

# Influence of Soil Environment on the Germinability of Constitutively Dormant Oospores of *Pythium ultimum*

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## ABSTRACT

Dormant, thick-walled oospores of *Pythium ultimum* were converted to thin-walled oospores after an incubation period of 1-10 weeks on nonsterile soil agar, in soil extract, or on soil saturated with water. Thin-walled, but not thick-walled, oospores germinated within 2 hours when placed on nutrient media or in fresh water, but not in situ. Thin-walled germinable oospores (average wall thickness, 0.53  $\mu\text{m}$ ) were readily differentiated from dormant thick-walled oospores (average wall thickness, 2.09  $\mu\text{m}$ ) by differences in thickness of the oospore walls and by their stainability with lactofuchsin. Thin-walled oospores, sporangia, and vegetative hyphae lost their viability when rapidly dried, but thick-walled dormant oospores survived rapid drying and

remained viable for a least 8 months. Conversion of dormant to germinable oospores was maximum at pH 7.0 in a soil saturated with water at 25 C. Damping-off of snapbean seedlings occurred in soil infested with thin-walled, germinable oospores, but not in soil infested with thick-walled, dormant oospores, until enough time had elapsed for conversion to germinable oospores. The resistant, thick-walled, constitutively dormant oospores and their conversion to germinable oospores in appropriate environments are significant to the survival of the species in soil and the etiology of plant diseases caused by this pathogen.

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*Additional key words:* survival, fungistasis, damping-off, *Phaseolus vulgaris*.

The importance of oospores in the survival of *Pythium* spp. is generally considered to be that of long-range persistence (9), enabling the pathogen to survive periods of adversity in a soil environment. The oospores of *Pythium ultimum* Trow seem capable of long-range survival because of their thick walls and their constitutive dormancy period. The dormancy of thick-walled oospores of *P. ultimum* is well documented (3, 5, 16, 20). Also, we have noted that oospores of *P. ultimum* are not germinable at first, but, after an extended period of incubation in soil extract, they gain the ability to germinate (2). In this regard, we follow the concepts of Sussman and Douthit (18) on the dormancy of spores: "constitutively" dormant oospores, which do not germinate because of the innate property of the dormant stage, we refer to as "dormant" or "thick-walled" oospores. On the other hand, the thin-walled, "exogenously" dormant oospores, which do not germinate because of an inappropriate environment (fungistasis), we refer to as "germinable" or "thin-walled" oospores.

The germination of *P. ultimum* oospores in vitro has previously been described. Trow (20) in the original description of *P. ultimum* and later Drechsler (4, 5) described the anatomical changes which occur when oospores convert from dormant to germinable in water-flooded cultures. In these reports, the germinable oospores are referred to as "ripe oospores" or "oospores converted to conidia" (20) and as "after-ripened oospores" (5).

We studied the germination of *P. ultimum* oospores to elucidate further the factors that affect their germinability, and to determine the importance of the conversion phenomenon in soil and on disease etiology.

**MATERIALS AND METHODS.**—*Pythium ultimum* isolate PuB5 (ATCC 26083) was used in all

experiments. Other isolates and species of *Pythium* used for comparison are indicated in the text.

Oospores were prepared from cultures in V8-cholesterol medium as previously described (2) and were used immediately after harvest or after 2 weeks of incubation in 1% nonsterile soil extract (2). Dormant oospores were usually applied to 2% water-agar disks, unless otherwise noted, dried within 30 minutes in rapidly moving ambient (22 C) air, and tested after various periods of dry storage (usually less than 1 month) at room temperature. Also, oospores in 0.5% water agar were applied to 22  $\times$  22 mm glass cover slips, dried, and stored before use. Conversion from dormant to germinable oospores was tested in about 10 ml of nonsterile soil extract, in 1% agar containing 50% soil (5 g soil + 5 ml 2% agar), in petri dishes, or on soil saturated with water, as indicated.

To test germinability of converted oospores, all preparations were transferred to Mircetich's pimaricin-vancomycin medium (MPVM) (14), a nutrient-rich medium that suppressed growth of bacteria and nonpythiaceous fungi derived from the soil extract. Incubation was usually at 24-30 C for the indicated time intervals. Spores were stained with acid fuchsin (0.03% in 85% lactic acid).

The soil used in all laboratory experiments was *Pythium*-free sandy loam (81% sand, 3% silt, 16% clay) from Beltsville, MD (pH 6.0) with a field capacity (FC) of 11.6% moisture.

