Chemical and Physical Soil Characteristics Related to Lysis of Oospores of *Pythium ultimum*

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ABSTRACT

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Thick-walled oospores of *Pythium ultimum* in agar films on glass slides were buried in soil collected from 11 cultivated fields in western Tennessee. The slides were retrieved after 21, 42, 63, and 84 days of incubation, and oospore morphology was determined. Mean oospore conditions for all 11 soils after 84 days were: 6% thick-walled, 39% thin-walled, and 55% lysed. Four percent of the lysed oospores had germinated before lysis. Significant differences in rates of conversion from thick to thin walls and lysis of thin-walled oospores occurred among the soils. Lysis of oospores was correlated positively with pH, C:N ratio, percent organic matter, and available P and negatively with percent clay. Rates of conversion from thick to thin walls were not significantly correlated with any of these soil variables. Significant correlations of thin-walled or lysed oospores were not found with concentrations of exchangeable Ca, Mg, or K, or with extractable Fe or Mn. From stepwise regression analysis, soil conditions that favored lysis were a high pH, along with high N and P contents. In greenhouse experiments, lysis was increased in an acid soil with a low level of available P by adding calcium hydroxide or calcium phosphate.

Oospores of *Pythium ultimum* Trow are produced in hypocotyls and roots of plants previously invaded by the fungus (17) and in plant tissue introduced into soil (7). Because of their constitutive dormancy and their thick walls, they are resistant to dessication and are believed to be primary long-term survival structures (10,15,17).

When placed in soil, thick-walled oospores of *P. ultimum* change to germinable thin-walled spores. This change is at first rapid with about 30% converted to thin-walled oospores in 48 hr. Thereafter, conversion to cells with thin walls is less rapid; 40% were converted in 7 days, and 60–85% were converted in 45 days (12,15). Rate of conversion is maximum at pH 7.0 in soil saturated with water at 25 C (15). Other soil characteristics or constituents affecting rate of conversion have not been determined.

In a study of longevity of *P. ultimum* in moist soil, Hancock (7) reported that after 6 wk of incubation many of the thin-walled spores were devoid of cytoplasm and some exhibited external cell wall erosion. In a preliminary study in our laboratory, we confirmed that lysis of oospores in soil is a significant factor in survival of the fungus (*unpublished*). The objectives of the present study were to determine if the rates of oospore wall conversion and lysis differed among soils and, if so, to determine the relationships of these changes to certain chemical and physical soil characteristics.

MATERIALS AND METHODS

Soil samples were collected from sites in 11 cultivated fields in western Tennessee in April 1985 and again in October 1985. Four of the fields were on Ames Plantation at Grand Junction, TN; two were on the Milan Experiment Station at Milan, TN; and five were on the West Tennessee Experiment Station at Jackson, TN. The previous crops on six and four of the fields were cotton and soybeans, respectively. One field was clean fallowed the previous year and in the summer of 1985. All of the soils were loessial, wind-deposited soils characterized by high silt and low clay contents (12). Fifteen or more soil subsamples from a 30-m^2 area of each field were taken with a hand trowel to a depth of 8–15 cm. Subsamples were bulked and stored at room temperature in sealed plastic bags for up to 1 wk before experimentation.

Incubation of oospores of *P. ultimum* in soil. A culture of *P. ultimum*, designated B6-1 (ATCC56081) and originally isolated from a diseased cotton seedling from a field in western Tennessee, was used in this study. Pathogenicity, oospore production in liquid culture media, and rates of conversion of B6-1 oospores from thick to thin walls were similar to other isolates of *P. ultimum* from cotton seedlings grown at different locations (11). Procedures for incubating oospores in soil have been described (11). Briefly, oospores in liquid culture were separated from hyphae through blending, sieving, and centrifugation and were then suspended in 2% agar at 42 C. Microscope slides previously dipped into the suspension and coated with agar films of oospores were buried in soil in plastic pots in a growth chamber with alternating 12-hr periods of 24 and 18 C. To maintain a high level of moisture, the pots of soil were watered to saturation whenever the exposed soil surface began to appear dry. Weeds were removed as soon as they emerged from the soil surface.

Determination of morphology of oospores in soil. At 21-day intervals, during a period of 84 days, four slides were removed from each soil, and the surfaces containing oospores were washed to remove soil particles. The oospores were stained with 0.03% acid fuchsin in 85% lactic acid and examined microscopically. Oospores on four agar slides were also stained and examined before incubation in soil. Two hundred oospores chosen at random on each slide were classified as being thick-walled, thin-walled, germinated, or lysed. This procedure was made with the 11 soils collected in April 1985 and repeated with collections of the 11 soils made in October 1985.

