The Sporangium of Pythium ultimum as a Survival Structure in Soil

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ABSTRACT

Sporangia of *Pythium ultimum* persisted for 11 months in air-dried and moist field soil with little or no decrease in either the rate or percentage germination. The *P. ultimum* population, as estimated by plate count, remained stable throughout this period. Sporangial germination occurred in soil in 1.5 hr, and reached a maximum of about 80% 3 hours after the addition of nutrients. Germ tube growth was independent of soil moisture contents between field capacity and near saturation. Germlings grew through moist soil at about 300 μ/hour, and were capable of maintaining this rate provided adequate nutrients were available.

Upon cessation of germling growth, germ tube and/or hyphal cell walls lysed. Two survival mechanisms capable of countering lysis were observed: (i) at low nutrient levels the protoplasm retracted from the tip of the germ tube, forming septa as it moved into the parent sporangium; and (ii) at high nutrient levels terminal and/or intercalary sporangia were formed. Both the parent and secondary sporangia were capable of regermination and germination, respectively. Quantitative population estimates, subsequent to sporangial germination and cell wall lysis in soil, indicated that these two survival mechanisms were capable of maintaining the *P. ultimum* population.

Results indicate that sporangia of *Pythium ultimum* function as major survival structures and inocula in cultivated soils. Phytopathology 61:157-164.

Pythium spp. survive in air-dry soil for periods of 2 to 12 years (10, 25), but the survival structure(s) (oospores, mycelia, zoospores, or sporangia) has not been adequately demonstrated.

The ephemeral nature of mycelium is well documented (12, 27), and probably is not important in long-term survival in soil, although it may be of major importance in spread of this organism after initial host colonization.

The role that motile zoospores play in the survival of *Pythium* spp. is not clear. Luna & Hine (13) demonstrated that zoospores of *P. aphanidermatum* encyst rapidly upon introduction into soil which did not contain a natural population of *P. aphanidermatum*. The organism was recovered 7 days after seeding with zoospores. This indicates that zoospores may survive as resistant structures (23).

Thick-walled oospores are commonly considered the fungal unit capable of long-term survival in soil (7, 10). Such spores are frequently observed in infested host tissue (3, 9, 14, 28). They survive for long periods of time (3, 9, 24) and germinate when stimulated (3, 4, 9, 23, 26).

Sporangia, which are also frequently observed in infested host tissue (3, 5, 14, 18), may function as survival structures of the sphaerosporangiate species of the genus *Pythium*. Agnihotri & Vaartaja (1) recently stated that sporangia of *Pythium ultimum* could persist for long periods of time in soil in the absence of a host, but provided no evidence other than citing the report that *Pythium* spp. were recovered from a naturally infested soil that was air-dried for 2 years (25). That sporangia may be important in survival is suggested by Warcup's observation (26) that all colonies of *Pythium* spp., isolated by the soil plate method, originated from spores. He did not determine, however, whether such colonies developed from oospores or resting sporangia. Warcup later reported (28) that *Pythium* was isolated only from chlamydospores present in decomposing roots. Bainbridge (3) further implicated sporangia in the survival of *P. ultimum* by demonstrating that sporangia, when collected from infested pea root segments buried in field soil for 5 months, germinated when placed in a nutrient medium.

Knowledge of the propagule responsible for the survival of *P. ultimum* in soil is vital to the understanding of its disease cycle. This is also necessary for conducting studies on pathogenicity, as use of other propagules such as mycelium may have little relevancy to the pathogen's activity in nature.

This investigation was undertaken to determine the role of sporangia of *Pythium ultimum* Trow as a survival structure in soil. Special attention was given to those biological characteristics, rapid germination and high growth rate (8), which contribute to the effectiveness of this pathogen as a damping-off pathogen. A preliminary report has been published (19).

MATERIALS AND METHODS.—Three agricultural sandy loam soils from the Salinas Valley were used. Soils A and B (Chular series) and soil C (Salinas series) had pH values of 7.3, 6.1, and 5.9, permanent wilting points (PWP) of 3.6, 6.3, and 6.1% moisture, and field capacities (FC) of 15.7, 20.2, and 18.4% moisture, respectively.

Four virulent *Pythium ultimum* Trow isolates obtained from diseased bean and cotton seedlings and soil B and C were used throughout. Isolates were maintained on potato-dextrose agar (PDA) and transferred monthly.