To study the effect of soil moisture on conversion of dormant oospores, we adjusted the soil to various moisture levels: soil saturated with water (0 bars), FC (about -1 bar), 75% FC (-4 bars), 50% FC (-7 bars), 25% FC (-39 bars), and air dried soil (-93 bars). The soils were allowed to equilibrate for 2 days after adjustment. Dried, dormant oospores on agar disks or oospores applied to

cover slips were placed on the surface of the soils in petri dishes, with the oospores in close contact with the soil surface, and incubated at room temperature (22 C) for 1-6 weeks. Soil-water potential was measured with a model HR-33 dewpoint microvoltmeter and a model C-51 sample chamber psychrometer (Wescor, Inc., Logan, Utah).

The effect of pH on oospore conversion was determined similarly on the surface of water-saturated soil previously adjusted to various pH levels with  $\text{CaCO}_3$  or  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$  and equilibrated before use. Incubation was at room temperature for 2-8 weeks.

Oospore conversion in natural soil and in the greenhouse was studied with soil from Salisbury, MD (sandy loam: 78% sand, 11% silt, 11% clay; 1.42% organic matter; pH 5.1; and FC of 6.9%). The soil was used either untreated or was treated with aerated steam for 2 hours at 80 C. Soils were infested with freshly harvested or dried oospores without the soil-extract treatment. Oospores for assay and direct examination in natural soil were added at the rate of 350,000/g of soil (equivalent dry weight). The high inoculum density was intended to facilitate examination of oospores in soil. Oospores for study of damping-off in relation to oospore conversion were added at the rate of 500 oospores/g of steamed soil. The latter inoculum density was considered to approximate natural levels. Ten bean (*Phaseolus vulgaris* L. 'Topcrop') seeds were planted per pot and incubated at 21 C in water-bathed temperature tanks. After 2 weeks, damping-off was assessed and the soils were incubated fallow for 2 more weeks after removal of the plants. A final test for damping-off was performed, again with the use of 10 seeds per pot and a 2-week incubation period.

Populations of *Pythium* in infested soils were assayed at 20 C by a dilution-plate frequency test, with the use of gallic acid medium (GAM) (13).

Experiments were repeated two or more times, and each included at least three replications.

**RESULTS.**—*Conversion of thick-walled dormant oospores to thin-walled germinable oospores.*—Thin-walled *P. ultimum* oospores were observed in preparations of thick-walled oospores flooded with nonsterile soil extract (Table 1, Fig. 1-A, B) or with sterile or nonsterile water. Only thick-walled oospores were observed in static cultures in V8-cholesterol medium or in

nondisturbed cornmeal-agar cultures, even after 8 weeks of aging. Conversion of oospores resulted in a decrease in percentage of thick-walled oospores and an increase in thin-walled oospores (Table 1). Germination of thin-walled oospores did not occur in the soil extract, but only after the thin-walled oospores were removed, rinsed with water or placed on MPVM or GAM (Fig. 1-G, I). The rate of germination paralleled the percentage of thin-walled oospores present (Table 1). Rapid drying in ambient air prevented the germination of thin-walled oospores and sporangia (Table 1). In contrast, oospores of *P. aphanidermatum* (Edson) Fitzp. (ATCC 26081) did not convert to thin-walled spores, had a high germination rate, and were not adversely affected by rapid drying (Table 1).

The thin-walled germinable oospores of *P. ultimum* resembled sporangia or conidia (Fig. 1-F), but were still enveloped in an oogonial wall (Fig. 1-B, E), occasionally with antheridia still attached (Fig. 1-C). In addition, they stained deep-red with lactofuchsin (Fig. 1-B, E), as did sporangia, whereas dormant oospores remained nonstained (Fig. 1-A, D).

Dormant and germinable oospores were readily distinguished from each other by the contrasting thickness of their walls (Fig. 1-A, B, D, E, J) especially when viewed microscopically with plane-polarized light (Fig. 1-H). Alteration of the intensity of birefringence of light through the thin walls was apparent. Wall thickness of dormant and germinable oospores and of sporangia were 2.09  $\mu\text{m}$  (range 1.53-2.60  $\mu\text{m}$ ), 0.53  $\mu\text{m}$  (range 0.24-1.18  $\mu\text{m}$ ), and 0.57 (range 0.24-1.18  $\mu\text{m}$ ), respectively.