Chemical and physical analyses. Soil subsamples for analyses were air dried, ground with a mortar and pestle, and passed through 2-mm-, 1-mm-, or 0.5-mm-mesh sieves, then stored in sealed plastic bags at 5 C. Particle size analysis was made with the Bouyoucos hydrometer method (3), and texture designations were assigned according to Luntz (16). Measurements of pH were made with 1:1 (w:v) 0.01 M $CaCl_2$ solutions of the samples with an Accumet 750 Selective Ion Analyzer fitted with a combination glass electrode (19). Carbon content was determined with a Leco Model IR 12 Infrared Carbon Analyzer for samples oxidized at 2,000 C. Organic matter content was calculated by multiplying the carbon content of each sample by 1.724. Total nitrogen was determined with the standard macro-Kjeldahl method (4).

Exchangeable Ca, Mg, and K were extracted with a neutral normal ammonium acetate solution at pH 7.0 with a vacuum extractor (1,9). Fe and Mn were extracted with a rapid citrate-dithionite procedure (8). Ion concentrations of Fe, Mn, Ca, Mg, and K in extracts were determined by atomic absorption spectrophotometry with a Perkin Elmer model 5000 Spectrophotometer. Available phosphorus was extracted with Mehlick I extractant (0.05 M HCl + 0.0125 M H_2SO_4) and determined by molybdovanadate colorimetry with a Technicon Autoanalyzer (18).

Soil amendments. Soil pH was adjusted by adding pulverized $Ca(OH)_2$ to air-dried soil that had been screened through a 2-mm-mesh sieve. After thorough mixing, the soil was watered to saturation, incubated in plastic bags for 24 hr, and air dried. This wetting, incubation, and drying procedure was repeated twice. Available P levels were adjusted by adding $Ca(H_2PO_4)_2 \cdot H_2O$ to soil followed by the mixing, wetting, and drying procedure used for pH adjustments. In experiments to determine the fate of oospores incubated in amended soils, pH and available P levels were determined at 21-day intervals.

Data analyses. Data were analyzed with the general linear model procedure of the Statistical Analysis System (SAS 1982, SAS Institute Inc., Cary, NC). Statistics to determine differences or correlations among variables were applied according to regression analyses ($P = 0.05$). Data were also analyzed by stepwise multiple regression procedures ($P = 0.15$). For experiments on modification of soil conditions, analyses of variance of data were performed, and treatment means were separated with Duncan's new multiple range test.

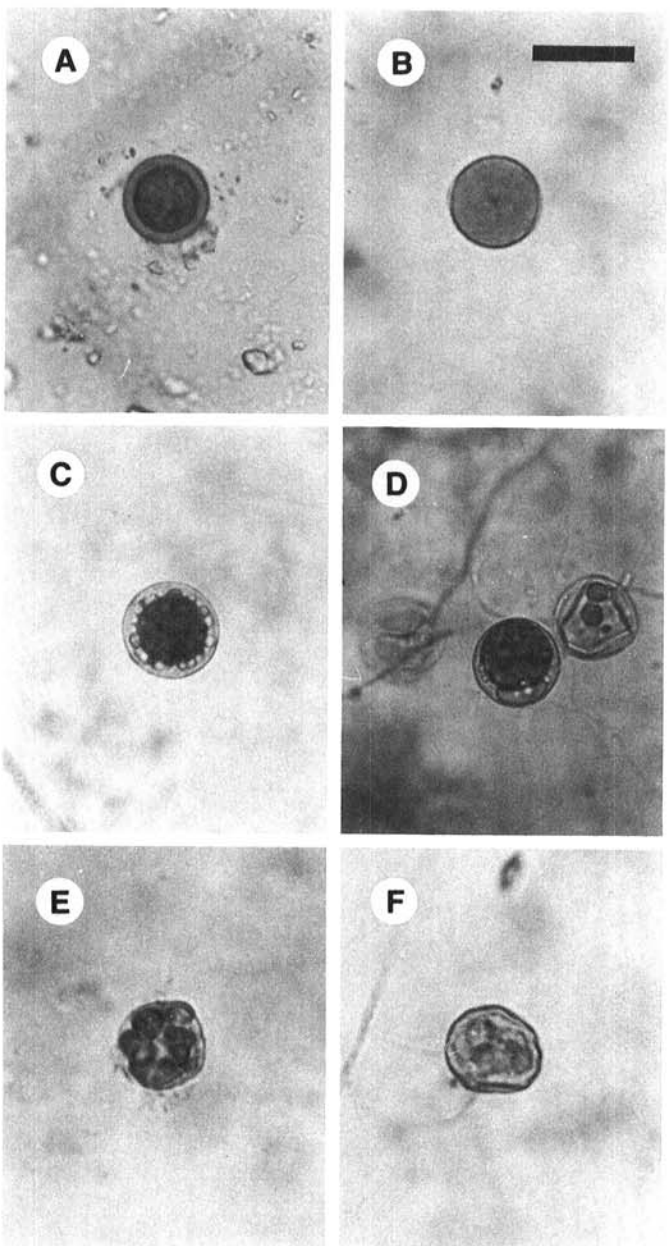

Fig. 1. Oospores of *Pythium ultimum* in water agar films on glass slides retrieved from soil and stained with lactofuchsin. **A,** Thick-walled spore. **B and C,** Thin-walled spores. **D,** Thin-walled spore and lysed spore. **E and F,** Lysed spores. Scale bar represents 20 μm.