Preparation of pathogen-infested soil.—Two methods were used. Five-mm discs were removed from 2-day-old PDA cultures of each *Pythium* isolate and placed in petri dishes containing (i) 20-ml sterile distilled water and 2 rolled oat seeds; and (ii) 20 ml of 3% rolled oat broth. The first medium supported abundant oospore and sporangium production and sparse vege-

tative growth, whereas the latter medium supported abundant vegetative growth and little or no spore production during the 2-week incubation period at 24 C. Mycelial mats were removed, washed in sterile tap water, placed in 30-g samples of soils A and B, and incubated for 2 weeks at 24 C. At the end of the incubation period, one-half of each soil sample was air-dried (ca. 0.5% moisture on a dry wt basis) and stored in stoppered glass vials. The remaining half was stored in petri dishes kept in a moist chamber. No attempt was made to maintain a constant soil moisture. The soils were, however, never allowed to dry and were periodically moistened with sterile distilled water with an atomizer. Immediately prior to experimental use, aliquots of the moist soil were dried to 2-3% moisture on a dry wt basis.

Quantitative determination of the *Pythium ultimum* population in the three soils used was estimated by the method of Stanghellini & Hancock (20). Soils A and B had a *P. ultimum* population of ca. 2.0×10^5 propagules/g of soil, and soil C had a natural population of *P. ultimum* of $3,814 \pm 442$ propagules/g of soil.

Method of observation of pathogen behavior in soil.—Pathogen behavior in soil, unless otherwise specified, was observed by a modification of the method of Nash et al. (17). Aliquots of infested soil were placed in glass vials and diluted 1:3 with sterile distilled water. The sample was then gently agitated and 0.1 ml of the soil suspension immediately removed, placed on a glass slide, smeared, and dried over a bunsen flame. The preparation was stained with 0.1% acid fuchsin in 85% lactic acid and observed microscopically at ×100 and 430.

Measurement of spore germination, germling growth and germling survival in soil.—Two methods were used to evaluate spore germination, germling growth, and germling survival in soil.

1) One-g samples from pathogen-infested soils A and B were placed in wells of a porcelain spot plate and amended with 0.3 ml of either nutrient solutions of known concn made from reagent grade chemicals or seed coat exudate. Seed coat exudate was obtained in the following manner: Three g of bean seed coats (*Phaseolus vulgaris* L. 'Pinto'), removed from dry seeds in pieces, were placed in 30-ml sterile distilled water and incubated for 30 min at 24 C. The supernatant was centrifuged at 10,000 *g* for 10 min, filtered through a 0.22 μ Millipore filter, and stored at 5 C. Exudate was analyzed for water-soluble carbohydrate, amino acid, and glucose concn by the anthrone (16), ninhydrin (15), and glucostat (Worthington Biochemical Corp.) methods, respectively. All extractions were replicated 3 times.

Bean seed coat exudate contained ca. 4,000 μg total carbohydrate, 214 μg amino acid, and 210 μg glucose/g of seed coat. Control plates were moistened with distilled water. Amendments resulted in a soil moisture of ca. 28% on a dry wt basis, pF 1.7 (pF = log [cm of water suction]). Plates were incubated at 12 and 24 C. Both soil and amendments were preincubated for 24 hr at the above temp prior to mixing.

2) Two-g samples of pathogen-infested soil A and B were placed in a Haines' apparatus (21). Either distilled water or nutrients of known concn were used to make the continuous liquid column. Soil samples were then saturated and immediately subjected to various suctions, the entire process taking ca. 15 min to reach equilibrium.

Preliminary studies showed that soil sample size was not a critical factor, and therefore 1- to 2-g soil samples were used throughout this study unless otherwise specified. Aliquots of treated soils were prepared and observed as described. All experiments were replicated 3 times and repeated periodically throughout an 11-month period.

Percentage germination, measured by determining the percentage of spores with germ tubes, was expressed as the average of three counts, 50 spores/count for each treatment.

Germling growth rate in soil was determined by (i) measuring and averaging the lengths of 75 germ tubes/treatment at hourly intervals after the initiation of germination; and (ii) measuring germ tube growth of 10 single germlings at 10-min intervals after the initiation of germination. The latter measurements were made by placing aliquots of amended soil suspensions on a glass slide and measuring the growth by means of an ocular micrometer at a magnification of ×430.

RESULTS.—*Microscopic examination of P. ultimum-infested soil.*—Microscopic examination of artificially infested soils after 2 weeks' incubation at 24 C showed that all the mycelium was lysed and only oospores and sporangia remained (Fig. 1-A). Sporangia ranged from 15.1-27.6 μ (average 23.4 μ) in diam. Wall thickness ranged from 0.7-1.0 μ. Oospores ranged from 10.0-24.1 μ (average 16.5 μ) in diam. Wall thickness ranged from 1.8-2.3 μ. The ratio of sporangia to oospores was ca. 30:1 as estimated by microscopic observation of soil smears. Differentiation between oospores and sporangia was also facilitated by the different staining properties of sporangia and oospores by acid fuchsin. Sporangia stained much more intensively than oospores.

Examination of soils A and B, infested by the two methods, revealed no morphological differences between oospores and sporangia formed on mycelial mats prior to soil infestation and those formed in soil during the incubation period.

