Environmental Influences on the Passive Survival of *Pythium ultimum* in Soil

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ABSTRACT

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Population densities of germinable propagules (GP) of *Pythium ultimum* declined exponentially (death rate) at progressively higher rates at increasingly higher temperatures (9, 15, 21, and 27 C) during the incubation of culture-produced sporangia in raw sandy loam soil. The average death rates of GP at each temperature were about 40% higher at initial matric potentials (ψ_m) of −0.4 bar than at −3 bar. When soil was infested with a mixture of culture-produced oospores and sporangia (7.3:1) the death rates of GP were lower under comparable conditions than when soil was infested with sporangia. However, death rates were higher at 9 or 27 C than at 15 and 21 C, but they were always higher in the soils initially adjusted to −0.4 bar than to −3 bar ψ_m. Differences in death rates of GP between intermediate temperatures (15 and 21 C) and temperature extremes (9 and 27 C) could reflect differences in the effect of temperature on oospore ripening, eg, higher rates of conversion of dormant oospores to GP at 15 and 21 C would reduce the overall death rates of GP at these temperatures. Yet, when dormant oospores alone were incubated in soil under the same conditions, no obvious direct relationships were found between ψ_m or temperature and oospore conversion to GP. Air-drying and remoistening of soil previously infested with oospores and incubated for 6 mo usually stimulated increases in the conversion of oospores to GP.

Additional key words: oospores, soil matric potential, sporangia, temperature.

Pythium ultimum Trow is distributed in cultivated soils throughout temperate regions of the world where it may cause pre- and postemergence damping-off of seeds and seedlings, and also root, stem, and fruit rots of many economically important crop plants. Inoculum increases in intensively cultivated soils are attributed primarily to saprophytic development (9,16,21). However, the ability to persist in soil for long periods is important when a colonizable substrate, or a host plant, is not available or when environmental conditions are not suitable for active growth and development.

In natural situations, inoculum of *P. ultimum* normally includes sporangia and oospores (3,21). Sporangia of *P. ultimum* were first shown to function as survival propagules in cultivated soils by Bainbridge (3). They were subsequently observed to persist in soil for several months (19). Freshly formed oospores usually will not germinate in the presence of nutrients (1). However, Lumsden and Ayers (15) showed that an increase of germinable propagules (GP) could occur if soils infested with oospores of *P. ultimum* were kept moist for several weeks. They demonstrated that up to 96% of constitutively dormant, thick-walled oospores were converted to germinable, thin-walled propagules in water-saturated soil at 25 C after 6 wk of incubation. They also found clear relationships between the soil environmental conditions (temperature, moisture, and pH) and rates of oospore ripening. However, their work was based primarily upon the incubation of oospores on agar plugs, placed on the surface of soil. Little is known about oospore ripening as it occurs in soil. Hancock (9,10) investigated the dynamics of changes of GP of *P. ultimum* in cultivated soils and noted a rapid and a slow phase of decline in their population densities. He attributed the rapid phase to direct losses in sporangia viability and the second phase to an equilibrium between declines of population densities of GP and increases from the conversion of dormant oospores to GP.

The purpose of the present study was to gain a better understanding of the role of sporangia and oospores in passive survival of *P. ultimum* in soil by studying the influence of temperature and soil moisture on changes in the population densities of GP in soils infested with both types of propagules.

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MATERIALS AND METHODS

The infestation of soil with mycelial mats of *P. ultimum*. The fungus (ATCC 32939) was grown in slants of a rolled oat agar medium (9) or in petri dishes in 10% potato-dextrose broth, for 17 days at 22–24 C under laboratory conditions. Mycelia growing on the liquid surface were removed with forceps from the plates and placed in field soil (Panoche sandy loam). The infested soil was initially saturated with water and then air-dried at 22–24 C for 48 hr to promote hyphal lysis. The infested soil was then ground and sieved through a 1.0-mm^2 (mesh size) screen. Microscopic observations of propagules in soil retrieved by the $CsCl_2$ flotation method (described later) did not reveal any viable hyphal remains; oospores and sporangia were present in a 7.3:1 ratio in soil infested with mats grown on rolled oat medium. In soil infested with mats grown on potato-dextrose broth only sporangia were present.

