

Longevity of *Pythium ultimum* in Moist Soils

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## ABSTRACT

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The initial rate of decline in natural soil populations of *Pythium ultimum* in the field was exponential. Following population growth in crop residues, an initial rapid population decline occurred over 2-3 mo with an average half-life ( $t_{1/2}$ ) of approximately 30 days. The average rate of decline after this initial phase was slower with a  $t_{1/2}$  of 125 days. Reproductive bodies formed in cotton leaves or from culture showed initial population declines ( $t_{1/2}$  = 25 to 30 days) for 2 to 3 mo in sandy loam or clay soils held at  $-70$  mbar matric potential and  $19 \pm 2$  C. These declines were followed by stable or increased

populations of *P. ultimum*. Population increases coincided with oospore ripening (conversion from endogenously to exogenously dormant spores). The decline in inoculum densities over the first few months (sporangia) was less evident and the subsequent increase in densities (oospore ripening) was more prominent if soils were sterilized prior to amendment with *P. ultimum* reproductive bodies. Microscopic observations of propagules from soil indicated that parasitic microorganisms aided the decline in soil populations of *P. ultimum*.

*Pythium ultimum* Trow rapidly colonizes and reproduces in virgin plant residues introduced into soil (3,4). It also reproduces in plant tissues killed during pathogenesis. Both sporangia and oospores are produced in plant tissues and are thought to play prominent roles as survival structures in soil (14). Yet, while *P. ultimum* is an important pathogen and widespread in cultivated soils in temperate regions, the characteristics of its survival in soil are not well understood.

Previous studies indicated that populations of *P. ultimum* are relatively stable for several months in soils when the moisture content is low and temperatures are moderate (4,16). However, two investigations with *P. ultimum* and other *Pythium* spp. suggested that population fluctuations observed under field conditions are a function of rapid increases of reproductive bodies in organic substrates followed by rapid losses in their viability (4,7). Soil moisture influenced losses in viability of propagules of *P. ultimum* in the laboratory (4) and may affect the patterns of decline in inoculum densities in the field.

This investigation is part of a study on soil factors that affect the behavior of *P. ultimum*. It focuses on the decline in reproductive bodies of *P. ultimum* in several California soils in the laboratory under moist conditions. It also considers the influence of the biotic environment on changes in soil populations of *P. ultimum*.

## MATERIALS AND METHODS

**Measurement of *Pythium ultimum* propagules.** The exogenously dormant inocula of *Pythium ultimum* in soil were measured by the soil-drop technique (15). Exogenously dormant propagules, or germinable propagules (GP), germinate once soil fungistasis is relieved in this test.

Three subsamples of 10 g of air-dry soil were taken from the mixed samples from each field site or experimental soil and mixed with 250 ml of water in a blender for 1 min. (If populations of *P. ultimum* are too high at this dilution, less soil may be used.) One-milliliter samples of soil suspensions, taken immediately after mixing, were pipetted dropwise around the periphery of four petri plates containing 2% agar. Plates were incubated for 20 hr at 22-24 C before being read. Populations are expressed on the basis of germinable propagules per gram air-dry soil.

**Field studies.** Sampling for *P. ultimum* from 10 commercial cotton fields in the western portion of the San Joaquin Valley was initiated in late 1972. Data on population changes of *P. ultimum* in individual field sites for over a 2-yr period were published with details of crop sequences, soil characteristics, and sample procedures (4). In this report, data for a 12-mo period (October, 1972 to September, 1973) were averaged at each sampling time and presented as the mean of GP per gram as a function of time. These data are of interest because they were collected in a period when there was a widespread, nearly synchronous, increase in populations of *P. ultimum* in a region where the agricultural and climatic conditions are very similar. Therefore, the propagules were similar in age and subjected to similar environmental conditions. Soil temperatures at a field station located within the sampling area were published (4). An unusual amount of rainfall occurred during the winter months and soils were at field capacity or above from December, 1972, through mid-February, 1973.

