Effect of Soil Water Potential on Disease Incidence and Oospore Germination of Pythium aphanidermatum

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ABSTRACT

Oospores of *Pythium aphanidermatum* germinated directly in asparagine-amended soils maintained at soil moisture levels ranging from saturation to −15 bars matric water potential. Percentage oospore germination and germ-tube growth rates were reduced at the lower soil moisture levels.

Colonization of alfalfa seeds, sown in soil containing a natural population of 80 viable oospores of *P. aphanidermatum*/g soil, occurred at all soil moisture levels except −15 bars matric potential.

Data indicate that wet soil conditions favor the activity of *Pythium* by increasing nutrient availability for oospore germination.

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Additional key words: soil moisture, soil-borne fungus, zoospores.

High soil moisture (field capacity and above) is usually associated with increased incidence of disease caused by *Pythium* spp. Such a condition is believed to act: (i) directly on the host by decreasing host vigor and increasing host exudation (3, 10, 15); (ii) by providing a suitable environment for the diffusion of host exudates necessary for fungal germination and/or growth (10, 17), growth apparently being unaffected by low oxygen conditions (3, 7); and (iii) by providing water-filled pores of a size required for zoospore production and dissemination (6, 9). The latter phenomenon is the more commonly accepted factor contributing to increased disease incidence by *Pythium* spp. as shown by the numerous papers concerning zoospore attraction to roots.

Although zoospores function as efficient infecting units when experimentally added to soil (2, 9, 12), neither their production from survival structures in the rhizosphere nor their significance as primary inocula in soil has been established. *Pythium* and *Phytophthora* oospores and/or sporangia contiguous to host tissue germinate directly (1, 2, 11, 13, 16), and are known to serve as primary inocula.

This investigation was undertaken to determine the influence of matric water potential on host colonization and oospore germination of *Pythium*

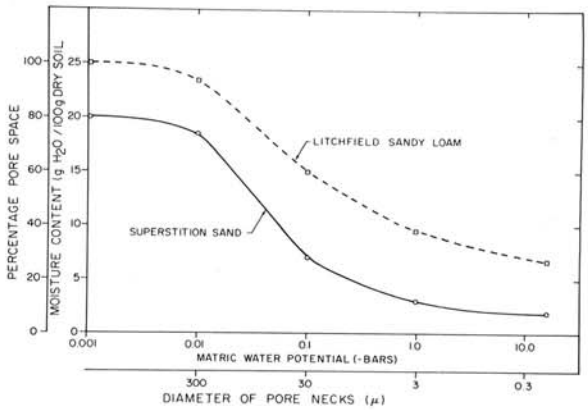

Fig. 1. Moisture characteristics (drying boundary curve) of Litchfield sandy loam soil and Superstition sand soil. Percentage pore space applies only to Litchfield sandy loam soil.

aphanidermatum (Edson) Fitzp. in field soil.

MATERIALS AND METHODS.—Superstition sand (SS), which did not contain a natural *Pythium aphanidermatum* population, and Litchfield sandy loam (LSL), which contained a natural population of 80 viable oospores of *P. aphanidermatum*/g soil, were used (16). Soils were stored at 24 C in polyethylene bags.

Pythium aphanidermatum was established in both soils as oospores (16). The resulting population was about 5×10^4 oospores/g soil (4). Artificially infested soils, each maintained at about −0.1 bar matric potential, were stored in polyethylene bags at 24 C.

Twenty-gram samples of the naturally- and artificially infested soils were placed on the sintered glass plate (medium porosity) of a Haines' apparatus (17) and in a pressure membrane apparatus; nitrogen was used as the compressing gas (14). The drying-boundary of the soils, as determined by the Haines' apparatus for −0.01 and −0.1 bars, and by the pressure membrane apparatus for −1.0 and −15 bars, shows the relationship between soil moisture and the corresponding matric water potential (8) (Fig. 1). After a 24-hr equilibration period, soil samples were removed from their respective apparati and placed in 50-ml glass beakers. Artificially infested soil samples from each moisture level were then amended as follows: (i) no treatment; (ii) brought to saturation with sterile distilled water (SDW); (iii) amended with 100 μg powdered anhydrous asparagine/g soil; and (iv) amended with 100 μg asparagine/g soil and brought to saturation with SDW. Beakers were then sealed with parafilm to prevent loss of moisture and incubated at 35 C. Oospore germination in soil was determined at hourly intervals, as described previously (16).

TABLE 1. Oospore germination of *Pythium aphanidermatum* after 24 hr exposure to 1 and 15 atmospheres of nitrogen gas[a]

	Oospore germination (%)[b]				
	Time (hr)				
Treatment	0	3	6	12	24
Ambient air	0	20	85	92	92
1 Atmosphere nitrogen	0	24	87	91	91
15 Atmospheres nitrogen	0	0	15	65	90

[a] Oospores, contained in a nylon mesh (10-μ) envelope, were placed in a pressure membrane apparatus and subjected to 1 and 15 atmospheres of nitrogen gas for 24 hr. Oospore germination did not occur during the 24-hr equilibration period.