The infestation of soil with constitutively dormant oospores. The fungus was grown on V-8-cholesterol liquid medium (1) for 21 days at 22–24 C under laboratory conditions. The cultures were then washed three times in sterile tap water, blended for 60 sec in a microblender (Eberbach Co., Ann Arbor, MI 48103), and sieved through two layers of cheesecloth to remove mycelial fragments. The filtrate was centrifuged at 4,340 *g* for 30 min, the supernatant was discarded, and the pellet was suspended in 10% potato-dextrose broth. The suspension was incubated at 24 ± 2 C for 48 hr and was then sieved through two layers of cheesecloth to remove the remaining mycelial fragments and germinating propagules. The filtrate was centrifuged at 4,340 *g* for 30 min, the supernatant discarded, and the pellet suspended in sterile tap water. This centrifugation procedure was repeated two more times in order to wash residual nutrients from the surface of spores. The oospore suspension was mixed with raw field soil (Panoche clay loam) to a final concentration of 2.5×10^4 oospores per gram of soil. The soil was air-dried, ground, and sieved through 1.0-mm^2 (mesh size) screen. Propagules of *P. ultimum* in these soils were not detected with the soil-drop method and only thick-walled oospores were noted in microscopic examinations of infested soil.

Increase of indigenous inoculum of *P. ultimum* in field soil. Soil samples were collected from two field sites naturally infested with *P. ultimum*: Davis (Zamora loam) and Boston No. 1 (Lethant sandy loam). Procedures for increasing inoculum density in these soils were described previously (13) and resulted in densities of 450 propagules per gram of air-dried soil in the Davis soil and 7,950 propagules per gram of air-dried soil in the Boston No. 1 soil.

Adjustment of matric potentials. Soil samples (30 g) infested with *P. ultimum* were placed within a ceramic plate extractor (Soil Moisture Equipment Co., Santa Barbara, CA 93105), saturated with water, and then held under pressure (3 or 0.4 bar) at 24 C for 15 hr. The soil samples were then placed within petri plates (60 mm in diameter), covered by two layers of self-sealing transparent 0.05-mm (2-mil) polyethylene film and held in place with rubber bands to reduce evaporation, and incubated at 9, 15, 21, and 27 ±1 C in temperature control cabinets. When adjusted to −0.4 bar matric potential (ψ_m), the water content of Panoche sandy loam was initially 21.1% (w/w). After 108 days at 9, 15, 21, and 27 C, respectively, the water content had decreased to 19.2 (−0.5 bar), 18.7 (−0.5 bar), 17.3 (−0.7 bar), and 13.3% (−3.5 bar). The water content of the soil adjusted to −3 bar was initially 14.6%, but after 108 days of incubation it had decreased to 12.3 (−5 bar), 11.6 (−8 bar), 8.9 (−15 bar), and 7.8% (−15 bar) at 9, 15, 21, and 27 C, respectively. Matric potentials were estimated from a moisture release curve (14).

Air samples were taken from the plates during the incubation with a microsyringe, and the concentration of O_2, CO_2, and N_2 were measured by gas chromatography (GC 8700, Carl Instrument Inc., Anaheim, CA 92801). The readings revealed no changes of concentrations of gases from day 3 of incubation throughout the experiment. The O_2 and N_2 concentration did not differ from ambient air, but CO_2 concentrations in the plates were estimated at about 0.1%, about threefold greater than that of ambient air.

Estimation of population densities of *P. ultimum* in soil. The soil drop method as described by Stanghellini and Hancock (18) and modified by Hancock (9) was used to measure GP populations.

Retrieval of sporangia and oospores from soil. Five-gram, air-dried soil samples were suspended in 10 ml of an aqueous cesium chloride solution ($CsCl_2$:H_2O, 1.2:1, w/v). The suspension was centrifuged for 5 min at 500 *g*, the supernatant was vacuum-sieved (through a 5-μm membrane filter), and the residue on the filter was washed onto a microscope slide. A drop of 0.5% aniline blue in lactophenol was added and samples of the suspension were observed with a microscope at ×160 or ×320.

RESULTS

Changes in population densities of GP of *Pythium ultimum* in soil infested with mycelial mats of the fungus. Table 1 contains results of regression analyses of data from three separate trials on the influence of temperature and ψ_m on the survival of GP of *P. ultimum* in soil. When sporangia were the original inocula, the graph of the logarithm of densities of GP versus sampling time yielded a straight line. This finding was similar to those described for other soilborne fungi (4) and *P. ultimum* (10). Thus, for ease of analysis, the actual GP population densities at each sample period were transformed to percentage of the initial population densities of GP (as estimated at day 0) and converted to logarithms. These transformed data were subjected to linear regression analyses, which yielded regression coefficients (*b*) and correlation coefficients (*r*). Half-lives ($t_{½}$) of GP were calculated from regression coefficients according to methods proposed by Yarwood and Sylvester (22).