Further field studies were carried on at the University of California's Kearny Field Station at Parlier. Data on inoculum densities was collected from soils from a grape vineyard and a fallow site where *P. ultimum* populations were elevated after grasses and herbaceous weeds were disked into irrigated fields (Hanford fine sandy loam) in April. Ten samples were taken monthly from the upper 15 cm with a tube sampler (2-cm inner diameter) from a 3 × 3-m area, combined, mixed, and assayed for *P. ultimum*.

**Laboratory studies.** Populations of *P. ultimum* were followed in several soils sampled from sites included in the field study in the San Joaquin Valley. Low soil populations of *P. ultimum* were increased either by amending soils with fresh organic matter and allowing a natural population buildup or by adding culture-grown propagules.

Crushed, dry, greenhouse-grown barley or cotton leaves were added at 1 g per 300 g of air-dry soil. Amended soils were mixed, wetted, and incubated at  $19 \pm 2$  C and  $-0.3$  to  $-1.0$  bar matric potential for 7-10 days. Following incubation, soils were air-dried and ground in a mortar, mixed thoroughly, and three subsamples were used to estimate populations of GP *P. ultimum*. These measurements were considered to be the initial propagule populations in survival tests. Propagules formed in culture were added to soil after freeing them from mycelium. An isolate of *P. ultimum* (ATCC 32939) was grown in a V-8 juice-cholesterol liquid medium (1) in 8.5-cm-diameter petri dishes at 22 C until reproduction was nearly completed (8-10 days). If only the fate of oospores was to be examined, cultures were rinsed several times with sterile water, placed on filter paper and dried with a stream of warm air as described by Lumsden and Ayers (6); this procedure

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kills sporangia, oogonia, and mycelia. If a mixture of viable sporangia and oospores was desired, the drying procedure was omitted. The culture mycelia were disrupted in a blender for 30 sec and the suspension of propagules and mycelial fragments were added to centrifuge tubes. After centrifugation for 10 min at 1,000 g, sediments were resuspended in water and the washing process was repeated twice. After the final washing, the sediments were suspended in small volumes of water and the suspensions were mixed thoroughly with soil. After incubation with moist soil for 3–5 days, the GP density was measured and, if necessary, the infested soil was diluted with noninfested soil to yield inoculum densities of  $3\text{--}5 \times 10^3$  GP/g.

At the start of survival experiments, soils were moistened to saturation and placed in clay pots (4 cm i.d.) fitted into covered capillary columns (39 cm i.d.) of sand: vermiculite (1:1, v/v) mixtures in a constant temperature room ( $19 \pm 2$  C). Pots were 34 cm above the water level in the reservoir (8 cm deep) at the base of the capillary columns. Tensiometric readings of soil matric potential ( $\Psi_m$ ) in clay pots were stable at  $-70$  millibar (mb) over the period of experimentation.

Data on the change of populations of GP were examined on arithmetic and semilogarithmic graphs. When linearity was observed in semilogarithmic plots, GP densities were transformed to logarithmic values and analyzed by linear regression as a function of time. Correlation coefficients ( $r$ ) and  $t_{1/2}$ s of GP were calculated by linear regression methods. The half-life concept as it applies to the survival of inoculum was thoroughly discussed by Dimond and Horsfall (2).

**Morphological changes of propagules in soil.** Culture-grown propagules of *P. ultimum* were added to autoclaved and untreated West Side Field Station (WSFS) soil at initial densities ranging from 3 to  $5 \times 10^3$  GP/g. Air-dried soils (1,800 g) were spread 2 cm thick in polyolefin film bags ( $48 \times 58$  cm; W. G. Whitney, Corp., Skokie, IL 60076) and autoclaved for 1 hr at  $1.05 \text{ kg/cm}^2$  (15 psi) and 121 C. Based on dilution plate counts using PDA and nutrient agar, autoclaving eliminated fungi and reduced bacterial populations from  $10^5$  to less than  $10^2$ /g soil. The initial ratio of oospores to sporangia was  $\sim 10:1$ . Soils were incubated at  $\Psi_m$  of between 0 and  $-70$  mb and at 23–25 C.

