

## Propagule Nature and Density of *Pythium aphanidermatum* in Field Soil

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### ABSTRACT

Oospores were the sole survival structure of *Pythium aphanidermatum* in naturally infested field soil. Population counts determined on a species-specific isolation medium from naturally infested soils of various types ranged from 10-250 oospores/g soil.

Enzymatic degradation of colonized organic matter

apparently resulted in liberation of dormant oospores which have an exogenous nutrient requirement for germination. No evidence was obtained for the presence of a constitutively dormant oospore population in naturally infested soils.

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*Additional key words:* soil-borne fungus, survival structure, population, selective medium.

*Pythium aphanidermatum* is a soil-borne fungus that causes root rots of various cultivated crops in Arizona (7). Indirect methods have implicated oospores as the primary survival structure of the fungus in field soil (1, 2, 7, 9). The purpose of this investigation was to determine the propagule density and the specific nature of the propagule responsible for persistence of *P. aphanidermatum* in naturally infested field soils.

**MATERIALS AND METHODS.**—Litchfield sandy loam, Guest clay, and Pomerene clay loam soils were collected from the Salt River Valley, Gila River Valley, and Cochise County in Arizona, respectively, and stored at 24 C in polyethylene bags.

Existing selective media (3, 4, 5) proved quantitatively unsatisfactory for determining the population of *P. aphanidermatum* Edson (Fitz.) in naturally infested soils. A medium was therefore developed which contained Difco cornmeal agar 17 g, pimarinic (Myprozine, potency 92.2%, American Cyanamid) 100 mg, streptomycin sulfate 200 mg, rose bengal 150 mg and benomyl 5 mg in 1 liter of water. Each antibiotic was added after autoclaving and cooling the cornmeal agar to 45 C. All antibiotics were prepared as stock solutions (1%) and stored at 10 C for no longer than 1 month.

Soil dilutions of 1:5, 1:10, 1:20, and 1:40 (g soil/ml 0.3% water agar) were used. Each dilution was mixed on a Vortex stirrer for at least 15 min and a 1.0-ml aliquot was dispensed evenly across the surface of the medium with the addition of 0.5 ml distilled water to facilitate distribution of the soil. Plates were incubated at 35 C, the optimum temperature for growth of *P. aphanidermatum*, for various time intervals after which the soil was washed from the agar surface under a stream of tap water, and colonies were observed.

**RESULTS.**—Our studies showed: (i) that oospores, sporangia, zoospores, and mycelial

fragments of *P. aphanidermatum* were all capable of greater than 90% germination and/or growth on the selective medium; (ii) that ca. 97% of a known oospore population was recovered from artificially infested field soil using the selective medium; (iii) that the selective medium was specific for *P. aphanidermatum* and prevented the growth of all other microorganisms from the naturally infested field soils tested; and (iv) that a linear relationship existed between the number of oospores of *P. aphanidermatum*/ml and the soil dilution (Fig. 1).

Dilution plates were incubated for 24 hr at which time colony sizes ranged from 3- to 5-mm diam and each one could be traced easily to its origin with the aid of a light microscope at X100. After 72 hr *P. aphanidermatum* colonies were characterized by a distinct red ring within the colonies (Fig. 2-A). Longer incubation periods usually resulted in colony overlap and therefore counting was difficult.

In soils tested over a 5-month period, 96% of the colonies examined originated from oospores (Table 1, Fig. 2-B). The remainder of the colonies could not be traced back to an originating propagule due possibly to dislodgement of the propagule from the agar surface during the washing phase. Direct microscopic examination showed that oospores were not found in association with organic matter either on dilution plates or in organic matter separated from soil prior to dilution studies. These observations were made using Arizona soils which had been fallow 4 to 6 wk.

A Litchfield sandy loam with a known history of disease caused by *P. aphanidermatum* and which was cropped to potatoes 1 month prior to sampling, had a population of 60-80 oospores/g, while Pomerene clay loam cropped to alfalfa contained 40-50 oospores/g soil. Guest clay, which had been continuously cropped to sugar beets for 3 consecutive years, contained populations up to 250 oospores/g soil.

In addition to cultivated soils of Arizona, species

specificity of the selective medium was demonstrated also in noncultivated Gila River sand as well as cultivated soils from Sonora, and Sinaloa, Mexico, where populations ranged from 10-27 oospores/g soil.

No change in the oospore population was observed in soils stored for a 5-month period at 24 C.

**DISCUSSION.**—The propagule of *P. aphanidermatum* found responsible for persistence of the fungus in field soil, as previously postulated (1, 2, 7, 9), was shown by direct observation to be the oospore. Oospores of *P. aphanidermatum* in field soils were not embedded in organic matter; they apparently survive in soil as free propagules. Oospores, however, have been observed frequently in newly colonized host tissue and in soil adjacent to colonized host tissue.