Rates of decline of GP in sporangia-infested soil increased (eg, half-lives decreased) as temperature increased in soils at both ψ_m (Table 1). However, the half-lives were consistently shorter in the soils with initial ψ_m of −0.4 bar than of −3 bar. The r^2 values determined in regression analyses were each significant at $P = 0.05$.

The rates of decline in densities of GP in soil infested with a mixture of oospores and sporangia (7.3:1) were generally lower than in the soils infested only with sporangia (Table 1). While the half-lives were again shorter in soils initially set at −0.4 bar than at −3 bar ψ_m, the ratios of half-lives of GP between the wetter and dryer soils were consistently higher than those determined with sporangia-infested soil. Moreover, the relationship between declines in GP and temperature in the soil infested with oospores and sporangia differed from that found in sporangia-infested soil: half-lives at 15 and 21 C were longer than those at the same ψ_m at 9 and 27 C. However, three of the four r^2 values determined at 15 and 21 C were not statistically significant, indicating a greater variability in rates of decline of GP at these temperatures.

Ripening of dormant oospores mixed in raw soil. Oospore ripening (conversion from dormant to germinable propagules) was evident in moist soil after 30 days of incubation at all treatments tested (Table 2). However, the incidence of ripening (proportion of germinable oospores to total oospores) was always below 5% even after 121 days of incubation. While there were no clear relationships between the conditions of incubation and the magnitude of oospores ripening, there was a tendency for more ripening in the wetter soils and at the higher temperatures (Table 2). However, when the initial ψ_m was −0.4 bar, the increases in densities of GP were consistently highest at 21 C.

Direct microscopic observations were made of the oospores that were separated from soil by the $CsCl_2$ technique. Samples were taken from infested soil that had originally been moistened to −0.4 bar and incubated for 150 days at 9, 15, 21, and 27 C. Two samples were taken from each treatment, and 80–100 oospores from each sample were observed for signs of hyperparasitism. Our observations showed no evidence of hyperparasitism, and less than 4% of the oospores in all samples showed signs of deterioration.

The effect of remoistening infested soil on subsequent changes in population densities of *P. ultimum*. Soil samples infested with mixtures of sporangia and oospores (7.3:1) incubated for 220 days in moist soil were remoistened. The GP population densities after the original 220 days of incubation at 9, 21, and 27 C were 852, 1,830, and 25, respectively, when soil moisture was −3 bar and 575, 200, and nondetectable, respectively, when soil moisture was at −0.4 bar. The soil samples were remoistened and adjusted to a ψ_m of −0.4 bar, stored at 21 C for an additional 60 days and the final GP

TABLE 1. Half-lives and coefficients of linear regression and correlation of changes in population densities of germinable propagules (GP) of *Pythium ultimum* in soil infested with sporangia or mixtures of oospores and sporangia[a]

Temperature (C)	Initial matric potential (−bar)	Sporangia			Oospores and sporangia[b]		
		b (day^{-1})	$t_{½}$ (day)	r^2	*b* (day^{-1})	$t_{½}$ (day)	r^2
9	0.4	−0.0040	76	0.3861*[c]	−0.0036	84	0.8087*
	3.0	−0.0029	105	0.2480*	−0.0012	251	0.1967*
15	0.4	−0.0072	42	0.6292*	−0.0014	220	0.0621
	3.0	−0.0067	45	0.6183*	−0.0008	391	0.0643
21	0.4	−0.0087	34	0.7980*	−0.0025	121	0.1784*
	3.0	−0.0064	47	0.4721*	−0.0009	354	0.0409
27	0.4	−0.0177	17	0.8481*	−0.0100	30	0.7198*
	3.0	−0.0112	27	0.9773*	−0.0062	49	0.5747*

[a] Linear regression was performed after the semilogarithmic transformation of data on changes in population densities of GP. Half-lives ($t_{½}$) were calculated from coefficients of linear regression (*b*) before rounding-off to four decimal places using the following equation: $t_{½} = 0.301\ b^{-1}$. Soils infested with sporangia and mixtures of oospores and sporangia were incubated for 50–220 days, respectively.