Small amounts of soil were retrieved from incubation containers at 2 wk intervals during the first 2 mo of incubation. The soil-smear technique (10) (without the acid fuchsin stain), and the use of phase-contrast optics provided a satisfactory means of directly viewing propagules.

## RESULTS

**Field studies.** Composite data from field studies in the western portion of the San Joaquin Valley showed a sharp decline in densities of GP of *P. ultimum* during the winter and spring months following the general increase in soil populations that had occurred during the previous autumn (Fig. 1). The initial decline in densities of GP followed an exponential pattern for 3 mo (Fig. 1, inset). A second, slower decline phase followed the initial phase and was also exponential. Values of  $r$  for regression analyses of both phases (ln GP vs time) were  $>0.95$ . Germinable propagules in the first and second phases of decline had  $t_{1/2}$ s of 30 and 125 days, respectively.

Data collected from the two sites at the Kearny Field Station, where soil populations of *P. ultimum* had increased after the vegetation was disked into soil in April, showed an initial exponential GP decline pattern similar to that found in the other field study; they had  $t_{1/2}$ s of 26 and 15 days. Further agricultural activities at these sites precluded following the fate of these propagules for longer than 3 mo.

**Laboratory studies.** Following the enhancement of naturally formed propagules after amendment of soils with cotton leaves, the decline in GP densities of *P. ultimum* was followed for several months at a constant moisture ( $\Psi_m = -70$  mb) and temperature ( $19 \pm 2$  C).

Figure 2 shows that an exponential decline in populations occurred in an exponential fashion only for the first 3 mo in soils

from the Lindemann (Mocho clay) and WSFS (Panoche sandy loam) sites, where GP densities were followed for 12 mo. The  $t_{1/2}$  of GP for both soils during this period was  $\sim 25$  days. After 3 mo, the decline in GP densities halted abruptly in both soils and there were no significant patterns of change over the next 9 mo. Densities of GP fluctuated between 100 and 300/g during this second period. Regression analyses of the data collected during the last 9 mo of this experiment showed either positive or negative correlations ( $r = 0.226$  or  $-0.179$ , respectively) when plotted as a function of time; these correlations were not significant ( $P > 0.1$ ).

In other experiments with soil from the WSFS site, where either cotton or barley leaf amendments enhanced soil populations of *P. ultimum*, the initial declines in GP followed the exponential pattern ( $t_{1/2} = 32$  to 42 days) (Fig. 3A). However, after the first 2 mo, diversions from the simple exponential decline were noted. An obvious increase was apparent in one of the experiments in which cotton leaves were used and slight increases or temporary leveling-off patterns were seen at GP inoculum densities of  $\sim 1,000$  propagules per gram of soil in the other tests.