Germinable oospores that had lost their oogonial wall were often difficult to distinguish from terminal sporangia because of their similar size, wall thickness and stainability [diameter 19.8  $\mu\text{m}$  (range 17.6-22.4  $\mu\text{m}$ ) and 20.1  $\mu\text{m}$  (range 14.2-25.7  $\mu\text{m}$ ), respectively]. To avoid confusion of the sexual and asexual stages, mycelial mats were dried within 30 minutes in rapidly moving ambient air (22 C) to kill mycelia and the relatively few sporangia that had developed in the original culture medium. After the mats were subjected to the lytic action of nonsterile soil extract, the resulting spore preparations consisted entirely of oospores.

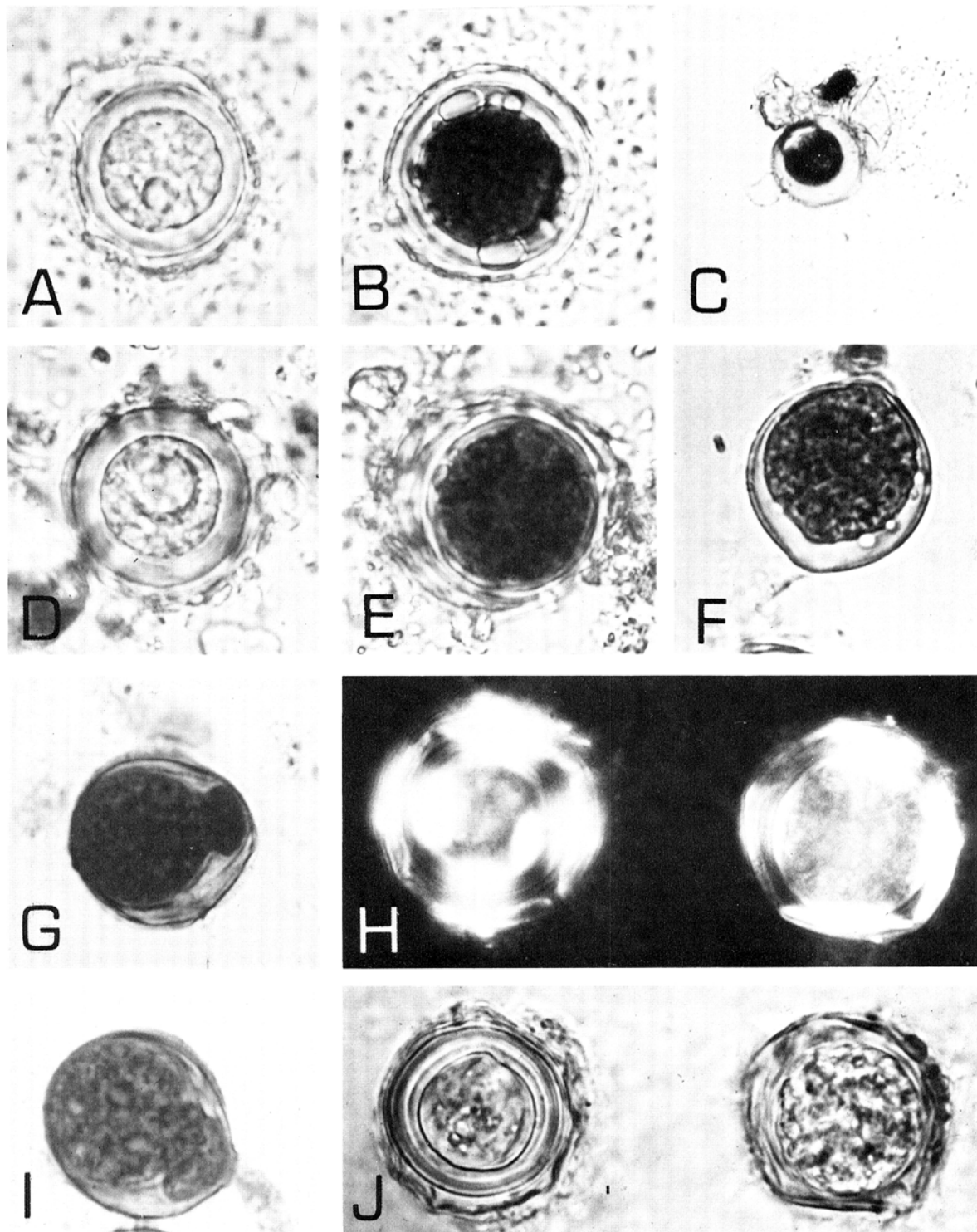
The degree by which nonsterile soil extract affected conversion of dormant oospores of several different

