no discoloration or rot of roots or stems, 1 to 5 = increasing degrees of root and stem rot, and 6 = plants dead. Plant heights of tomato and wet wt of rye plants were also taken.

To study seed infection at various temperatures, soil infested with the *Pythium* spp. as described in the pre-emergence studies was placed in 25 × 9-cm plastic containers covered with a plastic lid provided with six 2-mm apertures to allow gas exchange. Each container was filled with 2 cm of infested or noninfested (control) soil seeded with tomato (250 seed) or rye (175 kernels), then the seeds were covered with 1 cm of soil. Moisture was adjusted to field capacity, and the containers were placed in incubators at 4-C increments between 15 and 35 C. Controls consisting of seeds planted in noninfested soil were maintained at 27 C. After 24-, 48-, 72-, and 96-hr incubation, a quadrant of soil containing seed was removed, placed on a metal sieve, and washed with running water to remove soil around seed. Forty seed from each treatment combination were surface sterilized in a 0.525% sodium hypochlorite solution for 1 min, rinsed in distilled water, and plated on pimaricin streptomycin agar (100 ppm pimaricin, 100 ppm streptomycin sulfate, and 2% agar) that served as a selective medium inhibiting all but pythiaceous fungi (4). Per cent germination of seed selected at random for plating was recorded. Plates were incubated at 28 C for 24-48 hr, and seeds yielding *Pythium* colonies were recorded as being infected. *P. aphanidermatum* could be easily distinguished from *P. myriotylum* by the more rapid growth and more surface mycelium produced by the former. All studies were made at least twice.

RESULTS.—In the in vitro studies, both *Pythium* species grew best at 35 C with some growth at 15 and 43 C (Fig. 2). *P. aphanidermatum* colonies attained a greater diam at 43 C than did *P. myriotylum*, whereas the converse was true at 11 C. Generally, *P. aphanidermatum* grew slightly faster than did *P. myriotylum*.

In the pre-emergence study, almost 100% loss of rye stands occurred at 27-35 C 14 days after seeding in soil infested with either organism (Fig. 1-A). *P. aphanidermatum* reduced stands by 30% at 19 C, but no reduction occurred at 15 C. Similar results were obtained in *P. myriotylum*-infested soil. The greatest difference between the two *Pythium* spp. occurred at 23 C, where *P. aphanidermatum* eliminated 90% of the plants, whereas *P. myriotylum* eliminated only 25%.

Virulence of *P. aphanidermatum* to tomato was similar to that for rye. Damage to tomato increased with increasing soil temperature to 27 C, where 100% loss in stand occurred (Fig. 1-B). Stand counts were not reduced at 15 C by *P. aphanidermatum*; however, *P. myriotylum* caused approximately 20% reduction in stands at 15 and 19 C. Virulence of *P. myriotylum* to tomato increased from 19 to 35 C, where nearly all plants were killed.