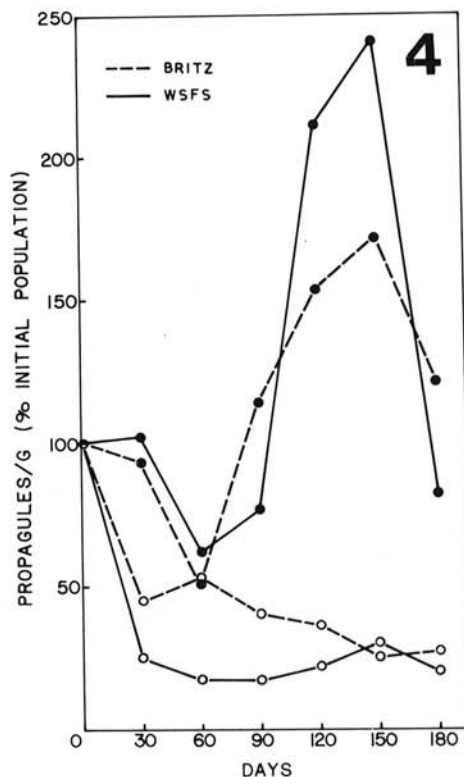
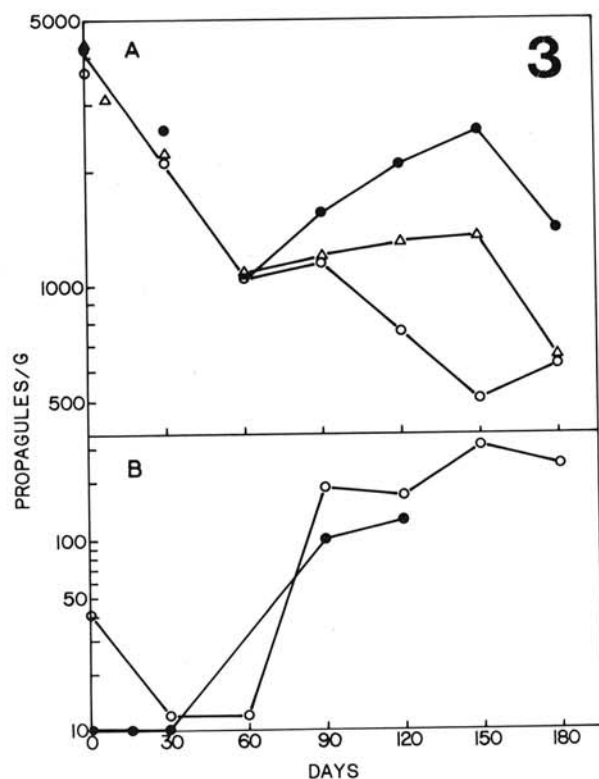
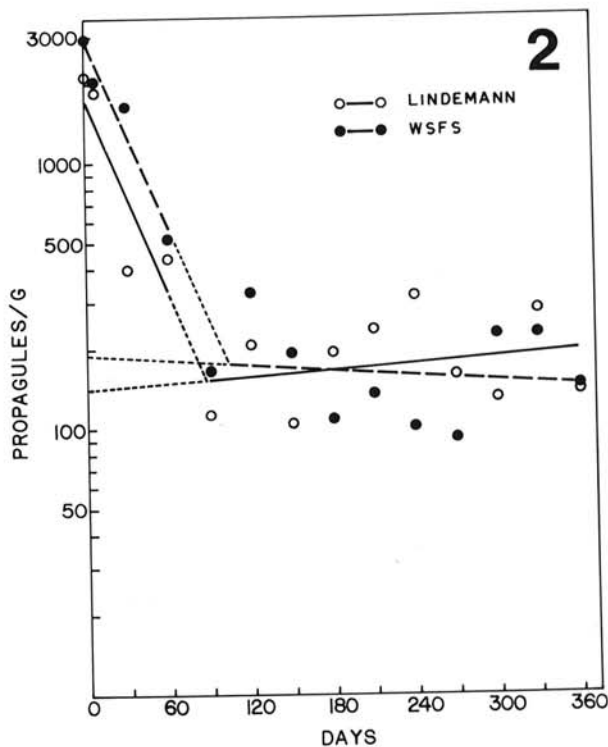
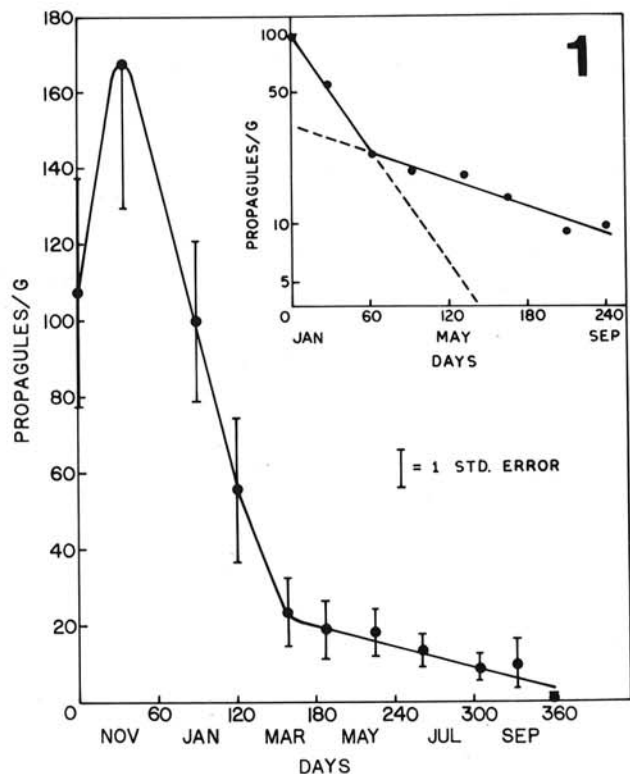
When culture-grown oospore preparations (dried with warm air) were added to soil from the WSFS site, the initial GP densities were low or declined to low levels during the first 2 mo (Fig. 3B). However, densities of GP increased over the next several months. The increase in GP in oospore amended soils (Fig. 3B) coincided with the increase in GP of *P. ultimum* in soils amended with leaves (Fig. 3A).