TABLE 1. Percentage of thick-walled and thin-walled oospores of *Pythium ultimum* and *P. aphanidermatum* and their germination after 2, 4, and 6 weeks of preincubation in lytic nonsterile soil extract

Incubation time in soil extract (weeks)	Oospores with:		Germination <sup>b</sup>	
	Thick <sup>a</sup> walls (%)	Thin <sup>a</sup> walls (%)	Before drying (%)	After drying (%)
<i>P. ultimum</i>				
2	97.4	2.6	6.1	0
4	75.0	25.0	27.3	0
6	35.0	65.0	66.6	0
<i>P. aphanidermatum</i>				
2	100.0	0	84.5	95.0
4	100.0	0	93.7	99.0
6	100.0	0	78.7	81.0

<sup>a</sup>Thick-walled oospores had wall thickness > 2  $\mu\text{m}$ , thin-walled had wall thickness < 1  $\mu\text{m}$ .

<sup>b</sup>Incubated 20 hours on pimaricin-vancomycin medium at 24 C.



**Fig. 1—(A to J).** Spores of *Pythium ultimum* obtained from V8-cholesterol cultures. **A, B**) Dormant, thick-walled and germinable, thin-walled oospores, respectively, on soil agar for 2 weeks and stained with lactofuchsin ( $\times 1,545$ ); **C**) stained, thin-walled oospores with antheridium attached ( $\times 659$ ); **D, E**) dormant, thick-walled and converted, germinable, thin-walled oospores, respectively, in soil ( $\times 1,545$ ); **F**) stained, thin-walled sporangium or conidium ( $\times 1,545$ ); **G**) thin-walled oospore beginning germination ( $\times 1,545$ ); **H**) thick- (left) and thin-walled (right) oospores viewed with plane-polarized light ( $\times 1,545$ ); **I**) germinated thin-walled oospores ( $\times 1,545$ ); **J**) nonstained oospores identical to those in (H), but viewed with transmitted light ( $\times 1,545$ ).

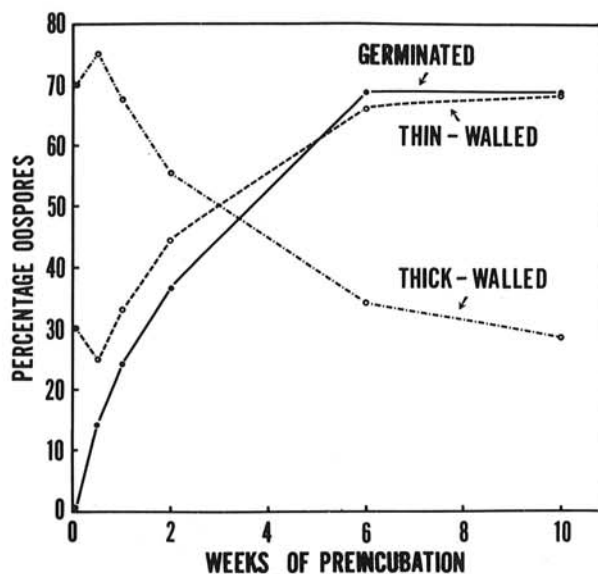


Fig. 2. The percentage of thick-walled dormant oospores in relation to thin-walled germinable oospores, as affected by time of incubation on 50% soil agar at 25 C. Germination of thin-walled oospores was determined on pimarcin-vancomycin medium after 20 hours of incubation at 20 C.

TABLE 2. Percentage germination of *Pythium ultimum* after various periods of storage on dried 2% water-agar disks immediately after storage and after 2 weeks on moist 50% soil agar

Storage time <sup>a</sup> (weeks)	Germination <sup>b</sup> (%)
0	58
24	62
32	63
44	34
48	9
59	8
60	10

<sup>a</sup>Stored at 25 C on dried 2% agar disks.

<sup>b</sup>Incubated on pimarcin-vancomycin medium at 30 C for up to 24 hours, after 2 weeks of preincubation on soil agar. Disks of comparable age not preincubated on soil agar had 0% germination.

isolates of *P. ultimum* to thin-walled germinable structures was compared after a 2-week incubation period at 20 C. Isolates PuB5 and PuA6 from Salisbury, MD, and isolates PuNJE and PuNJL from New Jersey yielded 66%, 22%, 43%, and 19% germinable oospores. The results indicated that conversion was a general characteristic of the species, but that the rates of conversion by various isolates may differ.