Influence of seed coat exudate on spore germination of P. ultimum.—Sporangia germination started 1.5 hr after the soil was supplemented with seed coat exudate, and reached a max of $82 \pm 3\%$ after 3 hr at 24 C (Fig. 3, top). Oospore germination was not observed throughout this study, and the ratio of sporangia (germinated + ungerminated) to oospores (ungerminated) remained the same. Ninety-six per cent of the sporangia germinated with only one germ tube, the remainder with two. Although branching was frequently observed only after initial extension of the germ tube (Fig. 1-B), bifurcation was also noted upon emergence of the germ tube from the sporangium. No germination occurred in moist soil amended with distilled water. Germination of sporangia in the air-dried soil when amended with dis-

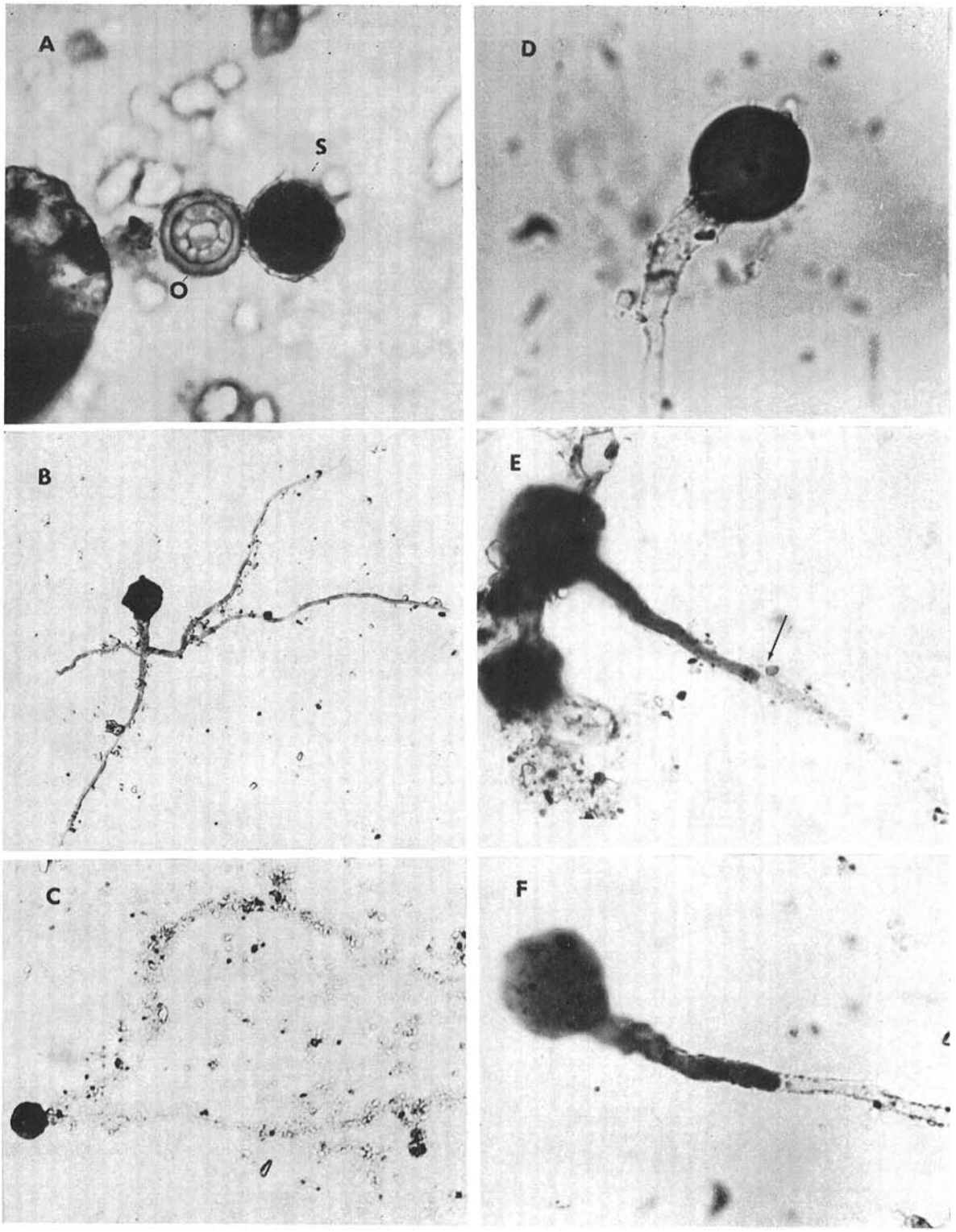

Fig. 1. **A)** Oospore (O) and sporangium (S) of *Pythium ultimum* in infested soil. (×430) **B)** Germinated sporangium of *Pythium ultimum* 2 hr after amending infested soil with bean seed coat exudate. (×320) **C)** Partially lysed germ tube walls of *Pythium ultimum* sporangium 24 hr after amending soil with 300 µg glucose/g soil (×280); and **D)** 4 hr after amending soil with 15 µg glucose/g soil. (×800). **E)** Retraction of protoplast with septum formation (×800), shown by arrow; and **F)** without septum formation (×800) in sporangial germlings of *Pythium ultimum* prior to germ tube wall lysis.