The behavior of mixtures of culture-grown oospores and sporangia was followed in autoclaved and untreated portions of two soils. No attempt was made to prevent contamination of autoclaved soils after reinfestation with *P. ultimum*. Patterns of change in GP were similar in soils from the Britz (Oxalis clay) and WSFS sites when soils were treated identically (Fig. 4). Untreated soils showed the initial decline pattern in GP found in previous experiments and after 3 mo the population decline halted. The patterns of change in the autoclaved soils were quite different. There was little decline in GP during the first months, but after the first 3 mo there was a large increase in GP densities in both soil types.

When propagules formed in culture were added to untreated soils to give initial GP densities in the range of 50 to 300 GP/g, subsequent changes in inoculum densities were relatively small over a 6-mo period. The initial declines in GP densities, seen when higher densities were used, were not apparent. However, small (approximately twofold) increases in GP densities were observed between the third and fourth months of incubation.

**Morphological changes of propagules in soil.** During the first 2 wk of incubation, there were no marked differences between the morphology of oospores in autoclaved or untreated soil; most of them had thick walls. Sporangia were difficult to locate at the inoculum densities used. Approximately 6 wk after soils were infested, morphological differences were apparent between oospores incubated in the two soils. Almost half the propagules (50 propagules were observed in each sample) in the autoclaved soils had thin walls with the rest possessing thick walls. About the same proportion of propagules in the untreated soils were thin-walled but  $> 50\%$  of these were devoid of cytoplasm (Fig. 5). External erosion of cell walls also occurred in a small proportion of the oospores in untreated soils. Wefts of fine yellow-brown fibrillar material were observed surrounding some oospores. In one experiment, extensive wefting occurred and after 4 wk of incubation it was difficult to locate intact propagules on soil-smears-on-slides preparations.

When oospores and sporangia were incubated in free water with small amounts of soil, a significant number of sporangia lost their cytoplasm after 2–3 wk of incubation. During this period, a number of sporangia contained swarms of small, highly motile rod-shaped ( $\sim 0.5 \times 1.0 \mu\text{m}$ ) bacteria. After incubation for a month, thin-walled oospores were affected similarly. Bacteria also were occasionally in thick-walled oospores. In a few cases these microbes were observed escaping through clefts or pores in the propagule walls.