**Rate of conversion and germination.**—Oospores incubated in soil extract for 2 weeks were dried rapidly on agar disks, remoistened, and incubated on soil agar in a humid atmosphere at room temperature. At periodic intervals, some of the disks were removed, stained, and examined for percentage of thick- and thin-walled oospores. Other replicate disks were placed on MPVM and examined for oospore germination after 20 hours at 20 C.

At first, 30% of the oospores were thin-walled, but none of these dried oospores germinated (Fig. 2). Some oospores were germinable after 3 days of preincubation on soil agar, whereas the percentage of thin-walled oospores appeared to decrease slightly. This decrease resulted from deterioration and lysis of thin-walled oospores that had been killed by drying. After 1 week, the percentage of germinable oospores paralleled that of newly-converted thin-walled oospores. From 6 to 10 weeks, more than 65% of the thick-walled oospores had been converted to thin-walled spores, almost all of which appeared to be germinable.

The effect of time on the germination of thin-walled oospores was tested. Signs of beginning germination (Fig. 1-G) were evident after 1 hour of incubation on MPVM at 25 C. Generally, 97-100% of the thin-walled oospores had completed germination in less than 2 hours of incubation on MPVM or in 0.1% potato-dextrose broth (Fig. 1-I). Nearly 100% germination occurred also when oospores were transferred to distilled water, but the incubation time for complete germination was more than 3 hours.

The longevity of dried dormant oospores was tested (Table 2). Oospores stored on dried agar disks at 25 C for various time intervals were remoistened and incubated on soil agar for 2 weeks. Oospores kept dry until the germination test did not germinate. In contrast, some oospores on disks incubated moist for 2 weeks germinated even after 60 weeks of dry storage. However, the rate of apparent conversion to germinable oospores and later germination decreased sharply after 32 weeks of storage. The germination rate of oospores stored 24 and 32 weeks was equivalent to that of nonstored oospores.

**Effect of temperature on germinability.**—Dried dormant oospores on agar were tested in two experiments for conversion to germinable oospores in soil extract at various temperatures. Thin-walled oospores were counted after 3, 4, and 6 weeks of incubation. Maximum

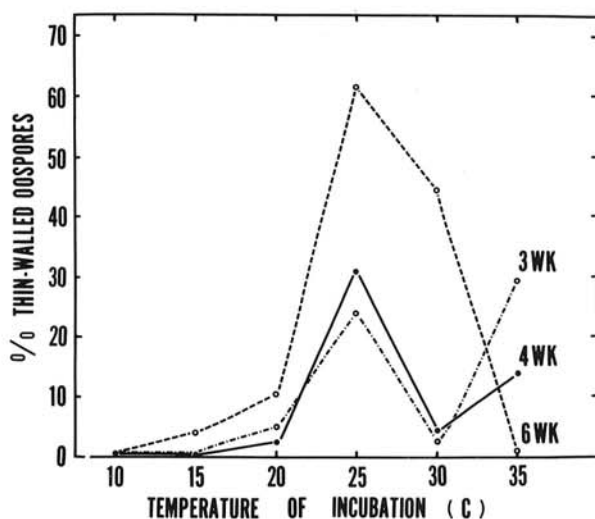


Fig. 3. Effect of temperature on the conversion of dormant, thick-walled oospores of *Pythium ultimum* to germinable thin-walled oospores after 3, 4, and 6 weeks of incubation in 1% nonsterile soil extract. After 6 weeks, the percentage of germination equalled the percentage of thin-walled oospores.



conversion occurred at 25 C, with a dramatic increase in thin-walled oospores to 60% of the total in 6 weeks (Fig. 3). Very little conversion occurred at 15 and 20 C and almost none at 10 C. Conversion decreased at 30 C. Many thin-walled oospores appeared after 3 weeks of incubation at 35 C, but the numbers that stained intensely diminished rapidly with time. This temperature appeared to injure the oospores and resulted in lysis in the soil extract. At the end of 6 weeks, the percentage of stained, thin-walled oospores was comparable to that of oospores that germinated.