tilled water probably resulted from the release of stimulatory substances following rewetting of air-dried soil. To test this possibility, three 10-g samples of air-dried and moist infested soil were shaken in 10 ml sterile distilled water for 10 min and centrifuged at 10,000 *g* for 10 min. The respective supernatant fluids were passed through a 0.22 μ Millipore filter and stored at 5 C. Aliquots from each were then used to supplement the moist infested soil. No germination occurred when this soil was supplemented with the supernatant from moist soil, whereas 4% germination occurred when supplemented with the supernatant from the air-dry soil. Analysis of the supernatant fluids for the soluble carbohydrates (16) showed that moist soil contained 12.6 ± 3.5 μg/g soil, whereas remoistened air-dried soil contained 34.6 ± 4.6 μg/g soil.

Twenty-five individual germlings were isolated by flotation, transferred to water agar, and incubated for 12 hr at 24 C. Single hyphal tips were then transferred to oatmeal agar for identification of *Pythium* spp. All isolates were identified as *P. ultimum*.

Germination tests were conducted monthly over an 11-month period. No decrease in either the percentage or rate of sporangial germination was noted in soils maintained in the moist or air-dry condition.

Influence of soil moisture, temp, and nutrient concn on sporangial germination, germling growth, and survival in soil.—Sporangia germinated within 1-1.5 hr after amending artificially infested soils with various concn of glucose, and reached a max after 3-hr incubation at 24 C at 50- and 100-cm suction. Percentage germination increased with increasing nutrient concn up to a level above which no further increase occurred (Fig. 3, center). At 12 C, sporangia germination started 4 hr after incubation and reached a max of 72.4 ± 4.1% after 5.5-6 hr at 50-cm suction.

Ninety-four per cent of the sporangia germinated with one germ tube, the remainder with two. Little or no lateral branching of the germ tube was observed during the first 5 hr of incubation at 24 C.

Individual germ tubes grew at a rate of 324 ± 36 μ/hr at 24 C. The growth rate was apparently independent of both initial nutrient concn and soil moisture contents from near saturation (50-cm suction) to FC (100 cm suction) (Table 1). At low nutrient levels (less than 15 μg glucose/g soil), germ tube growth ceased within 2.5-3 hr after germination. At nutrient levels greater than 15 μg glucose/g soil, germlings were

TABLE 1. Influence of soil moisture and various nutrient levels on germ tube lengths of *Pythium ultimum* in soil after 2-hr incubation at 24 C

	Avg length of 75 germ tubes at cm water suction	
μg Glucose/g soil	50	100
	μ	μ
7.5	128.7 ± 56.2	
15.0	141.3 ± 45.3	136.9 ± 53.2
30.0	147.7 ± 46.4	140.1 ± 46.2
300.0	149.5 ± 47.4	147.6 ± 48.1
600.0	135.7 ± 63.7	

able to maintain the growth rate for extended periods of time. After 3 hr, germ tube lengths of 382.2 ± 121.6 μ were recorded. Germ tubes subsequent to 3-hr incubation were dislodged from the sporangia during the preparation of soil smears, and consequently only a few intact germ tubes could be measured. Germ tube lengths exceeding 1,200 μ, however, were of common occurrence after 5-hr growth, indicating that the growth rate was sustained provided adequate nutrients were available. Rate of germ tube growth at 12 C was not assessed directly. Hourly measurements of germ tube lengths over a 3-hr period subsequent to germination gave an average growth rate of 96.9 ± 17.2 μ/hr. Cessation of growth at glucose concn below 15 μg/g soil occurred 7-10 hr after germination.

Subsequent to cessation of growth, lysis of germ tubes and/or mycelium occurred (Fig. 1-C, D). Germ tubes in various stages of lysis were observed 3-4 hr after amending soil with glucose concn below 30 μg/g soil. In general, lysis occurred from the tip of the germ tube, the protoplasm apparently retracting and forming septa during retraction into the parent sporangium (Fig. 1-E). The formation of septa, however, was not always observed. In germ tubes less than 100 μ in length, retraction occurred without septa formation (Fig. 1-F). Regardless of whether or not septa were formed, the sporangium was capable of regermination upon supplementation of additional glucose to the soil (Fig. 2-A). Both the rate of regermination and germ tube growth were the same as previously determined. Addition of nutrients prior to complete protoplasmic retraction indicated that the retraction process was a reversible phenomenon. Resumption of protoplasmic growth proceeded until encountering a previously formed septum, then branched at right angles to the septum (Fig. 2-C, D). Direct observation of the retraction phenomenon in germlings without septa formation in unstained soil smears proved unsuccessful. Germlings in various stages of protoplasmic retraction were observed, but instead of continued retraction into the parent sporangium, the protoplast resumed forward growth, filling the previously evacuated germ tube. This apparent reversal may be attributed to a number of factors ranging from a physiological response due to changes in light and/or temp or reduced competition resulting from soil dilution during the preparation of soil smears.

