

## Factors Affecting Production and Germination of Oospores of Three *Pythium* Species

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### ABSTRACT

Cholesterol stimulated production and maturation of oospores of *Pythium aphanidermatum*, *P. ultimum*, and *P. myriotylum*. *P. myriotylum*, especially, produced abnormal sexual structures in the absence of sterol. Temperatures of 15-35 C were satisfactory for oospore production by *P. aphanidermatum* and *P. myriotylum*, whereas 10-20 C were more favorable for *P. ultimum*. Nonsterile soil extract induced lysis of mycelial mats of these fungi within 1-2 weeks, leaving oospores free of hyphae. Freshly harvested oospores of *P. aphanidermatum* germinated (27%) on a nutrient medium within 18 hours. Air-drying or aging for 1-2 weeks in nonsterile soil extract increased germination (80-90%). Oospores of *P. ultimum* were dormant, at first, but became increasingly germinable with time of exposure to nonsterile

soil extract. Germination exceeded 90% after about 6 weeks in soil extract. Oogonia or immature oospores of *P. myriotylum*, as well as mature oospores, germinated in low numbers (approximately 12%) in 18 hours from fresh cultures. Air-drying reduced germination to < 1%. Isolated single oospores germinated a few at a time over a period of 20 days, reaching a total of about 6%. Germination of dried oospores of *P. myriotylum* was increased slightly by preincubation of the spores on nonsterile soil agar for 7 weeks. Freezing and thawing, heat shock, and exposure to light and to bean tissue leachates and root exudates or fresh plant tissue did not break the dormancy or stimulate germination of oospores of *P. myriotylum*.

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*Additional key words:* *Pythium aphanidermatum*, *P. myriotylum*, *P. ultimum*.

Oospores of *Pythium* spp. are commonly considered to be resistant structures permitting long-time survival of the species under adverse conditions and in soil (4, 9, 16, 17). Some of the conditions that favor oospore development and subsequent germination are known for certain species such as *P. aphanidermatum* (Edson) Fitzp. (1, 3, 17, 18), but are unknown for many others (11). Moreover, some investigators have reported immediate germination of freshly produced oospores of certain species (6). Others have observed high germination rates of the same species only after passage of the oospores through the digestive tract of snails (18). Oospore dormancy in pythiaceus fungi is still poorly understood and has hampered genetic, as well as ecological, studies (20).

During an ecological study of microbial populations in a field plot of snapbeans, we found disease caused by three species of *Pythium*: *P. aphanidermatum*, *P. ultimum* Trow and *P. myriotylum* Drechs. The first two species were readily isolated from the field soil by selective plating methods (10). However, *P. myriotylum* was only rarely recovered, even from soil with known high potential for *P. myriotylum* blight of snapbeans. Because of the apparent dissimilar behavior of the surviving propagules of the different *Pythium* spp., a comparative study was undertaken of the nutritional and physiological conditions that favor their oospore production and germination.

