Germination In Vivo of Pythium aphanidermatum Oospores and Sporangia

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ABSTRACT

Oospores of *Pythium aphanidermatum* were capable of either direct (production of germ tubes) or indirect (production of zoospores) germination in field soil, depending on the presence or absence of an exogenous source of nutrients. In the presence of nutrients, oospores germinated exclusively by a germ tube which continued elongation and either terminated in a sporangium prior to lysis or penetrated the host directly. Zoospore production from germinating oospores in soil occurred in the absence of exogenous nutrients and was restricted to the surface water of saturated soils.

Sporangia, formed in soil prior to germ-tube lysis, germinated directly upon addition of a substrate to moist soil but did not persist for more than 2 days in air-dry soil.

Oospores and not zoospores apparently are the major root-infecting units in field soil.

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Additional key words: soil-borne fungus, fungistasis.

Thick-walled oospores, with few exceptions (9), are commonly considered the primary propagule of *Pythium* spp. capable of long-term survival in soil (7, 8). Although their survival capabilities have been verified by direct (1, 10, 12, 13) and indirect (7) evidence, evaluation of their behavior and role as primary inocula in field soil has not been elucidated.

Barton (2) provided the first and only direct evidence that oospores of a *Pythium* sp. (*P. mamillatum*) were stimulated to germinate in response to root exudates in field soil.

This investigation was undertaken to determine factors which initiate germination of oospores of *Pythium aphanidermatum* (Edson) Fitzp. and their behavior in field soil.

MATERIALS AND METHODS.—The isolate used (P-1) was obtained from a diseased sugarbeet and maintained on V-8 juice agar, transferred monthly.

The soils used, Superstition sand (SS), and Litchfield sandy loam (LSL) had pH values of 8.2 and 7.7, water contents of 2.3 and 6.5% (w/w) at −15 bars, 7.1 and 15.3% at −0.1 bar, and 20.3 and 25.4% at 0 bar (saturation), respectively. The soils used in these experiments were stored at 24 C in polyethylene bags.

Pythium aphanidermatum was established in both soils as oospores, using aqueous suspensions of snail-ingested oospores, obtained as previously described (11). The resulting population was approximately 5×10^4 oospores/g soil as estimated by the method of Burr & Stanghellini (3). Soils were allowed to dry to about −0.1 bar after infestation and stored in petri dishes kept in a moist chamber until used.

One-gram aliquots of oospore-infested soil were placed in wells of a porcelain spot plate and amended, unless otherwise specified, with either sterile distilled water (SDW), tap water (TW), nutrient solutions of known concn made from reagent-grade chemicals, or bean seed exudate, and incubated at 24 and 35 C. Seed exudate was obtained as previously described (9). Amendments resulted in a saturated soil condition.

Direct observation of oospore behavior in soil was accomplished by microscopic examination of stained and nonstained soil smears (9) at × 100 and × 430. Percentage oospore germination was expressed as the average of three counts, 50 oospores/count for each treatment. All experiments were replicated three times and repeated periodically over a 4-month period.

RESULTS.—Oospore germination began in soil in 3 hr and reached a maximum of 92% 6 hr after soil was amended with either bean seed exudate or nutrients of known concn (Fig. 1B). No germination occurred in soils amended with SDW or TW (Fig. 1A).

Percentage oospore germination increased with increasing nutrient concn up to a level (25 μg asparagine/g soil or 40 μg glucose/g soil) above which no further increase occurred. All oospores germinated with the production of a single germ tube (Fig. 1B). Sustained germ tube growth was related to nutrient concentration; at low nutrient levels (less than 100 μg glucose or asparagine/g soil) germ-tube growth ceased within 8 hr after germination, while at higher nutrient levels (greater than 1,000-2,000 μg glucose or asparagine/g soil, or bean seed exudate) hyphal growth continued for periods up to 48 hr. Upon cessation of growth, at both high and low nutrient levels, lobate sporangia were formed (Fig. 1C). Complete lysis of the hyphae and/or germ tubes occurred within 96 hr and various-shaped lobate

sporangia were observed lying free in the soil (Fig. 1E). Production of new oospores was not observed.

Sporangia-infested soils were dried to about −0.1 bar and divided into two samples. One sample was amended with bean seed exudate and the other with SDW or TW. Approximately 80% of the sporangia germinated by the production of one to three germ tubes within 1.5 hr after amending infested soils with bean exudate (Fig. 1F), and continued to grow and form mycelia. Sporangia did not germinate in soils amended to saturation with SDW or TW.

The ability of lobate sporangia to persist in soils was determined by air-drying (0.3% moisture) a portion of the sporangium-infested soil for 2 days. Both the air-dried sample and a sample maintained at −0.1 bar were amended with bean seed exudate. Sporangia germinated only in the soil maintained at −0.1 bar.