**Figs. 1-4.** 1, Time course of population density (means of 10 sites; vertical bars = 1 standard error) changes of naturally formed germinable propagules of *Pythium ultimum* in fields in the San Joaquin Valley during 1972-1973. *Inset:* Data transformed to natural logarithms were plotted on a semilogarithmic scale. In each phase (0-60 days and 60-240 days) the lines were fitted by linear regression. 2, Time course of population changes of germinable propagules of *Pythium ultimum* after their formation in two cotton-leaf-amended soils. Soils were incubated for 1 yr at  $-70$  millibar matric potential and  $19 \pm 2$  C. Lines were fitted by linear regression techniques. 3, Time course of population changes of germinable propagules of *Pythium ultimum* in a Panoche sandy loam soil (West Side Field Station) over a period of six months at  $-70$  millibars matric potential and  $19 \pm 2$  C. **A,** Data from three experiments where propagules were formed in cotton-leaf (●—●, ○—○) or barley-leaf (△—△)-amended soil. **B,** Data from two experiments where culture-formed oospores were mixed with soil. 4, Time course of population changes of germinable propagules of *Pythium ultimum* after culture-formed propagules (sporangia and oospores) were mixed with autoclaved (●) or untreated (○) soils Britz = Oxalis clay and West Side Field Station = Panoche sandy loam). Soils were incubated for 6 mo at  $-70$  millibar matric potential and  $19 \pm 2$  C.

## DISCUSSION

The initial rates of decline of GP of *P. ultimum* under controlled conditions were similar regardless of the soil source and type or whether propagules were formed in culture or organic substrates. It was interesting that these rates of decline resembled those measured under field conditions where soil moisture and temperatures were variable.

Initial rates of decline in GP under high propagule densities apparently reflect the death of sporangia of *P. ultimum*. Sporangia are formed in organic residues and are germinable when fungistasis is relieved. While oospores are also formed in plant substrates by *P. ultimum* within a few days of colonization, they are initially thick-walled and dormant (6). Measurements of GP densities of *P. ultimum* by the soil-drop technique depends on the ability of the propagule to germinate. Since only thin-walled, exogenously dormant oospores can germinate and significant oospore conversion takes several weeks under optimum conditions, the initial estimations of densities of GP are assumed to represent mainly sporangia.

After the 2–3 mo of rapid exponential decline in GP densities, the pattern of change of GP densities was always altered significantly. In the field experiments the rates of decline in GP were reduced substantially during this later period; in laboratory tests the decline in GP densities either was halted or the GP densities temporarily increased before declining further. Oospore conversion to germinable thin-walled structures may account for the change in the decline pattern noted in the laboratory studies and may also account for the reduction in the rate of GP decline in the field. However, it is likely that several factors contribute to changes in the rates of decline of GP.

Delayed increases in GP densities after 2–3 mo were more evident in some experiments than in others (Figs. 2 and 3A). Differences between proportions of exogenous and constitutively dormant propagules formed in colonized organic substrates and soil physical and biotic factors are likely to account for this variability. Variations in agricultural practices, soils, and climate also can be expected to affect GP populations in the field. For example, while an oospore conversion-linked increase in GP was not evident in studies on soil population changes in the San Joaquin Valley (4), Watson (18) observed a pattern of population

changes of *P. ultimum* in the Salinas Valley which may have reflected oospore conversion to germinable propagules.

Population densities of *P. ultimum* in soil in the laboratory usually declined to or remained at 100–300 GP/g. It is interesting that these levels are consistent with soil populations of *P. ultimum* frequently reported for cultivated soils (8,12). The stability of these populations suggests that factors responsible for decline at 19 C and  $-70$  mb  $\Psi_m$  are density dependent and are relatively ineffectual when inoculum densities decline below certain levels.

Biological factors apparently influence the patterns of population changes of *P. ultimum* in soil. When mixtures of freshly formed oospores and sporangia were added to untreated soils, the typical pattern of GP decline occurred. However, when aliquots of these propagule mixtures were added to partially sterilized portions of these soils, the patterns of GP changes were different. Oospore conversion to germinable propagules was evident in the autoclaved soils, but not in the untreated ones. The absence of the initial decline in GP also was noted in autoclaved soils. These differences point to the effect of soil organisms on the survival of *P. ultimum*.