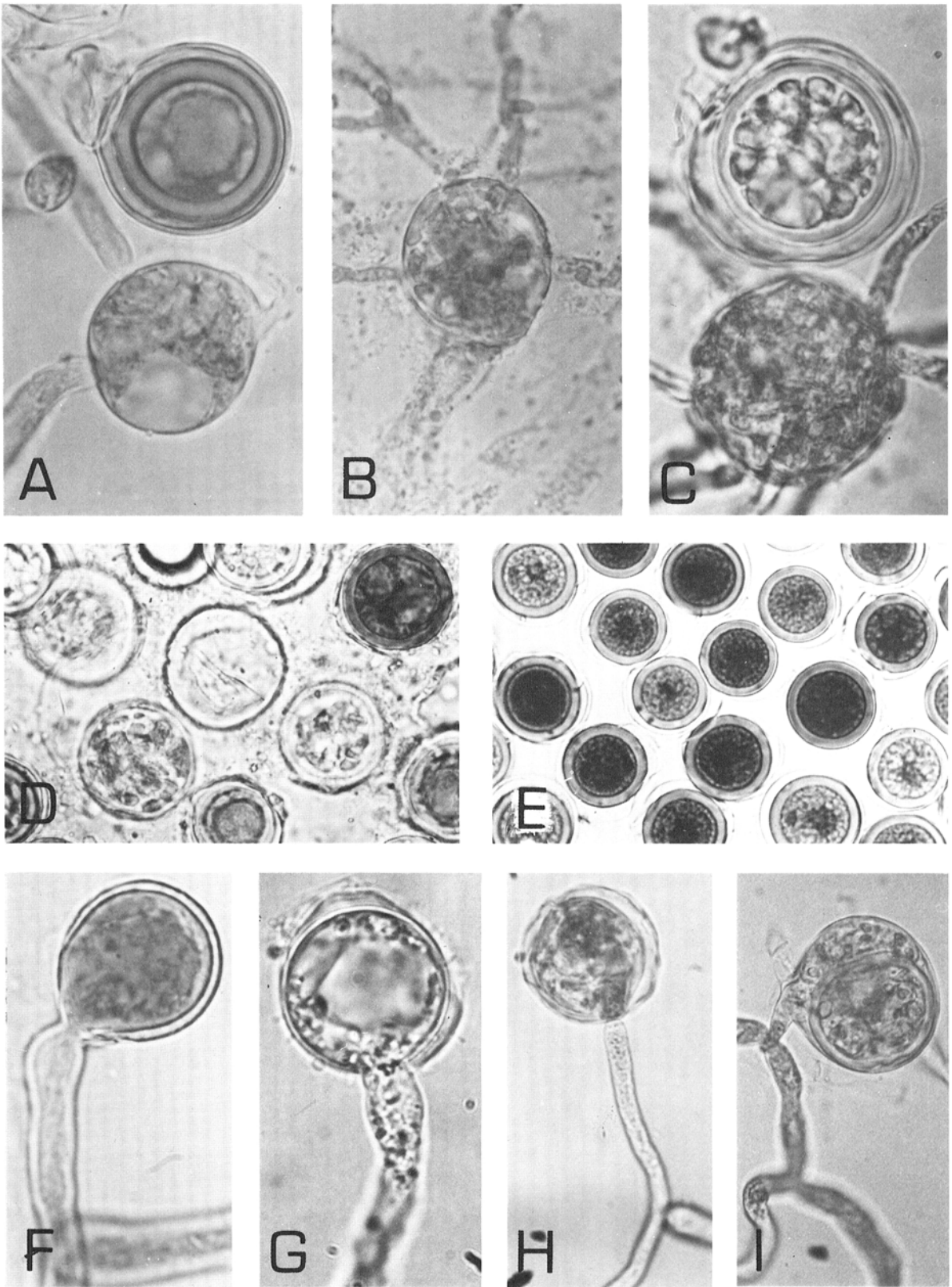
**MATERIALS AND METHODS.**—Oospores of *P. aphanidermatum* (ATCC 26081), *P. myriotylum* (ATCC 26082), and *P. ultimum* (ATCC 26083) were obtained routinely from 1- or 2-week-old culture mats grown in V8-cholesterol broth (200 ml V8 vegetable juice, 800 ml distilled water, and 2.5 g CaCO<sub>3</sub> solution clarified by centrifugation at 13,200 g for 30 minutes). Cholesterol (30 mg/liter) was added from a 1.5% solution in 95% ethanol

before the medium was sterilized at 121 C for 30 minutes. Cultures were grown in 12 ml of medium in sterile petri dishes and were incubated in the dark at 20 C for *P. ultimum*, and 25 C for *P. aphanidermatum* and *P. myriotylum*.

The effect of sterol concentration on oospore production was determined in V8-juice broth and in the sterol-free synthetic medium of Schmitthenner (15). Cholesterol was added at several concentrations from an ethanolic solution before sterilization.

Oospore suspensions were prepared from freshly harvested mycelial mats, from mats dried in open petri dishes within 30 minutes in a flow of air at 22 C, and from wet or dried mats that had been subjected to the lytic action of nonsterile soil extract. Soil extract was prepared from 10 g of fresh soil stirred into 1 liter of tapwater and was filtered through Whatman No. 1 paper after standing at 25 C for 3 days. Mats in petri dishes were covered with the filtered soil extract for 1 or 2 weeks. Lysed or nonlysed mats were rinsed in tapwater and then comminuted in a Tekmar Tisumizer for 30 seconds. Suspensions from nonlysed mats were passed through two layers of cheese cloth and one layer of lens paper to remove most mycelial fragments. Oospores were counted in a hemacytometer, and the suspensions were adjusted to appropriate concentrations and applied to dried agar disks, to membrane filters, or directly to germination media.