Since the mode of oospore and sporangium germination in the presence of an ephemeral

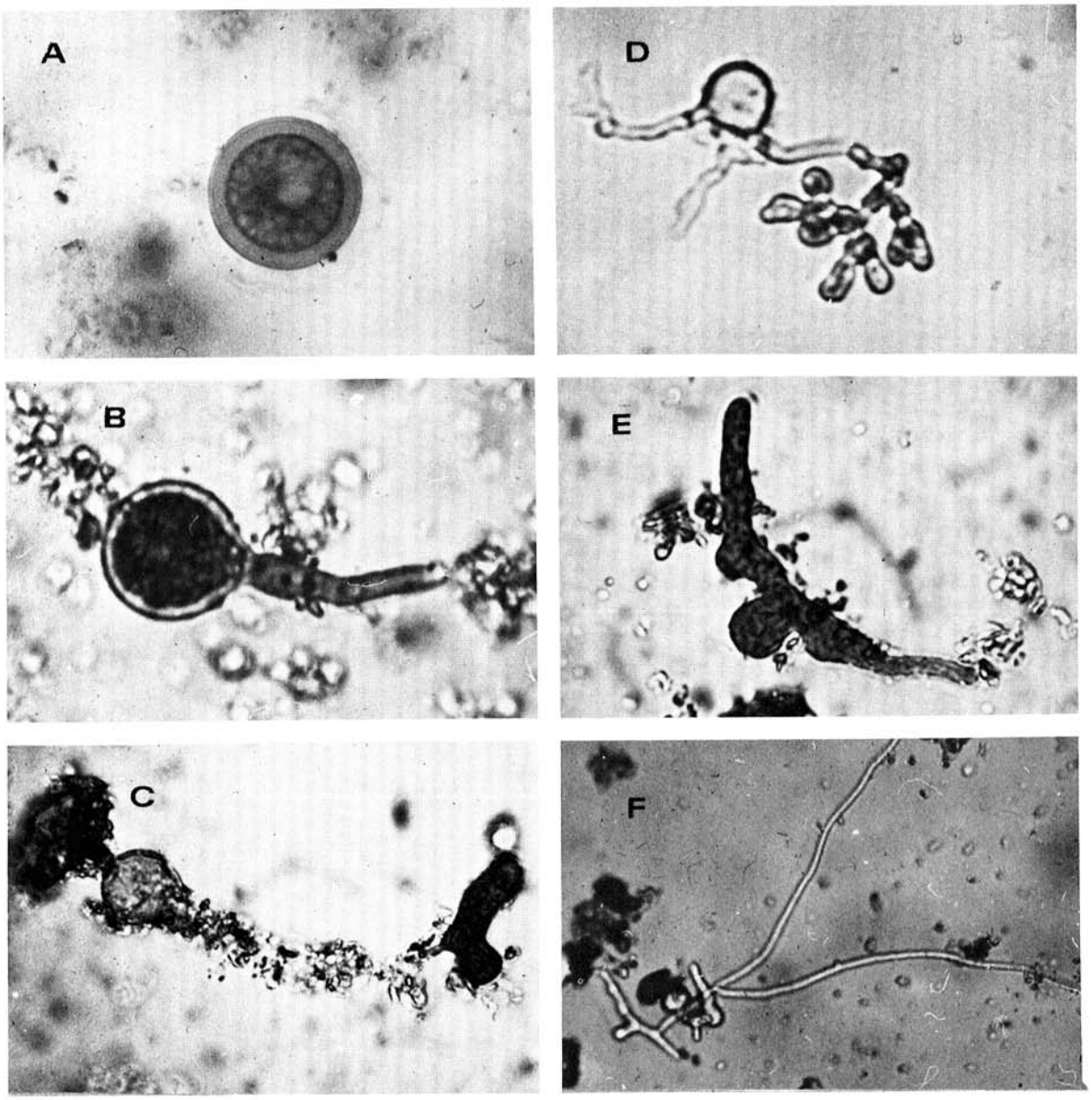

Fig. 1. Photomicrographs. **A)** Nongerminated oospores of *Pythium aphanidermatum* in infested soil (× 800). **B)** Germinated oospore of *P. aphanidermatum* 3 hr after amending infested soil with bean seed exudate (× 800). **C)** Lobate sporangium of *P. aphanidermatum* formed prior to complete lysis of oospore germling (× 400). **D)** Lobate sporangia of *P. aphanidermatum* formed in liquid culture (× 400). **E)** Lobate sporangium of *P. aphanidermatum* lying free in soil after complete lysis of oospore germling (× 800). **F)** Sporangia of *P. aphanidermatum* germinating directly 2 hr after amending infested soil with bean seed exudate (× 200).