[b] The mixture of oospores and sporangia were in a ratio of 7.3:1.

[c] Asterisk (*) indicates statistically significant at $P = 0.05$.

population densities were determined. The samples that initially had been stored at 27 C and −0.4 bar had a final density of 125 GP per gram of air-dried soil. The results of the other treatments are presented as the ratios between the final and the initial (before the second storage period) GP population densities (Fig. 1). Significant ($P = 0.05$) increases in GP population densities were found after the second storage period for soils that had been initially stored at −3 bar and 21 C (175% increase); at −3 bar and 27 C (810% increase); and at −0.4 bar and 21 C (330% increase). However, no significant increases were found in soils initially stored at 9 C.

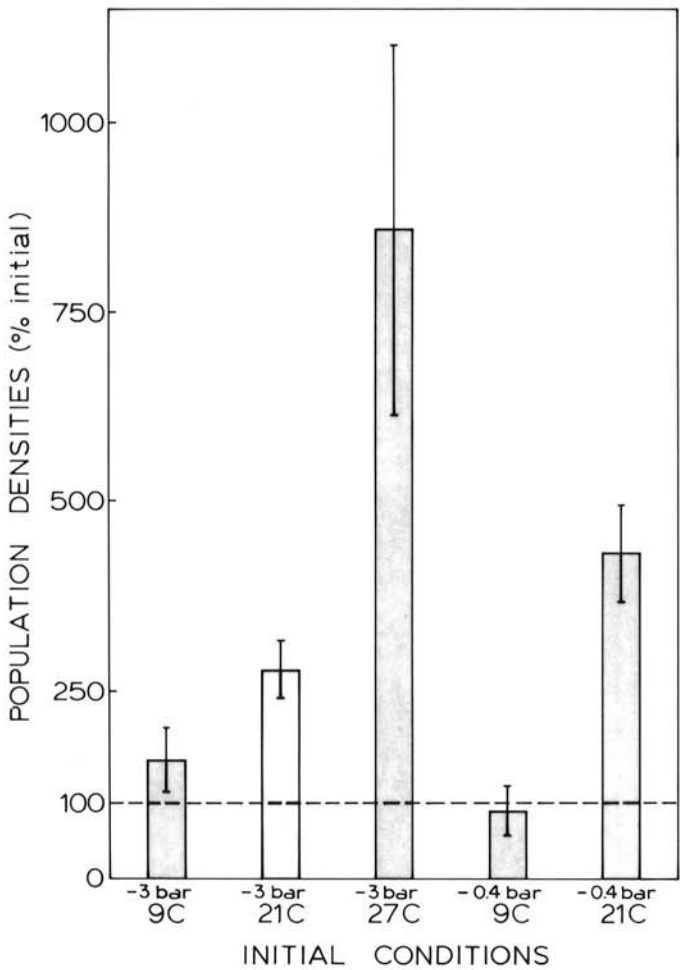

Fig. 1. Changes in *Pythium ultimum* germinable propagule densities after a second moistening and storage period. The soil (Panoche sandy loam) was originally infested with mats containing sporangia and oospores, and was initially incubated at different temperatures and moisture regimes for 220 days. Soils were remoistened to −0.4 bar (initial level) at 21 C for a further 60 days. The final germinable propagule densities are presented as the percentage of the densities measured just before the second storage period. The horizontal dashed line represents the initial densities and the bars represent ±1 standard deviation.

Passive survival of *P. ultimum* in naturally infested soils. Population densities of naturally formed GP of *P. ultimum* were increased in two soil types by the cotton leaf amendment procedure (13) and the soils were then moistened to −0.4 bar and incubated at 9, 15, 21, and 27 C for 92 days. The changes of GP population densities in the two soils during the incubation period are shown in Table 3. In Boston No. 1 soil (Lethant sandy loam) there were increases in GP population densities when the soil was incubated at 15 and 21 C, apparently reflecting the ripening of dormant oospores. Significant increases in population densities of GP of *P. ultimum* above the original densities did not occur in the Davis soil. However, the pattern of decline and subsequent gain of GP strongly suggested oospore conversion to GP was occurring. The increase in GP at 15 C was significant at $P = 0.05$.