Oospores germinated over a wide temperature range when incubated for 2 hours on MPVM. Thin-walled oospores incubated at 16-30 C, at 5 C intervals, had a germination rate of 97-100%. No germination occurred at 5 or 10 C during this period. After 16 hours of incubation, about 70% germination occurred at 10 C. Temperatures above 30 C resulted in greatly reduced germination rates.

**Effect of soil moisture on germinability.**—Oospore preparations with about 10% conversion to thin-walled spores after 2 weeks in soil extract were applied to glass cover slips and rapidly dried as before. The oospores were placed in direct contact with soils equilibrated at various soil-water potentials and were incubated at 25 C. Thin-walled spores were counted after 1, 2, and 6 weeks of incubation. Dormant, thick-walled oospores at -93 and -39 bars did not change during the 6 weeks of incubation (Fig. 4). The percentage of thin-walled oospores increased with increases in the soil moisture and with time. There was maximum conversion of oospores (to 96% thin-walled) in the saturated soil incubated for 6 weeks.

Germination of the thin-walled oospores was maximum also with spores converted in saturated soil. Nearly 100% of the thin-walled spores in saturated soil germinated on MPVM. In contrast, thin-walled spores dried before incubation in the soils with minimal moisture (air dried and 25% FC) did not germinate. These spores did not lyse even after 6 weeks because of the dryness of the soils in which they were incubated.

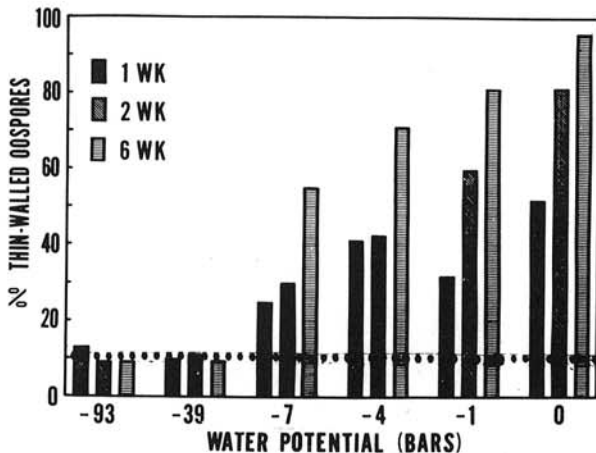


Fig. 4. Effect of soil-water potential on the percentage of thin-walled oospores of *Pythium ultimum* converted from thick-walled oospores after 1, 2, and 6 weeks of incubation at 25 C on soil adjusted to saturation (0 bar), field capacity (FC) (about -1 bar), 75% FC (-4 bars), 50% FC (-7 bars), 25% FC (-39 bars), and air dried soil (-93 bars). About 10% of the oospores were thin-walled when the experiment began (dotted line).

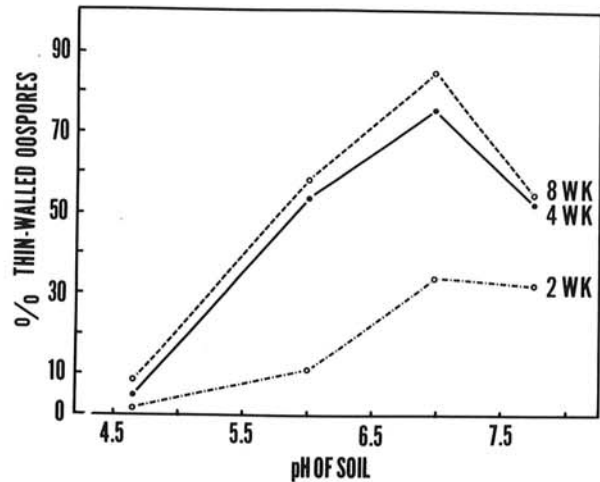


Fig. 5. Effect of soil pH [adjusted with  $\text{CaCO}_3$  or  $\text{Al}_2(\text{SO}_4)_3$ ] on the percentage of thin-walled *Pythium ultimum* oospores in preparations incubated on pH-adjusted soils at 25 C for 2, 4, and 8 weeks. The initial preparation contained all thick-walled dormant oospores.

TABLE 3. Conversion of dormant (thick-walled) *Pythium ultimum* oospores to germinable (thin-walled) oospores in field soil and on 2% soil agar

Incubation period (weeks)	Germinable propagules per gram moist soil <sup>a</sup>	Germination <sup>b</sup> (%)
0	0	0
1	273	37
2	1,144	52
3	40,560	73

<sup>a</sup>Fresh oospores (350,000/g soil) were added. Soil was dried, remoistened, and incubated at 25 C. Zero-time assay was made at time of remoistening. Propagules were assayed by a dilution-plate frequency method on a selective medium.