Under nutrient conditions capable of supporting extensive hyphal growth (Fig. 2-E) (greater that 30 μg glucose/g soil), sporangia were completely evacuated of protoplasmic contents, and a septum was formed within the first 25 μ of the germ tube length. Successive septa were then formed as the protoplast moved forward. Evacuated sporangia collapsed during preparation of soil smears for microscopic examination. Lysis was first observed under such nutrient conditions only after 10-12 hr incubation, and consisted of missing intrahyphal segments. Prior to complete lysis, however, both terminal and intercalary sporangia were formed (Fig. 2-G). Although the latter were sparsely formed under low nutrient conditions, large numbers were observed in soil after 24-hr incubation at high nutrient conditions (Fig. 2-F). The secondary sporangia (Fig. 2-B) germi-

nated in 1.5-2 hr, and germ tubes grew at about the same rate as parent sporangia when supplemented with additional glucose. It appeared that the secondary sporangia were smaller than the parent sporangia. Measurements of 100 germinating sporangia were made after three successive germinations in a single soil sample amended with 300 μg glucose/g soil every 24 hr. A progressive decrease in sporangial size was noted after each successive germination (Fig. 3, below).

Studies using naturally infested soil C.—Microscopic

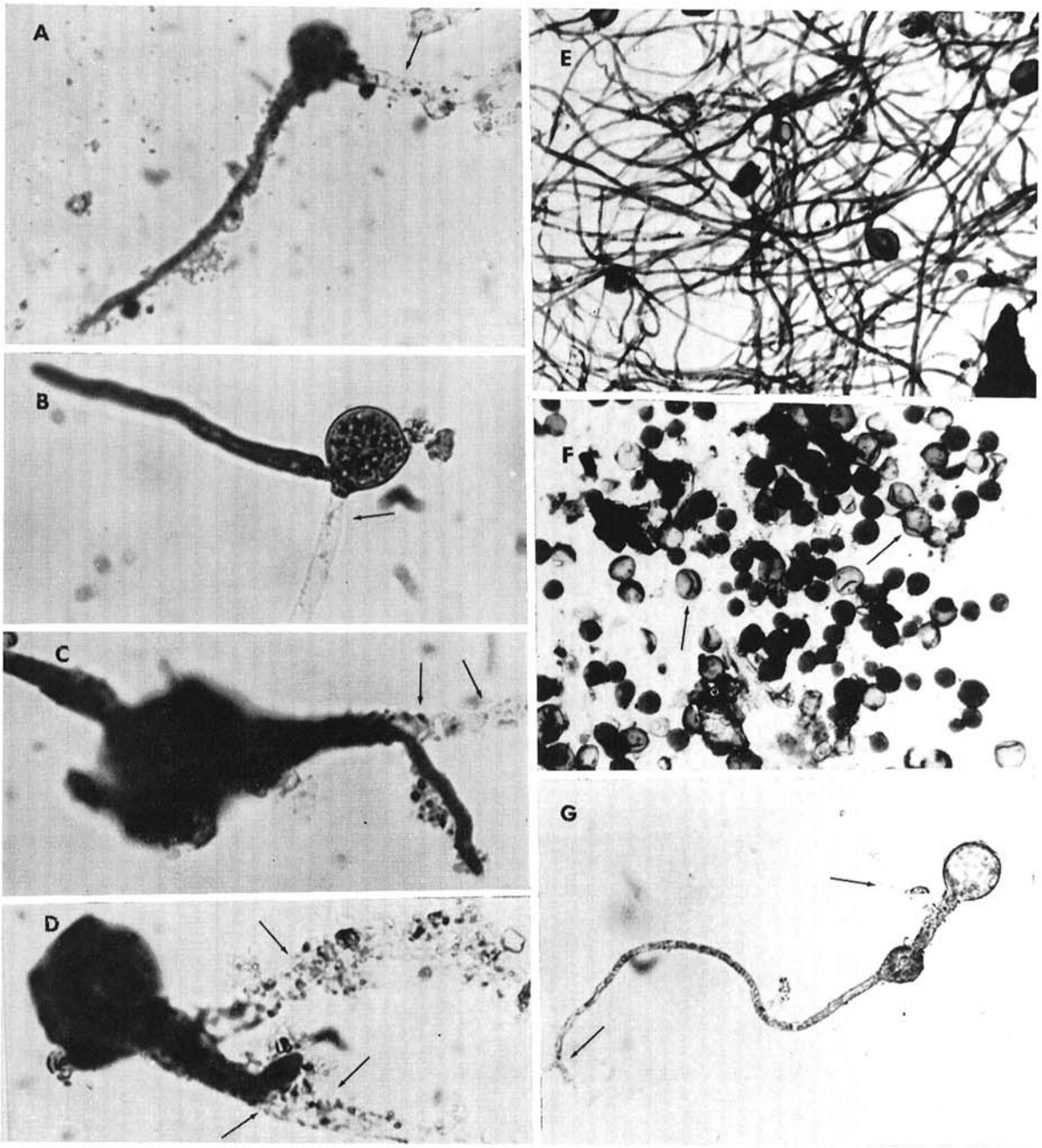

Fig. 2. **A)** Regermination of *Pythium ultimum* sporangium upon addition of glucose to soil after evacuation of the protoplast and incomplete wall lysis of the first germ tube (arrow). (×520) **B)** Germinated secondary terminal sporangium formed in soil. (×640) Evacuated germ tube of parent sporangium still attached (arrow). **C, D)** Reversal of protoplasmic retraction after the addition of glucose prior to complete lysis of evacuated germ tube walls. Growth resumed until encountering a previously formed