Dried agar disks consisted of 15-mm-diameter circles cut from 2% water agar plates. The disks were allowed to air dry, then drops of oospore suspensions were applied to each, and the preparations rapidly dried within 30 minutes in a flow of air. After remoistening with water, disks containing dried oospores were applied directly to germination media or allowed to age on the surface of nonsterile soil agar plates prepared by mixture of 5 g fresh soil with 5 ml of 1% water agar. The plates were kept in



**Fig. 1**-(A to I). Photomicrographs of *Pythium* spp. oospores or oogonia: **A**) Germinated oogonium and nongerminated mature oospore of *Pythium aphanidermatum* ( $\times 1,200$ ); **B**) germinated oogonium of *P. ultimum* ( $\times 1,200$ ); **C**) germinated oogonium and nongerminated oospore of *P. myriotylum* ( $\times 1,200$ ); **D**, **E**) nongerminated oospores of *P. myriotylum* and *P. aphanidermatum*, respectively, in nonsterile soil extract stained with lactofuchsin ( $\times 620$ ); **F**, **G**, **H**) germinated mature oospores of *P. aphanidermatum*, *P. ultimum* ( $\times 1,200$ ), and *P. myriotylum* ( $\times 665$ ), respectively; **I**) oospore of *P. myriotylum*, with oogonial wall invaded by hypha from adjacent germinated structure of this fungus ( $\times 920$ ).

moist chambers to prevent drying.

In certain experiments, oospores of *P. myriotylum* were obtained from infected plant tissue as follows: snapbean seedlings (*Phaseolus vulgaris* L. 'Topcrop') were grown aseptically in sterile battery jars containing 100 ml of 2% water agar. Five-day-old seedlings were inoculated with mycelia of *P. myriotylum* and incubated under artificial light at 30 C. After 20 days, the roots of infected plants were removed from the agar and comminuted in a small amount of water. Crystalline commercial pectinase (10 µg/ml) was added, and the mixture was incubated for 30 minutes at 25 C, to further macerate plant tissue. The preparation was blended for 1 minute and filtered through cheesecloth. The oospores were washed three times with distilled water by centrifugation and were used for germination tests directly or after drying on agar disks.

Germinability of oospores was generally tested on Mircetich's pimarinic-vancomycin agar medium (MPVM) (12), a nutrient-rich selective medium. It permitted rapid growth of these *Pythium* spp. (10) and suppressed growth of bacteria and nonpythiaceus fungi derived from the nonsterile soil extract. Other specialized media, cited in the text, were also used in certain experiments in an attempt to induce dormant oospores of *P. myriotylum* to germinate. The identity of *Pythium* colonies was routinely verified by transfer of mycelia to an identification medium (10).

To arrive at an accurate estimate of the low germination rate of *P. myriotylum* oospores, we used the following procedure: a dilute oospore suspension prepared from mats that had undergone lysis for 14 days in nonsterile soil extract was spread over the surface of water agar plates. One thousand oospores that appeared mature were selected with the aid of a microscope and marked with a single-sporing device (13). Ten oospores were transferred with a needle to each MPVM agar plate in a pattern to allow maximum distance between each spore. The plates were incubated in a moist chamber at 30 C and examined daily for 25 days. We cut out colonies that formed from germinated oospores, to prevent the spread of the culture over the remaining nongerminated spores.

**RESULTS.**—*Effect of sterol on oospore production.*—In initial experiments on germination of oospores of the three *Pythium* spp., we used oospores from cultures grown for 7 to 14 days in filtered V8-broth without supplemental sterol. Although considerable

numbers of oospores developed on this medium, there were many apparently immature oospores and unfertilized oogonia, especially in mats of *P. myriotylum*. These immature structures, recognized by the absence of a distinct oosphere and thick oospore wall, germinated readily within 24 hours when placed on MPVM or other nutrient media (Fig. 1-A, B, C). But they did not do so if they were quickly dried in air before being applied to the medium. Cholesterol,  $\beta$ -sitosterol, or other related sterols are required for optimum oospore production by many *Pythium* spp. (8). Therefore, the effect of supplementary sterol on production and maturation of oospores of the three *Pythium* spp. in V8-broth was tested.