DISCUSSION

With soils infested with sporangia of *P. ultimum*, progressively more rapid exponential declines of GP occurred at progressively higher sets of temperatures between 9 and 27 C. This relationship was in contrast to behavior of a mixed inoculum containing oospores and sporangia where it was found that the rates of decline of GP were highest at 9 and 27 C and lowest at 15 and 21 C. The longevity of *P. ultimum* was shorter at warmer soil temperatures in a previous study with mixed inocula where it was found that the rates of decline of GP were higher at 32 than at 16 C (9). Inclusion of more temperature sets shows that the population densities of GP can be most stable at moderate temperatures under certain soil moisture conditions. Findings in this study with sporangia or mixed inocula support the conclusion that *P. ultimum* survives poorly where mean soil temperatures are high (>30 C) (9).

Although soil moisture was not held constant during the incubation of propagules in soil over 5–7 mo, the trends strongly indicate that the longevity of *P. ultimum* was increased under the drier conditions. This is consistent with preliminary findings (9) and observations that population densities of GP may remain stable for months in soil in the laboratory (6,10,19).

Longevity of inoculum of *P. ultimum* is likely to be modified greatly by different soil abiotic and biotic factors such as found in a previous investigation of the saprophytic development of *P. ultimum* in soil (14). In the field, there were remarkable differences in fluctuations of the population densities of GP at different sites (9). Indeed, the formation of inocula and the proportions of oospores and sporangia are likely to be significant sources of variability in the field. Because the half-lives of GP in soils infested with oospores and sporangia are actually the net effect of the death rates of the original sporangia and the maturation rates of ripened oospores, the "stability" of inocula in soil is a complex function.

TABLE 2. The population densities of germinable propagules (GP) of *Pythium ultimum* in soil infested with dormant oospores[a] and incubated at different temperature and moisture regimes

Incubation time (days)	GP per gram of soil maintained at matric potentials of:							
	−0.4 bar[b] and				−3 bar[c] and			
	9 C	15 C	21 C	27 C	9 C	15 C	21 C	27 C
0	n.d.[d]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30	50.0 ± 17.1[e]	191.7 ± 33.2	770.0 ± 163.2	360 ± 86.2	65 ± 8.6	45 ± 6.7	310 ± 101.6	245 ± 36.3
80	110.0 ± 22.2	104.2 ± 22.4	554.2 ± 142.2	220 ± 42.6	75 ± 17.1	108 ± 22.8	n.d.	n.d.
121	66.8 ± 16.6	237.5 ± 27.1	837.5 ± 248.4	290 ± 16.6	25 ± 12.3	495 ± 162.1	245 ± 72.6	195 ± 26.4

[a] 2.5×10^4 oospores per gram of air-dried soil.
[b] The water matric potential was initially adjusted to −0.4 bar, and after 108 days of incubation had decreased to −0.5, −0.5, −0.7, and −3.5 bar at 9, 15, 21, and 27 C, respectively.
[c] The water matric potential was initially adjusted to −3 bar, and after 108 days of incubation had decreased to −5, −8, −15, and −15 bar at 9, 15, 21, and 27 C, respectively.
[d] n.d. indicates that GP populations of *P. ultimum* were not detected.
[e] Mean ± standard deviation.

TABLE 3. Influence of temperature on the changes in population densities of germinable propagules (GP) of *Pythium ultimum* when inocula were formed by colonization of cotton leaves in soil[a]

Incubation time (days)	Source of soil[b]							
	Boston No. 1				Davis			
	9 C	15 C	21 C	27 C	9 C	15 C	21 C	27 C
0	100.0 ± 20.8[c]	100.0 ± 20.8	100.0 ± 20.8	100.0 ± 20.8	100.0 ± 20.4	100.0 ± 20.4	100.0 ± 20.4	100.0 ± 20.4
14	67.6 ± 46.5	57.5 ± 25.0	72.6 ± 31.6	78.0 ± 22.0	47.3 ± 22.4	51.8 ± 17.8	72.2 ± 33.3	56.7 ± 20.2
45	82.4 ± 12.8	124.8 ± 22.9	114.2 ± 21.1	39.6 ± 6.3	80.7 ± 7.6	54.5 ± 24.9	64.0 ± 27.8	59.6 ± 29.8
92	53.5 ± 13.7	189.6 ± 19.2	154.1 ± 33.1	38.7 ± 14.9	77.8 ± 25.6	105.6 ± 48.4	120.0 ± 40.9	100.0 ± 14.7

[a] Field soil samples were amended with cotton leaves in a procedure described in reference 14 to provide high soil population densities of GPs of *Pythium ultimum*. Soil moistures were initially adjusted to −0.4 bar.