septum, then branched at right angles to the septum. Partially lysed evacuated germ tubes and septum indicated by arrows (×1,000). **E)** Mycelial growth of *Pythium ultimum* 12 hr after amending soil with 300 μg glucose/g soil. **F)** Secondary spore formation and complete mycelial lysis in the same soil after 24-hr incubation. Collapsed primary sporangia indicated by arrows. (×200) **G)** Intercalary sporangium by *Pythium ultimum* formed prior to complete lysis of evacuated germ tubes (arrows). (×400)

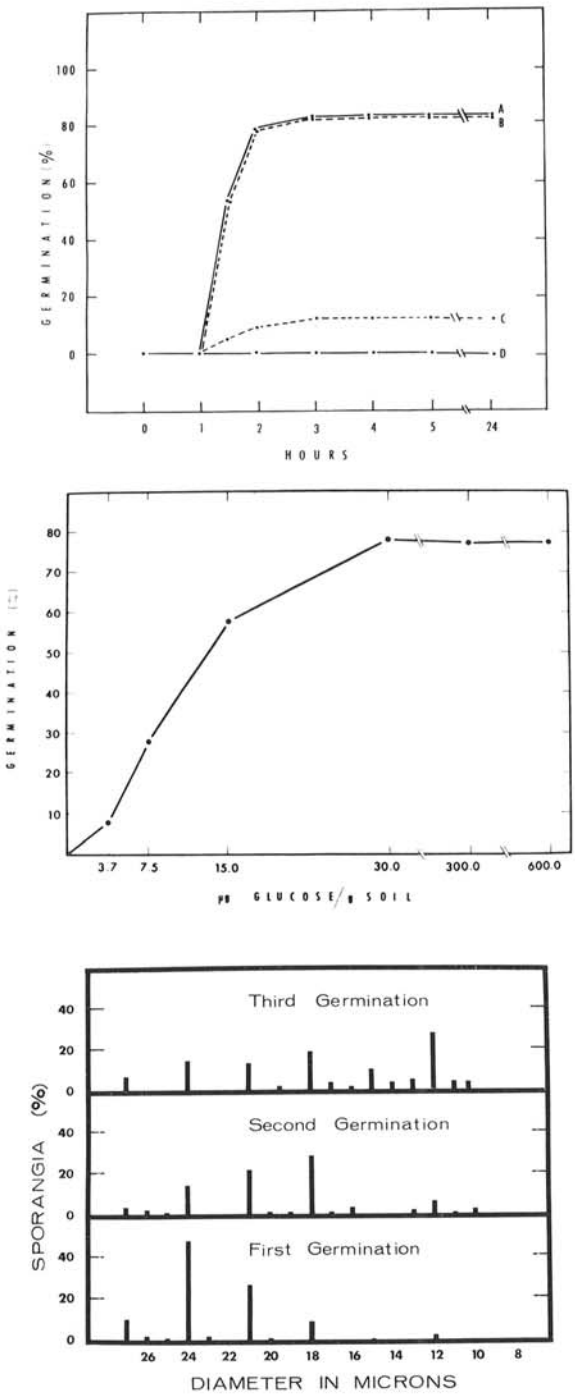

Fig. 3. (Top) Rate and percentage germination of *Pythium ultimum* sporangia from moist and air-dry soil after amending soil with seed coat exudate (A and B) and sterile distilled water (C and D). A and D = soil maintained moist: B and C = soil maintained air-dry prior to the addition of seed coat exudate. (Center) Influence of various glucose concn on percentage germination of *Pythium ultimum* sporangia in soil after 3-hr incubation at 24 C. (Below) Sporangial size distribution of *Pythium ultimum* after three successive germinations in a single soil sample amended with 300 µg glucose/g soil. Measurements were obtained from 100 germinating sporangia after successive additions of glucose at 24-hr intervals.

examination of naturally infested soil revealed the presence of spherical, thin-walled, sporangial-like propagules. Fifty such spores were measured and had a mean diam of 24.7 µ with a range from 18-31 µ. Wall thickness ranged from 0.9-1.3 µ. Upon supplementation with seed coat exudate, these propagules germinated in soil within 2-3 hr. Fifteen germlings were recovered by flotation and transferred to oatmeal agar for identification. All isolates were identified as *P. ultimum*. No germination occurred when soil was amended with sterile distilled water. No thick-walled oospores were observed in this soil except for an occasional spiny oospore which had not germinated.