Supplementary cholesterol markedly stimulated oospore production by all three species (Table 1). Most striking, however, was the apparent influence of sterol on the maturation of oospores of *P. myriotylum*. Without supplementary sterol only 15% of the structures that formed in 11 days appeared to be mature, whereas with sterol, 87% seemed mature. An increased ratio of mature to immature oospores of *P. aphanidermatum* and *P. ultimum* was also obtained by adding sterol to the medium, but the effect was less pronounced than with *P. myriotylum*.

The effect of several concentrations of cholesterol on oospore production in a sterol-free synthetic medium is shown in Fig. 2. The lowest concentration tested (0.001 mg/liter) induced a response in all three species, but it was less than that induced by the more optimal concentrations of 1 and 30 mg/liter. The inclusion of sterol was essential for production of maximum numbers of mature oospores for studies of oospore germination. Therefore, 30 mg cholesterol/liter was added to all subsequent V8 medium preparations.

*Effect of temperature on oospore production.*—The influence of temperature of incubation on oospore formation was determined in V8-cholesterol broth. *P. aphanidermatum* and *P. myriotylum* produced oospores abundantly in the range of 15-35 C (Table 2). The variability of the microscopic counts and nonuniform distribution of oospores on the mats, however, made determination of temperature optima difficult. Oospores of *P. ultimum* were produced abundantly in the 10 to 20 C range, with few or no oospores produced at 25 C and higher temperatures. Accordingly, in subsequent experiments on oospore germination, we used oospores of *P. ultimum* produced at 20 C and oospores of *P. aphanidermatum* and *P. myriotylum* produced at 25 C.

TABLE 1. Effect of cholesterol on numbers and maturity of oospores produced by *Pythium* spp. in V8 broth<sup>a</sup>

Incubation (days)	Treatment	<i>P. aphanidermatum</i>		<i>P. ultimum</i>		<i>P. myriotylum</i>	
		(No./ml)	Mature (%)	(No./ml)	Mature (%)	(No./ml)	Mature (%)
3	Sterol	...	83	0	0	11,000	83
	No sterol	...	66	0	0	4,000	0
7	Sterol	533,000	83	335,000	89	385,000	82
	No sterol	452,000	67	53,000	58	137,000	5
11	Sterol	1,146,000	93	309,000	98	307,000	87
	No sterol	561,000	71	52,000	69	77,000	15

<sup>a</sup>Mats grown in clarified V8 broth with or without cholesterol (30 mg/liter) were comminuted in 10 ml water; oospores were observed and counted in a hemacytometer.

<sup>b</sup>Not determined.

*Lysis of mycelium by nonsterile soil extract.*—The three species of *Pythium* behaved similarly in nonsterile soil extract. Digitate sporangia of *P. aphanidermatum* and *P. myriotylum* and spherical, terminal and ovate, intercalary sporangia of *P. ultimum* formed during the first 2 days of incubation. Lysis of mycelium was observed after 2 days and zoospores were produced by sporangia of *P. aphanidermatum* and *P. myriotylum*, but not *P. ultimum*. The zoospores germinated and subsequently lysed. Lysis of the mycelial mats progressed. After 14 days of incubation, oospores were surrounded by a gelatinous mass of debris from disintegrated hyphae, with few, if any, hyphae remaining discernible. Occasional, freshly germinated oogonia and oospores of *P. myriotylum* were observed during the incubation period, but oospores of *P. aphanidermatum* and *P. ultimum* were not observed to germinate in the soil extract. Hyphae from freshly germinated structures quickly lysed, leaving oospores relatively free of mycelia.

Mats dried before treatment with soil extract followed a similar pattern of lysis. However, dried mats produced few or no sporangia in the soil extract, because the viability of the mycelia was destroyed. Because we desired the absence of sporangia, especially those of *P. ultimum*, to avoid confusion with oospores, we usually air-dried mats within 30 minutes in a rapid flow of ambient air in a fume hood before treatment with nonsterile soil extract in preparing oospores.

Lysis of mats permitted the development of many bacteria, protozoa, and, less frequently, filamentous fungi. These microorganisms did not interfere in our distinguishing the oospores of each species and usually did not affect their viability. However, mats of *P. myriotylum* supported the growth of a more highly varied microflora and microfauna than did mats of the other two species, and some oospores were occasionally invaded by fungi in the soil extract. Oospores of *P. myriotylum* from both lysed and nonlysed mats varied more in size, appearance, and internal organization (Fig. 1-D) than did the oospores of *P. aphanidermatum* (Fig. 1-E) and *P. ultimum*.