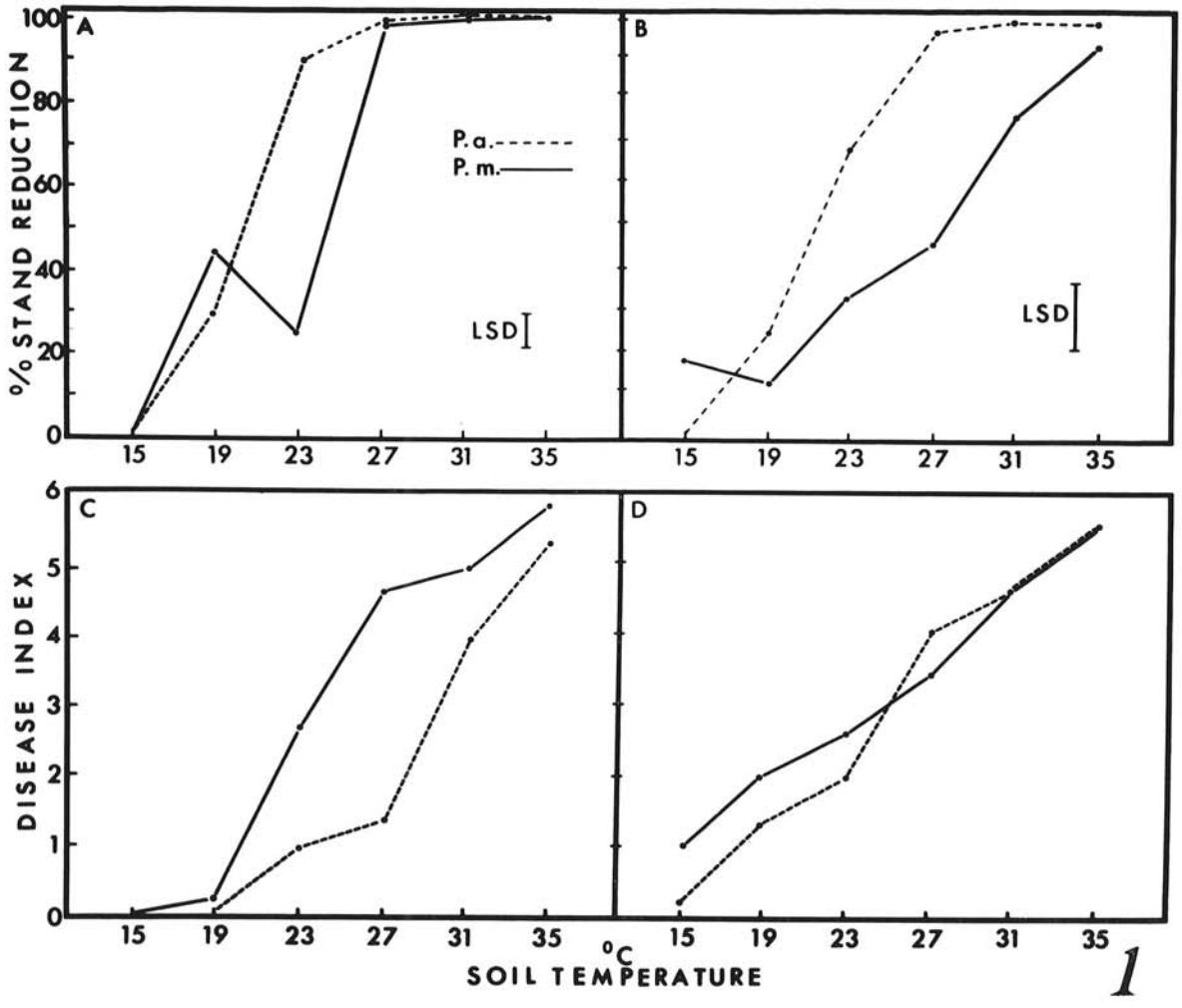
P. aphanidermatum caused little damage to established rye plants below soil temperatures of 31 C (Fig. 1-C). A significant reduction in fresh wt of plants was not detected at temperatures below 35 C (Fig. 3). Figure 3 indicates the detrimental effects of continuous soil temperatures above 27 C on growth of rye. It was only at these higher temperatures that *P. aphanidermatum* caused necrosis of the roots and crown areas and reduced plant growth. *P. myriotylum* significantly reduced plant growth at 23 C and above when compared to plants growing in noninfested and *P. aphanidermatum*-infested soil. No damage was observed at 15 C with either fungus.

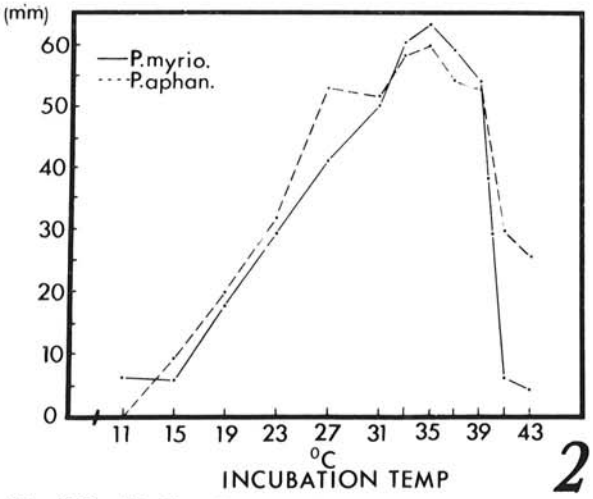
Both species caused most severe damage on established tomato plants at 35 C (Fig. 1-D). *P. myriotylum* was slightly more virulent than *P. aphanidermatum* at the lower temperatures, and caused some root discoloration at 15 C and moderate to severe rot at 23 C. Final plant height was reduced 52% at 19 C when compared with controls.

When seed were planted in infested soil and removed, *P. aphanidermatum* had penetrated 73% of rye kernels during 24-hr exposure at 35 C (Table 1). At 19 C, more than 50% of the kernels were infected during 96-hr exposure. Few kernels were infected at 15 C even after 96 hr. *P. myriotylum* infected rye kernels less rapidly than did the former species, with only 15% of the kernels being infected after 24 hr at 35 C. Kernels were not infected below 23 C. Compared with *P. aphanidermatum*, *P. myriotylum* infected relatively low numbers of kernels up to 48 hr regardless of soil temperature.