Effect of air-drying on the P. ultimum population in soil C.—Aliquots of soil C were air-dried at 24 C. The remaining soil was maintained at ca. FC and stored in plastic bags in the laboratory. Monthly estimates of the *P. ultimum* population were determined using the isolation technique. No significant changes were recorded in the *P. ultimum* population over a 7-month period from either air-dried or moist field soil C.

Quantitative estimations of the effect of air-drying on the *P. ultimum* population in infested soil A and B were not made. The isolation technique was not developed until 4 months after initial infestation of this soil, and the high population recorded at that time was unevenly distributed throughout the soil mass. But microscopic observation of soil smears prepared from both air-dry and moist infested soil throughout an 11-month period indicated that little or no changes in the propagule density occurred, since ca. 3 sporangia/microscopic field at ×100 were consistently observed.

Effect of various nutrient amendments and fluctuating moisture regimes on the natural population of Pythium ultimum in soil C.—Results from the studies using artificially infested soils demonstrated the behavior of *P. ultimum* in soil under various nutrient regimes. The overall effect of nutrient amendments on fluctuations in the *P. ultimum* population in soil was not known. Possible changes in the population as a result of soil amendments were determined using naturally infested soil C. This soil had a stable population of 3,814 ± 422 propagules/g soil (20).

One-g soil samples were placed in 125-ml flasks and amended with 0, 30, 300, and 3,000 µg glucose/g soil. Sterile distilled water served as a control. All amendments resulted in a saturated soil condition. After adding amendments, soils were incubated for 48 hr, then air-dried for a further 48 hr at 24 C. This procedure was repeated up to 3 times with individual 1-g soil samples. Estimations of the *P. ultimum* population were determined after each 48 hr air-dry treatment. Each treatment had three replicates and was repeated twice.

No germination occurred in the water controls and no germ tubes or hyphae were observed in soil smears prepared after each 48-hr air-dry treatment. Percentage germination was determined in soils receiving 30 and 300 µg glucose/g after the first and third germinations as follows: 1-g soil samples were placed in 125-ml flasks and amended with 30 and 300 µg glucose/g soil. Soils were incubated for 6 hr at 24 C, then diluted with 99 ml sterile distilled water. The suspension was shaken

for 2 min by hand. One-ml aliquots were removed and dispensed on solidified water agar in petri dishes. Plates were observed at ×30 magnification on a dissecting microscope, and individual germlings recovered using a microcapillary tube. According to previous population estimates, each ml of the 1:100 dilution should contain 38 ± 4 germlings. An average of 26.0 ± 4.3 and 17.3 ± 4.4 germlings was recovered from three 1-ml aliquots from three separate 1-g soil samples after the first and third germinations, respectively, in soil amended with 30 μg glucose/g soil, and an average of 28.1 ± 2.2 and 21.4 ± 6.4 germlings were recovered after the first and third germinations, respectively, in soil amended with 300 μg glucose/g soil. Upon recovery, germlings were transferred to water-agar plates and incubated for 12 hr at 24 C. Hyphal tips were transferred to oatmeal agar for identification. Ninety-two per cent of the germlings recovered were identified as *P. ultimum*.

Results presented in Table 2 indicate that germination and subsequent lysis does not result in a corresponding decrease in the *P. ultimum* population, and suggest that the survival mechanisms observed in artificially infested soil A were probably operating in the naturally infested soil C.

DISCUSSION.—We sought to determine the nature of the *Pythium ultimum* propagule responsible for survival in soil. Results provide direct evidence that sporangia of *P. ultimum* function as survival structures and as inocula in cultivated soils. Sporangia, morphologically identical to those produced in culture, are produced in soil and are capable of persisting for long periods of time in either air-dry or moist field soil in the absence of a host, with no decrease in either the percentage or the rate of germination. Population estimates revealed no significant changes in the *P. ultimum* population in soil maintained in either an air-dry or moist condition over a 7-month period. In addition, cyclic wetting and drying of individual soil samples did not affect the population. These results indicate that fluctuating or constant soil moisture regimes have little effect on the survival ability of sporangia. Although the effect of temp on sporangial survival in soil was not studied, Bainbridge (3) found that sporangia collected from infected pea roots buried in field soil over the hot summer months were viable, indicating that they were capable of withstanding high temp. That sporangia are capable of acting as survival structures in soil is strengthened by direct microscopic observation of sporangia in a naturally infested field soil.