Although oospores are apparently constitutively

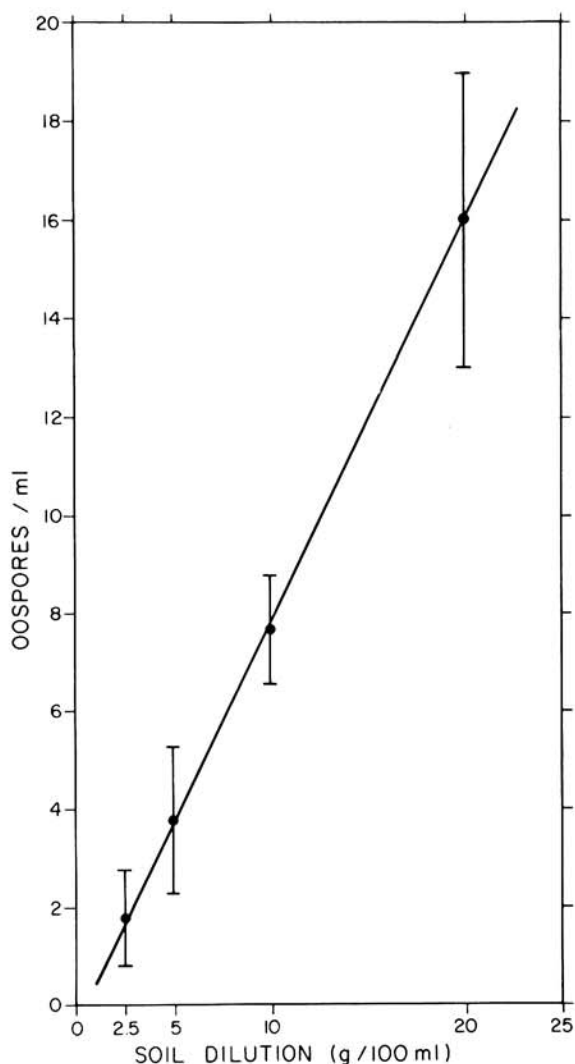


Fig. 1. Relationship between *Pythium aphanidermatum* population and soil dilution. The mean and standard error of 10 separate soil samples, each replicated 10 times, are plotted.

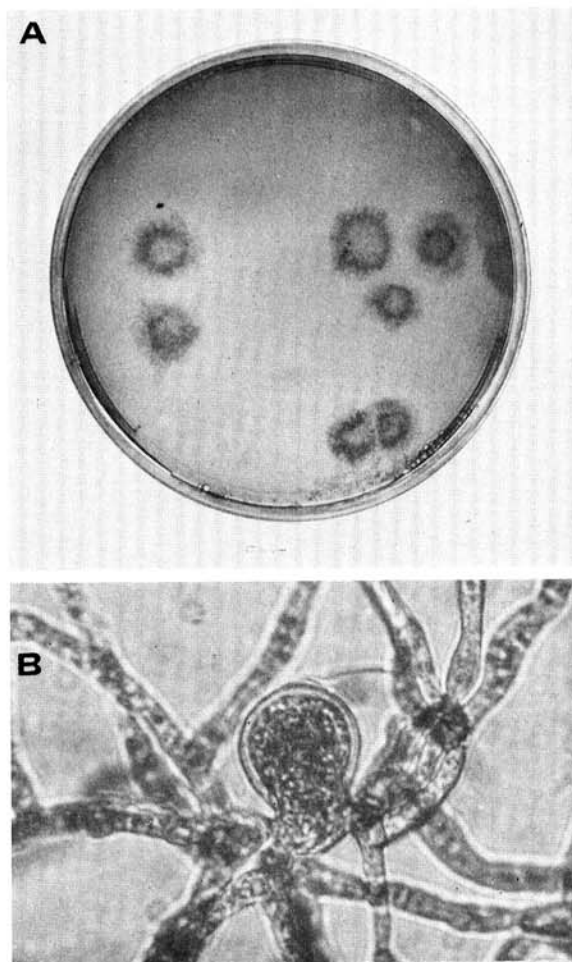


Fig. 2. Photographs of A) colony morphology and B) originating propagule (oospore) of *Pythium aphanidermatum* on a species-specific isolation medium after 72 hours incubation at 35 C.

dormant while embedded in colonized host tissue, their subsequent release through enzymatic degradation (6, 8) could alter oospore wall permeability thereby establishing a population having only an exogenous nutrient requirement for germination. Degradation of organic matter in Arizona desert soils occurs rapidly and soils usually have an organic content between 0.2-0.8%.

Interpretation of the significance of soil fungal populations frequently reported as propagules/g soil is difficult unless the exact nature of the propagules is known. Soil samples taken in the rhizosphere of infected plants may contain sporangia, zoospores, and mycelial fragments, all of which could give rise to colonies on a dilution plate. They may, however, have no significance as primary inoculum of the organism unless they can be shown to survive inter-substrate periods.

Media for the isolation of *Pythium* spp. have been developed with little emphasis placed on species

TABLE 1. Origin of colonies of *Pythium aphanidermatum* from naturally infested field soils on a species-specific isolation medium<sup>a</sup>

Experiment	Total colonies observed on five or more dilution plates	Colonies traced to oospores	Colonies traced to oospores (%)
1b	46	43	93.5
2b	50	48	96.0
3b	36	35	97.2
4b	12	12	100.0
5c	131	130	99.2
6d	43	40	93.0
7d	20	19	95.0
Total	338	327	Avg. 96.3

<sup>a</sup> Soil dilutions of 1:10 (g soil/ml 0.3% water agar) were incubated at 35 C for 24 hours on the selective medium.

<sup>b</sup> Litchfield sandy loam soil.

<sup>c</sup> Pomerene clay loam soil.

<sup>d</sup> Guest clay.

specificity (10). Since those media were shown to be quantitatively unsatisfactory for determination of the *P. aphanidermatum* population in Arizona soils, we developed a medium specific for *P. aphanidermatum* with the elimination of all other microbial growth. Besides being species-specific it also has the following desirable characteristics: (i) species specificity eliminates the need for isolation and identification of all colonies on the medium, a persistent problem with other less-selective media; (ii) allows identification of the originating colony propagule and subsequent isolation of pure single oospore isolates; (iii) the

linear relationship between oospores/g soil and soil dilution eliminates preparation of numerous soil dilutions when dealing with soils containing populations of *P. aphanidermatum* of unknown density.

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