<sup>b</sup>Fresh oospores (5,000) were added to a 2-cm diameter 2% water agar disk and dried. The disks were remoistened and incubated moist at 25 C. Percentage of germination was determined after incubation on a selective medium at 25 C for 16 hours. Zero-time germination rate was determined after remoistening and incubation on the selective medium as above.

**Effect of pH on germinability.**—Dormant oospores, dried on agar disks and on glass cover slips, were incubated in contact with water-saturated, pH-adjusted soil. Maximum conversion occurred at pH 7.0 after 8 weeks (Fig. 5). The conversion was reduced by pH 7.8, and fewer than 10% of the oospores were converted at pH 4.7.

Germination of converted oospores on pH-adjusted MPVM medium was high (98-100%) over a broad range of values from pH 4.5 to 7.5. Only 17.3% germinated at pH 3.5. Thus, pH appeared to influence conversion of the oospores more than their germination.

**Conversion of oospores in natural soil.**—A sandy soil artificially infested with 350,000 thick-walled oospores/g

was air dried within 30 minutes and then remoistened to about FC. Viable numbers of *P. ultimum* propagules were estimated at zero time and at weekly intervals following infestation by the dilution-plate frequency method. Oospores from the same preparation were also applied to water-agar disks (5,000/disk) and incubated on soil agar at 25 C. Germinable oospore numbers in the remoistened soil increased from 0 initially to 40,560/g in 3 weeks (Table 3). Likewise, the oospores on the surface of agar disks placed onto soil agar increased in their rate of germinability to 73% during the same period. The conversion of thick-walled oospores to thin-walled stainable oospores within soil, was verified by microscopic examination of stained smears of the soil on water agar. The appearance of the converted spores (Fig. 1-E) was identical to that of spores converted in soil extract.

*Relation of germinability to damping-off of bean seedlings.*—The conversion of thick-walled dormant oospores to thin-walled oospores appeared to be a prerequisite to their ability to incite damping-off of beans in soil. Steam-treated soil was infested with either dried, thick-walled oospores or with predominantly thin-walled oospores at an inoculum level of about 500/g. At 2 weeks, all of the bean seedlings damped off in soil containing thin-walled oospore inoculum, whereas there was no damping-off in soil containing thick-walled oospore inoculum. Continued, but fallow, incubation of the latter soils for 2 more weeks permitted the initially-dormant inoculum to incite significant damping-off (63%). Controls remained free of disease.

**DISCUSSION.**—Oospores are long-term survival propagules of *Pythium* spp. (9). This statement is corroborated by the long-term survival in dry storage of *P. ultimum* oospores (Table 2). Under favorable conditions in which the oospores might be protected by insulating layers of plant debris or soil or where moisture conditions are adequate, survival may be further prolonged. Long-term survival of a *Pythium* sp., perhaps related to *P. ultimum*, was shown also in air dried muck soil (10).

Because of the ability of constitutively dormant thick-walled oospores to convert to exogenously dormant thin-walled propagules, they are undoubtedly important, not only for survival, but also in the saprophytic and pathogenic activities of the fungus. Gradually with time, and in moisture-saturated soil (Fig. 4) at moderate temperatures (25 C) (Fig. 3), and at a pH near neutrality (Fig. 5), these dormant structures become germinable. They appear similar to vegetative sporangia. The terminal sporangia are about the same size and shape as germinable oospores with about the same wall thickness. Both stain readily with lactofuchsin. The similarity in general appearance between the two structures was noted by others (5, 20) and the wall thickness is comparable to previous dimensions reported for sporangia (16). One distinguishing characteristic is the oogonial wall that nearly always enveloped germinable oospores, but this wall is often difficult to see or may be lost.

Even more striking than their similar appearance is the similar behavior of germinable oospores and sporangia. As described for sporangia of *P. ultimum*, (16) thin-walled oospores germinate rapidly over a wide range of temperature (1) and pH characteristics that probably

contribute to the effectiveness of *P. ultimum* as a damping-off organism in competition with other soil microflora. The ability to germinate, however, depends on the fungistatic properties of the soil. Sporangia are susceptible to soil fungistasis (1, 16). Neither thin-walled oospores nor sporangia germinated in our studies, unless nutrients were supplied or the spores were transferred to distilled water. The ability to germinate in water raised the question of the nature of the fungistatic factor that prevents germination of *P. ultimum* propagules (1). Germination in a medium presumably devoid of nutrients suggests the presence of an inhibitor whose effect can be annulled by the addition of nutrients or by being washed away with water.