substrate, as used in the above tests, might differ in the presence of host organs, the following tests were conducted. Bean seeds, sugarbeet seeds, and 2-wk-old sugarbeet seedlings were employed as hosts. Two-gram samples of infested soil were placed in wells of a porcelain spot plate. A single bean seed, sugarbeet seed, or seedling was placed on the soil, which was brought to saturation with SDW and TW. Soil amended with SDW and TW served as controls. Direct microscopic examination of oospores and sporangia, germinating in soil adjacent to the respective host organs, was made at hourly intervals using the soil smear technique. Maximum oospore and sporangium germination (greater than 80%) occurred within 6 to 10 hr and 3 to 6 hr, respectively, in the immediate vicinity of the various host organs. Both propagules germinated directly, and vegetative growth continued until host colonization (24-36 hr). Host penetration, determined by microscopic examination of epidermal strips made from root and hypocotyl regions of sugarbeet seedlings, was accomplished by appressorium formation and/or direct hyphal penetration. Production of new oospores occurred only in colonized host tissue and in soil contiguous to host tissue.

Lack of zoospore formation from either oospores or sporangia in saturated soils is in contrast to previous reports (4, 5, 6). In previous work, germination was studied in water culture. Our preliminary studies showed that zoospore production from oospores of *P. aphanidermatum* readily occurred when oospores were placed in a liquid medium (SDW containing 20 μg Ca^{++}/ml and 20 μg asparagine/ml). Oospores germinated within 3 hr by the production of a short germ tube which either terminated in a lobate sporangium (Fig. 1D), which eventually produced a short evacuation tube and vesicle, or the protoplasm simply flowed into a vesicle from which 8 to 12 (avg 10) zoospores were released. Oospore and sporangium-infested soils were therefore amended with three times the volume of SDW or TW necessary to reach saturation (over saturation). Under such conditions ca. 30% of the oospores and 90% of the sporangia produced zoospores. The remaining oospores did not germinate. When infested soils were over-saturated with SDW or TW containing nutrient solutions, bean exudate, or bean seeds, however, only direct germination of the propagules occurred.

The effect of over-saturated soil conditions on zoospore production from oospores located at various depths in soil was determined by placing infested soil into a plastic cylinder 5-cm long and 1-cm diameter. Soil was poured into the cylinder, compressed to a depth of 4 cm, and sufficient water (SDW or TW) added until saturation occurred. The bottom of the cylinder was then sealed and a 1-cm layer of water maintained above the soil surface. Cylinders were incubated at 24 and 35 C. Zoospores were detected in the top 1 cm of water after 6 hr, reached maximum production (ca. 3,000/ml) after 24 hr, and were still motile after 96 hr. Germinated oospores, void of protoplasm, were observed in the surface water and in soil removed from the immediate soil-water interface. No zoospores were observed in saturated soil removed from the 1-, 2-, or 3-cm depths after a 6-, 24-, 48-, or 96-hr incubation period. Germinated oospores (ca. 10-20%) were found, however, at all soil depths but germ tubes (less than 100 μ in length) were filled with protoplasm and after 96 hr incubation was observed in various stages of lysis.

DISCUSSION.—Oospores of *Pythium aphanidermatum* were capable of either direct or indirect germination in field soil. Mode of germination, however, was governed by the presence or absence of an exogenous source of nutrients and presence or absence of free surface water in saturated soil. In the presence of nutrients, oospores germinated exclusively by germ tubes which continued to grow. These tubes eventually terminated in ephemeral sporangia or penetrated the host directly, without producing sporangia.

These results indicate that in addition to acting as the major survival structure of *P. aphanidermatum*, oospores, by germinating directly, also function as the primary infecting unit. Although oospores and sporangia are capable of producing zoospores in surface water of saturated soils or in water culture (4, 5, 6), neither their production from resting structures in the rhizosphere, nor their significance as primary root-infecting units under field conditions, has been demonstrated. Although zoospores may be produced at some distance from a root, their effectiveness as primary inocula would be considerably less than that of oospores germinating by a germ tube in the immediate rhizosphere. The function of zoospores may be limited to dissemination and chance colonization of above ground plant parts submerged during furrow irrigation or periodic flooding following thunderstorms.

Oospores of *P. aphanidermatum*, once they are stimulated to germinate in the absence of a colonizable substrate, are incapable of reforming a persistent survival structure in soil. This behavior, unlike that of sporangia of *Pythium ultimum* (9), suggests a possible method of biological control; namely a reduction in the inoculum density following incorporation of materials into soil capable of stimulating oospore germination in the absence of a host. Control, however, would depend upon: (i) oospore distribution in soil, (ii) quantity and quality of colonizable substrates in soil, (iii) the oospore density necessary for successful host colonization, and (iv) the physiological heterogeneity of the oospore population in soil.

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