The large proportion of empty propagules in untreated soils further indicates that biological agents are responsible for losses on propagule viability. Bacteria were observed within sporangia and ripened oospores in soil-water cultures of *P. ultimum* and their activities destroyed the host cytoplasm while leaving cell walls intact. However, because bacteria were not observed within either empty or intact propagules in soil, the cause of the loss of cytoplasm from soilborne propagules is uncertain.

Sneh et al (13) observed motile bacteria in oospores of *Phytophthora megasperma* var. *sojiae* on flooded soil and described effects of bacterial activities similar to those found in this study. Bacteria also were reported to attack oospores of *Phytophthora erythroseptica* in wet soil (17). These observations and the findings in this study suggest that bacteria are one of the principal predators of reproductive structures of the pythiaceae fungi in moist soil and deserve more attention.

The association of declines of propagules with high soil moisture at moderate temperatures and the relatively large distances between propagules in soil makes the involvement of motile antagonists or predators, (eg, bacteria, chytrids, or amoebae [11,13]) more likely than those depending on filamentous growth for mobility. Calculations according to the equation of McCoy and Powelson (9) indicate that evenly distributed propagules would lie about 700  $\mu\text{m}$  apart in soil when densities are 3,000 propagules per gram (soil bulk density is about 1.4  $\text{g}/\text{cm}^3$ ), an approximate initial GP density that was used in several experiments in this study. Filamentous mycoparasites would need to derive enough nutrients from propagules (15–24  $\mu\text{m}$  diameter) of *P. ultimum* to sustain growth between propagules in soil. Considering the metabolic energy and quantities of structural components necessary for linear growth and the efficiency of homing necessary, it would be unlikely that this could occur. However, in the field, where propagules are concentrated in colonized plant substrates, the involvement of filamentous fungi as parasites of oospores and sporangia is possible.

As this and other studies (5,13) show, propagules of *Pythium* are susceptible to attack by a number of predatory soil organisms. Nevertheless thick-walled oospores may increase the longevity of *P. ultimum* under field conditions, especially under adverse conditions. Low temperatures and soil moisture increase oospore ripening times (6), which may prolong the survival of the fungus during the cool seasons and periods of drought. The capacity of *P. ultimum* to regenerate its soilborne propagules also confers an advantage in survival (16). However, its characteristic behavior as an aggressive saprophyte and pathogen of juvenile tissues with a wide host range are probably more important to the success of *P. ultimum* as a soil inhabitant than the longevity of its reproductive structures.

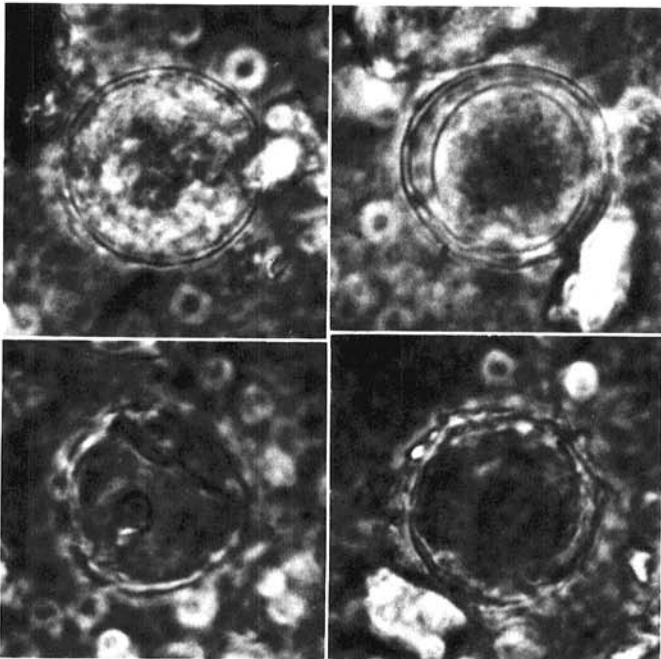


Fig. 5. Culture-formed propagules ( $\times 1000$ ) of *Pythium ultimum* on soil-smear slides after incubating for 6 wk in Panoche sandy loam soil at  $-70$  millibar matric potential and  $25 \pm 2$  C. Top left—normal sporangium; top right—normal thin-walled oospore; and bottom—empty thin-walled propagules.