[b] Boston No. 1 = Lethant sandy loam; Davis = Zamora loam.

[c] Mean percent (± standard deviation) of the initial germinable propagule (GP) density. In the Boston No. 1 sample, the initial GP population density was estimated as 7,950 propagules per gram of air-dried soil; in the Davis sample the initial GP population density was estimated as 450 propagules per gram of air-dried soil.

Given the possible ways in which environmental factors could affect these two different processes, optimal survival conditions should only be described in general terms, such as inoculum densities of *P. ultimum* are most stable in moderately dry soil at mild (15–21 C) temperatures. Yet, because of the heterogeneity of soils and the inocula of *P. ultimum*, exceptions to this generalization could occur.

The proportion of oospores of *P. ultimum* converted to GP in soil did not exceed 5%. A similar situation with *Pythium aphanidermatum* was observed by Trujillo and Hine (20), who found that less than 10% of the oospores in the soil were capable of germination at any one time, even under favorable nutritional and environmental conditions. Each of these findings is at odds with the report of Ayers and Lumsden (1) that an entire batch of oospores could ripen (up to 96%) within 6 wk of their introduction into moist soil. These conflicting results may be due to differences in methodology, ie, Lumsden and Ayers (15) followed the ripening of oospores incubated on agar plugs placed on the surface of soil, whereas we observed changes in densities of GP in soil infested with oospores. Indeed, our results are in reasonable agreement with Ayers and Lumsden (1) (see Table 3), who reported oospores ripening in raw soil (Table 3). Thus, these conflicting results can be best explained by differences in behavior of oospores on agar plugs and in raw soil.

Lumsden and Ayers (15) demonstrated progressively higher rates of oospore ripening as ψ_m were raised incrementally between −7 and 0 bar. They also found a sharp optimum for ripening at 25 C. Our results failed to show such clear relationships between soil moisture and temperature and the ripening of oospores. However, the densities of GP tended to increase to a greater extent in the soils incubated at the higher moisture regime (−0.4 bar) at the higher temperatures (21 and 27 C). Yet, the optimum temperature for increases in population densities of GP was closer to 20 than 25 C. Because the death rates of GP in sporangia-infested soils increase at progressively higher temperatures (Table 1), the data on ripening of oospores in raw soil may be subject to greater distortion as temperature is increased. Thus, it is possible that over any given time period, a greater proportion of the ripened oospores die at 27 than at 21 C, giving the impression that ripening is greater at 21 C.

The low degree of ripening or conversion to GP by cohorts of oospores in soil could have resulted from one of the following: (i) heterogeneous physiology, eg, the oospores were viable but ripened over indefinite time periods or (ii) the oospores had lost their viability or were subjects of hyperparasitism. When oospores were retrieved from soil by the $CsCl_2$ flotation technique, less than 4% of the thick-walled oospores appeared defective after 6 mo of incubation in moist soil. While hyperparasitism of oospores may be significant in very moist soils (2,10), it did not appear to be an obvious factor affecting survival in this investigation. Furthermore, the remoistening of soil (Fig. 1) stimulated a flush of increased ripening, which indicated that the pool of oospores in soil remained viable, but constitutively dormant, under original treatments. Why there were such large variations in responses to remoistening in terms of ripening among oospores exposed to the original soil treatments is uncertain.

Hyperparasitism of oospores of oomycetes is described frequently and could also be a factor in the passive survival of *P. ultimum* under some conditions (2,7,10–12,17). Contributions of hyperparasitism to death of propagules of *P. ultimum* in this study may have escaped detection because of methodology. However, physiological heterogeneity of oospores appeared to be the major factor in the stability of population densities of GP in soil. Our findings support the observations of Blackwell (5) and Garrett (8), who stressed that the heterogeneous ripening of oospores is an ideal passive survival trait because no single set of environmental conditions would allow complete germination, eg, the possibility for complete loss of inocula.

The ubiquitousness of *P. ultimum* is testimony to the adequacy of its methods of survival. Nevertheless, from the standpoint of its role as a pathogen, it may be possible to reduce the severity of diseases caused by *P. ultimum* through management of its soilborne inocula. Precautions to avoid saprophytic buildup of inocula (14) as well as prolonged exposure of soils to warm, moist conditions may contribute to control of diseases caused by this pathogen.

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