*Germinability of oospores.*—Freshly harvested oospores of *P. aphanidermatum* placed on nutrient media germinated within 18 hours from 20 to 45% in different experiments. Air-drying or treatment with nonsterile soil extract markedly increased the germinability of the oospores. The results of one experiment (Table 3) indicate an initial germination rate of 27% of moist oospores applied to MPVM; germination increased to 82% after the oospores were dried, remoistened, and tested. Incubation in nonsterile soil extract for 14 days promoted a gradual increase in germinability of the moist and dried oospores, until almost all were germinable. Germination of mature oospores of *P. aphanidermatum* was typically by a single germ tube (Fig. 1-F).

The effect of culture age on germination of *P. aphanidermatum* oospores was determined in a separate experiment. Fresh oospores from cultures harvested after 3, 5, 10, and 18 days germinated 0, 30, 46, and 43%, respectively on MPVM. Air-drying of 18-day-old oospores increased the germination rate to >90%, but did not influence the rate of germination of oospores from the younger cultures.

Freshly harvested oospores of *P. ultimum* did not

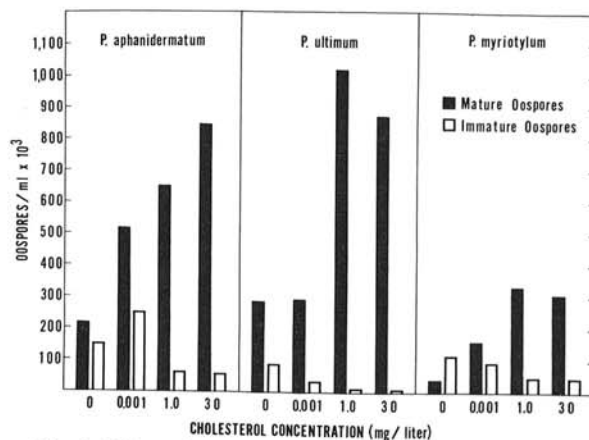


Fig. 2. Effect of several concentrations of cholesterol on oospore production and maturation of *Pythium* spp. in a synthetic medium.

TABLE 2. Effect of temperature on oospore formation of *Pythium* spp. in V8-cholesterol broth after 14 days

Temperature (C)	Oospores per field <sup>a</sup> (no.)		
	<i>P. aphanidermatum</i>	<i>P. ultimum</i>	<i>P. myriotylum</i>
5	0 <sup>b</sup>	0 <sup>c</sup>	0 <sup>b</sup>
10	12	16	6
15	32	18	10
20	38	11	28
25	41	0 <sup>c</sup>	16
30	44	0 <sup>c</sup>	16
35	42	0 <sup>c</sup>	33

<sup>a</sup>Mean of 10 microscope fields ( $\times 100$ ) counted for each of three replicate mats of each species.

<sup>b</sup>No growth.

<sup>c</sup>Growth, but no oospores present.

TABLE 3. Effect of drying and incubation in nonsterile soil extract on germinability of oospores of three *Pythium* spp.

<i>Pythium</i> sp.	Initial state	Germination in 18 hours on MPVM <sup>a</sup> (%) in soil extract after			
		0 days	3 days	7 days	14 days
<i>P. aphanidermatum</i>	Wet	26.9	42.0	64.0	90.2
	Dried	82.0	75.0	89.2	88.7
<i>P. ultimum</i>	Wet	0	0	0.7	4.8 <sup>b</sup>
	Dried	0	1.5	4.3	34.3 <sup>b</sup>
<i>P. myriotylum</i>	Wet	11.7 <sup>c</sup>	2.7 <sup>c</sup>	2.7 <sup>c</sup>	4.5 <sup>c</sup>
	Dried	0	0	0	0

<sup>a</sup>Percentage of oospores with germ tubes out of 200 oospores from each of two replicate mats on Mircetich's pimarcin-vancomycin medium (MPVM). Oospores of *P. aphanidermatum* and *P. myriotylum* were incubated at 30 C and oospores of *P. ultimum* at 20 C.

<sup>b</sup>After 6 weeks, oospores from both wet and dried preparations germinated > 90%.