## LITERATURE CITED

1. Ayers, W. A., and Lumsden, R. D. 1975. Factors affecting production and germination of oospores of three *Pythium* species. *Phytopathology*

- 65:1094-1100.
2. Dimond, A. E., and Horsfall, J. G. 1965. The theory of inoculum. Pages 404-415 in: K. F. Baker and W. C. Snyder, eds. Ecology of Soil-Borne Plant Pathogens. University of California Press, Berkeley. 571 pp.
  3. Garrett, S. D. 1956. Biology of root-infecting fungi. Cambridge University Press, London. 292 pp.
  4. Hancock, J. G. 1977. Factors affecting soil populations of *Pythium ultimum* in the San Joaquin Valley of California. *Hilgardia* 45:107-122.
  5. Hoch, H. C., and Abawi, G. S. 1979. Mycoparasitism of oospores of *Pythium ultimum* by *Fusarium merismoides*. *Mycologia* 71:621-625.
  6. Lumsden, R. D., and Ayers, W. A. 1975. Influence of soil environment on the germinability of constitutively dormant oospores of *Pythium ultimum*. *Phytopathology* 65:1101-1107.
  7. Lumsden, R. D., Ayers, W. A., Adams, P. B., Dow, R. L., Lewis, J. A., Papavizas, G. C., and Kantzes, J. G. 1976. Ecology and epidemiology of *Pythium* species in field soil. *Phytopathology* 66:1203-1209.
  8. Kraft, J. M., and Burke, D. W. 1971. *Pythium ultimum* as a root pathogen of beans and peas in Washington. *Plant Dis. Rep.* 55:1056-1060.
  9. McCoy, M. L., and Powelson, R. L. 1974. A model for determining spatial distribution of soil-borne propagules. *Phytopathology* 64:145-146.
  10. Nash, S. M., Christou, T., and Snyder, W. C. 1961. Existence of *Fusarium solani* f. *phaseoli* as chlamydospores in soil. *Phytopathology* 51:308-312.
  11. Old, K. M., and Patrick, Z. A. 1979. Giant soil amoebae, potential biocontrol agents. Pages 617-628 in: B. Schippers and W. Gams, eds. Soil-Borne Plant Pathogens. Academic Press, London. 686 pp.
  12. Pieczarka, D. J., and Abawi, G. S. 1978. Populations and biology of *Pythium* species associated with snap bean roots and soils in New York. *Phytopathology* 68:409-416.
  13. Sneh, B., Humble, S. J., and Lockwood, J. L. 1977. Parasitism of oospores of *Phytophthora megasperma* var. *sojae*, *P. cactorum*, *Pythium* sp., and *Aphanomyces euteiches* in soil by oomycetes, chytridiomycetes, hyphomycetes, actinomycetes, and bacteria. *Phytopathology* 67:622-628.
  14. Stanghellini, M. E. 1974. Spore germination, growth and survival of *Pythium* in soil. *Proc. Am. Phytopathol. Soc.* 1:211-214.
  15. Stanghellini, M. E., and Hancock, J. G. 1970. A quantitative method for the isolation of *Pythium ultimum* from soil. *Phytopathology* 60:551-552.
  16. Stanghellini, M. E., and Hancock, J. G. 1971. The sporangium of *Pythium ultimum* as a survival structure in soil. *Phytopathology* 61:157-164.
  17. Wynn, A. R., and Epton, H. A. S. 1979. Parasitism of oospores of *Phytophthora erythroseptica* in soil. *Trans. Br. Mycol. Soc.* 73:255-259.
  18. Watson, A. G. 1971. The effect of decomposing green crop residues on lettuce seedling injury in the Salinas Valley. Ph.D. thesis, University of California, Berkeley, 273 pp.