TABLE 2. Effect of glucose amendments and fluctuating moisture regimes on the *Pythium ultimum* population in a naturally infested field soil

Wet-dry[a] cycles	Soil amendments: μg glucose/g soil			
	0	30	300	3,000
	propagules/g soil × 10^{-2}			
1st	37.3 ± 3.6[b]	38.5 ± 6.3[c]	39.0 ± 4.5[e]	36.3 ± 5.1
2nd	35.0 ± 5.0	34.5 ± 5.5	36.1 ± 6.5	27.3 ± 5.2
3rd	37.8 ± 4.4	29.1 ± 4.5[d]	32.5 ± 3.8[f]	28.8 ± 3.1

[a] One-g soil samples were amended with various glucose concn and incubated for 48 hr, then air-dried for 48 hr when population estimates were determined. The procedure was repeated up to 3 times with individual soil samples.
[b] Mean and standard error of six replications.
[c] 60% Germination.
[d] 42% Germination.
[e] 67% Germination.
[f] 50% Germination.

Sporangia of *P. ultimum* apparently require no dormancy period, but germinate readily in soil when provided with exogenous nutrients. The low nutrient requirement for sporangial germination in soil, as shown in this study, and the high percentage of the *P. ultimum* population capable of germination in a short period of time, would, at first, appear to be detrimental to the survival of this organism. When viewed in regard to the ecology of this organism, however, these biological characteristics contribute to the effectiveness of *P. ultimum* as a damping-off organism. Furthermore, the rapid rate of germ tube growth through soil, which is apparently independent of the initial nutrient concn and soil moisture contents between saturation and field capacity, enables *P. ultimum* to reach its substrate before natural antagonism develops.

Failure of successful substrate colonization after sporangial germination does not necessarily result in a decrease in the inoculum density. Under nutrient conditions capable of stimulating germination but not supporting vegetative growth, formation of secondary sporangia and/or the retraction of the protoplast provides *P. ultimum* with two survival mechanisms capable of maintaining its sporangial population. Although intrahyphal protoplasmic retraction has been observed in cultural studies (5), its significance was not elucidated. The formation of secondary sporangia, functionally analogous to chlamydospore reformation (6), has not been previously reported in soil. Agnihotri & Vaartaja (1) reported that no resistant structures were formed in soil prior to the death of growing *P. ultimum* hyphae. They, however, employed only a single sampling time subsequent to sporangial germination in soil.

While quantitative population estimates after sporangial germination in soil provided indirect evidence that these two survival mechanisms were capable of maintaining the *P. ultimum* population, microscopic examination of soils showed a progressive decrease in the size of secondary sporangia after successive germination and reformation. Whether this decrease in sporangial size results in a decrease in the inoculum potential was not determined, but deserves consideration.

The term lysis, as used in this study, refers only to degradation of empty germ tubes and/or hyphal cell walls. Mycolysis, as defined by Lloyd & Lockwood (11), is the dissolution of both the protoplast and the cell wall. We did not observe protoplasmic dissolution. Empty germ tubes and intrahyphal segments were attributed to evacuation and conservation of protoplasm as described by Aragaki (2) rather than protoplasmic dissolution. Whether a similar phenomenon occurs in other fungi warrants investigation. Mere observation of empty germ tubes or empty intrahyphal segments does not preclude protoplasmic dissolution.

Thick-walled oospores are generally considered the primary fungal unit capable of long term survival in soil. Aside from their ability to survive in soil (3), little direct evidence has been reported concerning their role as inocula in soil. Studies on oospore germination are complicated by reason of their constitutive dormancy period, at least among the sphaerosporangiate *Pythium* spp., and the technical difficulty in differentiating between a sporangium and an oospore once germination has taken place (3, 7, 22). Quantitative information on the rate of oospore germination in soil and factors initiating germination are needed. Barton (4) 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 soil. Cultural studies (9) have shown that oospores of *P. debaryanum* and *P. graminicola* germinated in the presence of growing plant roots and solutions containing root exudates. In view of this scant information, evaluation of the relative importance of oospores as a primary source of inoculum in heavily cropped agricultural areas is not possible.

Evidence presented in this study indicates that sporangia of *P. ultimum* play a major role in the life cycle of this species. The ability of sporangia to persist under adverse conditions, coupled with their extraordinary capacity to avoid competition by rapid germination and rapid germ tube extension in response to low exogenous nutrients and by producing secondary sporangia and retracting and conserving protoplasm under starvation conditions, enables *P. ultimum* to occupy its soil ecological niche; that is, *Pythium ultimum* is well adapted as a primary colonizer of substrates possessing readily available sugars.

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