<sup>c</sup>Most structures of *P. myriotylum* that germinated (approximately 80%) were oogonia; the rest appeared to be mature oospores.

germinate at all, either from the wet state or after being dried (Table 3). However, their germination increased with time of incubation in soil extract, until a significant number germinated after 14 days (Fig. 1-G). Drying the oospores before the soil-extract treatment appeared to

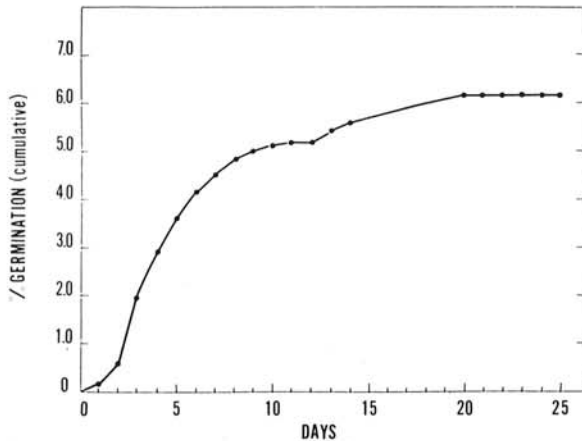


Fig. 3. Effect of incubation time at 30 C on germination of 1,000 isolated oospores of *Pythium myriotylum* on pimarcin-vancomycin medium.

hasten their rate of conversion to the germinable state. After 6 weeks in soil extract, oospores from both wet and dried preparations of *P. ultimum* germinated more than 90%.

Oospores of *P. myriotylum* germinated in very low numbers from freshly harvested mats (Table 3). Many of the structures that germinated appeared to be unfertilized oogonia or immature oospores (Fig. 1-C). These structures germinated by two (and often more) germ tubes, and they seemed to lack a distinct oospore. About 20% of the total germinating structures were judged to be mature oospores. These contained a thin-walled oosphere from which one or occasionally two germ tubes originated (Fig. 1-H). The germ tubes often traveled some distance in the oogonial cavity, before exiting through the oogonial wall. Air-drying the preparations completely prevented immediate germination of these structures when they were placed on the germination medium. Incubation of the dried oospores for 2 weeks in soil extract did not induce germination (Table 3). Oogonia or immature oospores of *P. aphanidermatum* and *P. ultimum* able to germinate could also be observed in very young cultures (3 days). These were absent in older cultures and also were killed by drying.

A phenomenon resembling germination was observed also with *P. myriotylum*. Hyphae from germinated structures often formed appressorium-like structures on adjacent free fertilized oogonia and appeared to invade and then exit through the oogonial wall of these oospores (Fig. 1-I). The hyphae invading the oogonium in Fig. 1-I was traced microscopically to its origin from a germinated oospore. This "self-invasion" was often difficult to distinguish from oospore germination.

*Delayed germination and dormancy of oospores of P. myriotylum.*—The effect of prolonged incubation on the germination of isolated, moist oospores of *P. myriotylum* is shown in Fig. 3. After 24 hours, only two oospores out of 1,000 on the plates had germinated. Continued incubation, however, permitted more oospores to germinate daily, until by 20 days, 6.2% had germinated. No more germinated from 20 to 25 days, possibly because of developing toxicity and microbial contamination of the aging agar plates.

Oospores that were dried on membrane filters before being placed on MPVM germinated at even lower rates than oospores kept moist, yet they displayed a similar delayed germination pattern (Fig. 4). Filter segments that contained 750 oospores/segment from mats lysed in soil extract developed *P. myriotylum* hyphal outgrowths on MPVM gradually with extended incubation up to 18 days. By 4 days, one or more oospores on most of the filter segments had germinated to give rise to a colony. Microscopic examination of the filters with growth indicated that on each filter one to several oospores per segment had germinated. Continued incubation up to 20 days allowed the germination of a few more oospores, until almost all of the segments had given rise to growth. Storage of the dried oospores for 4 months decreased the percentage of filter segments that developed colonies. Yet enough germinable oospores were present on 40% of the segments to give rise to growth during an 8-day incubation period on the germination medium.