[b] Percentage oospore germination on V-8 agar after treatment.

The effect of matric water potential on host colonization by *P. aphanidermatum* was determined by thoroughly mixing 20 alfalfa (*Medicago sativa* L.) seeds into 20-g samples of naturally infested LSL which were then compressed to their original volume. Alfalfa seeds were recovered from soil by collection on a 20-mesh sieve after 24, 48, and 96 hr incubation at 35 C, washed in running tap water for 1 hr, and plated on water agar contained in petri dishes. Five seeds, selected at random from each soil moisture level, were crushed and microscopically examined at × 430 for the presence of coenocytic mycelium within plant tissues.

RESULTS.—No significant differences were observed between oospore germination in the two soils. Oospore germination did not occur during the 24-hr equilibration period necessary to achieve the various matric water potentials. It was noted, however, that oospores subjected to 15 bars of nitrogen gas for 24 hr had a lag period of 6 hr after removal from the pressure membrane apparatus (PMA) before they were capable of germination (Table 1). The latter determinations were made by placing oospores, which were contained in a 10-μ nylon mesh envelope, on V-8 agar after their retrieval from the PMA.

Oospore germination did not occur in either nonamended soils maintained at the various soil moisture levels or in soils brought to saturation with SDW from their original soil moisture levels (Table 2). Maximum oospore germination (ca. 90%) occurred only in asparagine-amended soils maintained at −0.01 and −0.1 bars, and in all asparagine-amended soils brought to saturation with SDW. Germ-tube length in all the above soils averaged ca. 400 μ after 3 hr and 1,400 μ after 6 hr of incubation at 35 C. Oospores were also capable of germination in asparagine-amended soils maintained at −1.0 and −15 bars, but the rate and percentage both were reduced at these matric water potentials and germ-tube length was considerably less (ca. 800 μ at −1.0 bars and 300 μ at −15 bars after 36 hr incubation) than in asparagine-amended soils maintained at −0.01 and −0.1 bars.

Colonization of alfalfa seeds by *P. aphanidermatum*, which were sown in naturally infested SS, occurred at −0.01, −0.1, and −1.0 bars after incubation periods of 24 hr, 24 hr, and 48 hr, respectively. Microscopic examination

TABLE 2. Oospore germination of *Pythium aphanidermatum* in nonamended and asparagine-amended soils maintained at various matric water potentials

Soil treatment	% Oospore germination in soil at matric water potentials (−bars)			
	0.01	0.1	1.0	15.0
Unamended	1-2	0	0	0
Asparagine amended[a]	90-92[b]	85-91[b]	38-54[c]	6-10[d]

[a] 100 μg powdered L-asparagine was thoroughly mixed into soils maintained at the various matric water potentials.

[b] Maximum percentage oospore germination after 3-6 hr of incubation at 35 C.

[c] Maximum percentage oospore germination after 6 to 12 hr of incubation at 35 C.

[d] Maximum percentage oospore germination after 24 hr of incubation at 35 C. No oospore germination occurred in nonamended soils when saturated with sterile distilled water (SDW). Greater than 90% oospore germination occurred in asparagine-amended soils maintained at −1.0 and −15 bars within 3 to 6 hr when saturated with SDW.

revealed the presence of coenocytic mycelium within plant tissues prior to attempted isolation on water agar. Mycelium was not observed in seeds removed from soil maintained at −15 bars and *P. aphanidermatum* was not isolated from such seeds after 96 hr of incubation at 35 C. A matric water potential of −15 bars was apparently insufficient for alfalfa seed germination since it was noted that imbibition of water had not taken place. No attempt was made to record actual percentage of the alfalfa seed colonized by *P. aphanidermatum* at the various soil moisture levels since the possibility of secondary spread subsequent to primary colonization of a single seed could not be ruled out.

DISCUSSION.—The favorable influence of wet soil conditions on the activity of *P. aphanidermatum*, as well as other *Pythium* spp. (10, 17), is apparently due to solubilization and diffusion of host exudates and/or other exogenous nutrients necessary for overcoming fungistasis. Reduced availability of nutrients at low soil moisture levels, −1.0 and −15 bars, was apparently responsible for the low percentage oospore germination and reduced rate of both germ-tube growth and subsequent colonization of below ground plant parts since nutrients were present in soil in quantities sufficient to support maximum oospore germination.

Zoospore production and/or dissemination, even at high soil moisture levels (−0.01 and −0.1 bar) was not observed in any of the amended soil samples and only directly germinating oospores were recorded. Colonization of alfalfa seeds in naturally infested soil at −1.0 bar likewise ruled out zoospore infection since ca. 60% of the soil pores were drained of free-water and the remainder contained water-filled pores about 3 μ or less in diam, a size restrictive to zoospore movement even assuming that such water-filled pores were continuous in nature.

These results support and extend previous observations (3, 5, 7, 10) which indicate that soil moisture per se, over the range available to higher plants, is seldom limiting to fungal activity and that the association of increased incidence of *Pythium* spp. under wet soil conditions is attributable to increased nutrient availability as well as fungal tolerance of such moisture conditions.

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