An inhibitor may also be responsible for the constitutive dormancy of thick-walled oospores of *P. ultimum*. Although our studies did not involve the mechanism of oospore wall thinning and induction of germinability, they suggest the presence of an inhibitor that prevents germination—not merely related to the immaturity of oospores, as previously suggested (5). Oospores remained thick-walled and dormant in static cultures of the fungus and became germinable after rinsing, drying, and flooding with nonsterile soil extract or water. Moreover, conversion did not occur after many weeks of incubation at an unfavorable soil pH of 4.6 (Fig. 5), at temperatures below 20 C (Fig. 3), in dry soils (Fig. 4), or on dried agar disks (Table 2). The wall of the dormant, but not the germinable, oospore is impermeable to lactofuchsin (Fig. 1-A, D) (16), and undoubtedly also to other materials. Upon removal of an inhibitor, perhaps the oospore wall is catabolized, and permeability is greatly enhanced, allowing germination to begin when nutrients are adequate.

Apparently this increased permeability is also detrimental to germinable oospores, as well as to sporangia, by allowing rapid water loss with concomitant death. Unquestionably, rapid drying of both propagules destroyed their viability, but apparently did not affect dormant oospores (Table 1). In fact, rapid drying of oospore preparations was the means of confining our studies to dormant oospores, without the confusion that results from the presence of terminal sporangia. The effect of drying on germinable propagules of *P. ultimum* in soil is, however, unclear from the literature. Neither the rate nor percentage of sporangium germination of *P. ultimum* was affected in air-dried soils (16), and no harmful effects were noted on the survival of *P. ultimum* dried in the greenhouse and stored at -18 C (15). Neither of these reports indicated the rapidity of drying. We have noted a protective effect of slowly drying germinable oospores or infested soils over several days (Lumsden and Ayers, unpublished). Perhaps slow drying prevents rapid water loss and allows the propagules to resist desiccation.

*P. aphanidermatum* oospores are not adversely affected by rapid drying. In fact, our previous results (2) and the data in Table 1 suggest that germination of oospores of this species is enhanced by drying. Protection from desiccation may result from maintenance of thick oospore walls by *P. aphanidermatum*, even up to the time of germination. Unlike *P. ultimum* whose oospore walls may thin down well in advance of germination, thinning of *P. aphanidermatum* oospores has been associated only with the pregermination stage just prior to actual

germination (17). The oospore wall thickness and perhaps permeability may explain the constitutive dormancy of *P. ultimum* (3, 5, 16, 20), as opposed to the apparent lack of this type of dormancy in *P. aphanidermatum* (17).

Dormant *P. ultimum* oospores convert to germinable oospores readily in field soil (Table 3). Such conversion contributes to the incidence of damping-off of seedlings. Dormant oospores did not incite damping-off until enough time had elapsed for conversion. In contrast, germinable oospores could cause severe disease during the first 2-week incubation period.

The conversion of oospores depends on several soil environmental factors. These factors, including temperature, pH, and moisture, also affect the incidence of damping-off by *P. ultimum*. The additive effect of these factors on host susceptibility and on conversion of dormant to germinable oospores may influence the incidence and severity of disease. Moderate to cool temperatures usually favor disease development by *P. ultimum* (12, 19). Likewise, the conversion of oospores is favored by moderate temperatures, and their survival appears to be adversely affected by elevated temperatures (Fig. 3). In a similar manner, the incidence of damping-off is favored by neutral or alkaline soil reactions (7). The conversion of dormant, noninfective oospores to germinable, infective oospores occurs optimally near neutrality (Fig. 5). Finally, the severity of damping-off and root rot is enhanced by relatively high soil moisture levels (6, 8, 11, 12, 14), and conversion of oospores is most rapid in saturated soils (Fig. 4). Many of the above reports indicate no direct effect on the pathogen by these factors, but instead an effect on the host. A direct effect on the ability of *P. ultimum* oospores to germinate and cause disease could, however, influence the severity of damping-off by causing an increase in the effective inoculum density of the pathogen in soil.

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