Both *Pythium* spp. were weakly virulent to tomato seed, as indicated by the low recovery rates at most temperatures even after 96 hr (Table 1). Maximum infection occurred after exposure to *P. aphanidermatum* for 96 hr at 31 C. *P. myriotylum* infection was not detected for any exposure time in seed held at 31 and 35 C, and 10% was the maximum number of seed infected in any temperature-time combination.

DISCUSSION.—These studies indicate that *P. myriotylum* is similar to *P. aphanidermatum* in that both require high temperatures for maximum growth and infectivity. Optimum temperatures for mycelial growth in culture and disease development in rye and tomato were similar. The most significant difference between the behavior of the two *Pythium* species occurred at 23 and 27 C on established rye plants. At these temperatures, *P. myriotylum* caused considerable root and stem rot, whereas *P. aphanidermatum* caused little damage. This indicates that in the southern USA, *P. myriotylum* may be potentially more destructive to fall-seeded small grains, as it could continue to cause some damage even after the onset of cooler weather. It appears that *P. aphanidermatum* characteristically causes pre-emergence or very early postemergence





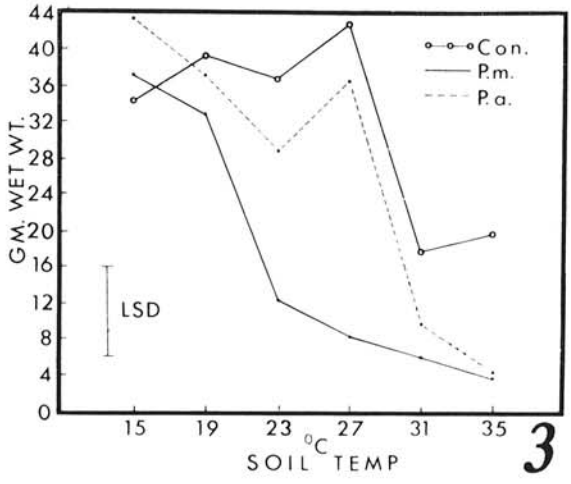


Fig. 1-3. **1)** The effect of soil temperature on pre- and postemergence damage to Wrens Abruzzi rye and Campbell 17 tomato 14 days after inoculations with *Pythium aphanidermatum* or *P. myriotylum*. **A, B)** Pre-emergence tests, per cent stand reduction of rye (A) and tomato (B). **C, D)** Postemergence tests, disease index (10) of rye (C) and tomato (D). **2)** Effect of incubation temperature on radial growth of *P. aphanidermatum* and *P. myriotylum* on V-8 juice agar. **3)** Effect of soil temperature on growth of Wrens Abruzzi rye as determined by wet wt 14 days after inoculations with *P. aphanidermatum* or *P. myriotylum*.

TABLE 1. Percentage of rye and tomato seed infected by *Pythium* spp. when incubated in infested soil at six temperatures for various periods

	P. aphanidermatum, hr				*P. myriotylum*, hr			
C	24	48	72	96	24	48	72	96
	% rye seed infected							
15	0	3	10	5	0	0	0	0
19	8	0	30	65	0	0	0	0
23	30	38	45	75	3	5	3	20
27	28	73	78	88	3	0	23	68
31	45	73	78	65	8	15	38	33
35	73	78	83	90	15	5	33	28
	% tomato seed infected							
23[a]	0	0	0	3	0	0	0	3
27	0	3	3	10	0	0	3	10
31	0	0	18	40	0	0	0	0
35	3	3	3	0	0	0	0	0

[a] No infected seed found at temperatures below 23 C.

damage, and causes damage to established rye plants only at high temperatures (31 C or above) also detrimental to the growth of rye. *P. myriotylum* causes considerable pre-emergence damage and is also capable of attacking well-established plants. The optimum temperatures for disease caused by *P. aphanidermatum* on tomato and rye were similar to those reported for other crops.

The seed infection studies showed that rye kernels were more readily invaded by *P. aphanidermatum* than by *P. myriotylum*. This more rapid infection by *P. aphanidermatum* may partially explain the increased pre-emergence damage caused by this organism over that caused by *P. myriotylum*. We do not have an explanation for this difference in their capacity to infect seed. Kraft & Erwin (6) showed that sugars and amino acids were exuded from mung bean (*Phaseolus aureus*) seed when incubated in water; these compounds reportedly increased the virulence of *P. aphanidermatum*. We do not believe that the failure of *P. myriotylum* to invade seed as readily as *P. aphanidermatum* is nutritional in nature, because both species grow similarly on a variety of culture media (8). In the present studies, the in vitro growth of *P. myriotylum* was slightly slower than that of *P. aphanidermatum*. It is possible that *P. myriotylum* does not colonize the soil as readily as does *P. aphanidermatum*. The nature of the resistance of tomato seed to fungal colonization was not determined.

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