To test the possibility that aging or maturing of the oospores in the moist state may be a prerequisite for germination we incubated small agar disks, each with approximately 37,000 dried oospores, on the surface of nonsterile soil agar at 25 C. At several weekly periods, 25 disks were removed from the agar and placed on the germination medium for 48 hours at 30 C. Results indicated that dried oospores that at first were not germinable were conditioned by preincubation on soil agar for 1 week or longer to germinate in low numbers on most of the disks. The percentages of disks that gave rise to *P. myriotylum* hyphae on MPVM after 0, 1, 2, 3, and 7 weeks preincubation on soil agar were 0, 80, 96, 92, and 100%, respectively. Microscopic examination of disk samples did not evidence any germination of oospores in situ on the soil agar. Disks with mycelial outgrowth after incubation on the germination medium had only a few

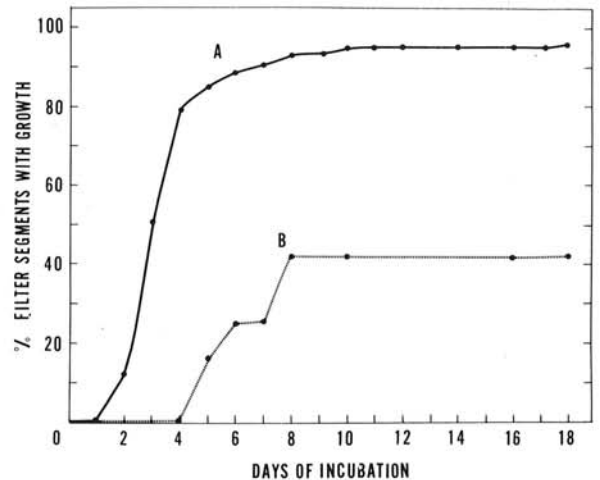


Fig. 4. Germination of dried oospores of *Pythium myriotylum*, as evidenced by outgrowth from membrane filters on pimarcin-vancomycin medium. (A) Each of 72 filter segments contained approximately 750 oospores from 2-week-old mats lysed in nonsterile soil extract for 2 weeks and air dried for 3 days before incubation on the medium at 30 C; (B) 12 filter segments of the same spore preparation stored dried at 20 C for 4 months before the germination test.

germinated oospores; most oospores were ungerminated.

Since oospores of *P. myriotylum* germinated at very low percentages, many experiments were conducted to induce oospore germination or to break their dormancy. The following agar media failed to support higher percentages of germination of oospores than MPVM; Difco lima bean, Difco potato-dextrose, V8-juice, gallic acid medium (10), water agar, bean stem leachate, bean sprout leachate, and bean seed decoction (filter-sterilized liquid from bean parts steeped in water, 50% by weight). Roots and stems of axenically-grown bean seedlings placed in water agar were equally ineffective in stimulating mass germination. Less than 1% germinated in 24 hours with freshly prepared oospore suspensions and by oospores aged for 6 weeks on soil agar in solutions of bean root exudates collected from axenically-grown bean plants (2). Germination was equally poor by oospores applied directly to roots and hypocotyls of bean plants growing in water agar and in sand culture.

Freezing followed by thawing, heat shock for 10 minutes at 5-degree intervals from 40 to 60 C, exposure to daylight and fluorescent light for varying lengths of time up to 8 hours or exposure of oospores in solution to a range of pH 2 to 8 before germinability tests—all failed to induce germination of the oospores at percentages greater than those without these treatments.

Oospores harvested from roots of bean plants infected by *P. myriotylum*, like those obtained from artificial culture, did not germinate en masse on MPVM or on growing plant roots or stems. These "natural" oospores germinated at a rate of approximately 0.05% within 48 hours on MPVM at 30 C. Continued incubation for 1 week permitted the germination of a low, but undetermined, number of additional oospores.

**DISCUSSION.**—Viewed in toto, the results indicate significant differences in the production and germinability of the oospores by the three *Pythium* spp. They differed in their requirement for sterol for maximum production of mature oospores, in their temperature requirements for oospore development, in their behavior during lysis in nonsterile soil extract, and in dormancy and germination of their oospores when nutrients were supplied. Apparently, the conditions that govern oospore dormancy and germinability vary markedly in different species of *Pythium*, and each may require individual attention before the behavior of these propagules in soil may be understood.

Supplementation of V8-juice medium, as well as the synthetic medium with cholesterol markedly stimulated oospore production and maturation by all three *Pythium* spp. Cholesterol concentrations of 1 mg/liter or greater appeared to favor maximum oospore development. Although a requirement for exogenous sterols for normal sexual reproduction has been shown for *P. aphanidermatum*, *P. ultimum* and many other Pythiaceae (7, 8), a sterol requirement has apparently not been reported for *P. myriotylum*. The most affected species, *P. myriotylum*, produced relatively few normal-appearing oospores in the absence of supplementary sterol, even in the V8-juice medium, which presumably contains some natural sterol. Drechsler (4) observed that filtered corn meal agar gave rise to more abortive or degenerating sexual structures of this species than agar

containing finely-divided corn meal. This observation may reflect the higher natural sterol content of the grain particles.

The use of nonsterile soil extract to induce lysis of mycelial mats of *Pythium* spp. appeared to be useful in our obtaining oospore preparations free of other *Pythium* propagules. Mats became completely lysed within 1 to 2 weeks leaving easily dispersed clumps of oospores in jelly-like masses. There was little or no germination of the oospores, except for a few germinating oospores of *P. myriotylum*, while they were in the soil extract. Fungistasis brought about by the developing microbial flora and fauna in the solution probably prevented germination. Moreover, the oospores so treated may be assumed to behave more like those released from organic matter under natural conditions in soil than those kept under axenic conditions in culture.

Germination of oospores of *P. aphanidermatum* from freshly prepared cultures on nutrient media has been reported by various workers to be 55-71% (1), 50% (15), 95% (6) and 20% (18). In the last report, oospore germinability was increased to over 90% by passage of the oospores through the digestive tract of water snails (18). In the results reported here, fresh oospores germinated from 25 to 45%, but reached 90% or more after various treatments. Three conditions appeared to favor increased percentages of *P. aphanidermatum* germination: (i) increased age of cultures from which the oospores were harvested, (ii) a period of aging or after-ripening in nonsterile soil extract, or (iii) drying before the oospores were tested for germinability. Although we have no adequate explanation for these effects, these results may explain the differences in germinability of *P. aphanidermatum* obtained by various workers who used different techniques and ages of harvest.

In contrast to those of *P. aphanidermatum*, oospores of *P. ultimum* freshly harvested from cultures were all dormant at first. Incubation of either moist or dried oospores in nonsterile soil extract promoted a gradual increase in their germinability with time, until by about 6 weeks nearly all were germinable. Thus, oospores of *P. ultimum* behaved qualitatively similar to those of *P. aphanidermatum*. Yet they appeared to reach the germinable state in the soil-extract treatment much more slowly than *P. aphanidermatum* oospores. The requirement for a period of after-ripening of the oospores of *P. ultimum* has been observed (5, 19).

The behavior of *P. myriotylum* oospores contrasted sharply with that of the other two species: only a few mature oospores (less than 1%) germinated immediately after harvest. Air-drying further reduced the numbers of immediately germinable spores. Prolonged incubation in soil extract or on nonsterile soil agar resulted in only marginal increases in the numbers of mature oospores able to germinate when placed on nutrient media. Oospores from infected plant tissue appeared to be as equally dormant as oospores from artificial cultures. Drechsler (4) described oospore germination in his original description of this species, but did not report on the frequency or abundance of their germination.

As a complicating factor in determination of the germination rate of *P. myriotylum* oospores, the morphology of the spores that germinated varied. Apparently, unfertilized oogonia or fertilized immature

oospores germinated readily. These structures were most abundant in media deficient in sterol and only in very young cultures of *P. aphanidermatum* and *P. ultimum*. We regarded them as nutrient-deficient artifacts of artificial culture, rather than true survival structures that would be formed under natural conditions. Oogonium germination of apparently immature oospores was reported in *Phytophthora infestans* by Romero and Gallegly (14). However, these authors apparently agreed later that these germinating structures were probably true oospores (cf. 20). Another confusion in our study was the oospore-invading structures from adjacent *P. myriotylum* hyphae, which often gave the appearance of oospores germinating.

Perhaps significant is the delayed germination pattern of single isolated mature oospores of *P. myriotylum* on MPVM. Although the germinating spores were few in total numbers at any one time, they appeared to reach a germinable state independently of the nutrient stimulus provided by the germination medium. These results, as well as the observed occasional germination of the oospores within the soil extract and their variability in morphology, suggest that these oospores behave as individuals in varying stages of dormancy. Further research is needed to establish whether random germination is the normal behavior pattern of *P. myriotylum* oospores or whether they will germinate en masse with the proper activating stimulus.

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