RESULTS

Fate of oospores in soil. Lysed oospores in agar films on glass slides retrieved from soil consisted of spores devoid of cytoplasm, those with eroded, broken, or flattened thin walls, or spores with walls completely disintegrated (Fig. 1). Most of the lysed oospores did not appear to be parasitized by soil microorganisms. Fewer than 5% of the lysed oospores were associated with fungal or actinomycete hyphae or visible bacterial cells. Only occasionally were spores (lysed) observed with identifiable germ tubes attached. During the 84 days of incubation, 4.1% of the lysed oospores had germinated before lysis. For brevity, we will use the term "lysed oospores" to include both germinated and nongerminated oospores. In mean values across all 11 soils, more than 70% of the thick-walled spores placed in soil had converted, and 12% had lysed in 21 days (Fig. 2). After 84 days of incubation, 55% of the spores had lysed and fewer than 6% remained thick-walled. Lysed thick-walled spores were not observed. Differences in oospore conversion and lysis varied significantly among the soils. After 84 days of incubation, 11% of the oospores remained thick-walled in soil J-2, but only 2% were thick-walled in soil A-4 ($P < 0.01$). Oospores in soil A-1 were consistently slower ($P < 0.01$) to lyse than oospores in soil J-4 (Fig. 3).

Relationships of oospore survival with chemical and physical soil characteristics. Mean values for C:N ratio and available P were slightly higher in October soil samples than in those collected in April; probably this reflected summer fertilization. Otherwise, values for physical characteristics and concentrations of chemicals in April and October samples were similar. All samples were high in silt content; the lowest contained 65% silt (Table 1). Most of the soils were about medium in fertility, but levels of available P varied from 3 to 45 μg/g. The soils were acid in reaction, varying from pH 6.5 to a strongly acid pH 4.3.

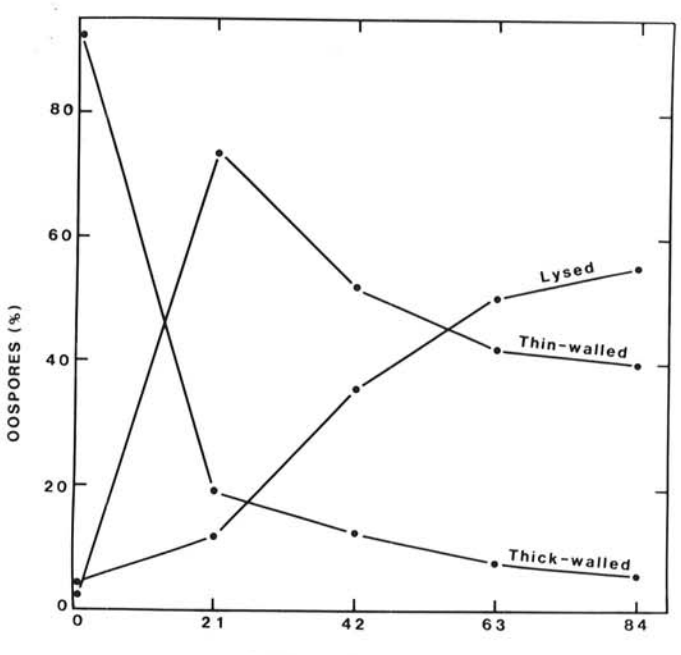

Fig. 2. Fate of oospores of *Pythium ultimum* in agar films on glass slides in soil. Data points are means for soil samples collected in April and October 1985 from 11 fields in western Tennessee.

Although there were significant differences among the soils in the quantities of thick-walled oospores remaining after 42 and 84 days of incubation, these differences were not correlated with any of the physical or chemical soil variables studied. However, percentages of thin-walled oospores were correlated positively at 42 and/or 84 days with percent clay and negatively with C:N ratio, available P, and pH (Table 2). Percentages of lysed oospores were correlated negatively at 42 and/or 84 days with percent clay and positively with percent organic matter, C:N ratio, available P, and pH. No significant correlations of thin-walled or lysed oospores were found with concentrations of total N, exchangeable Ca, Mg, and K, or with extractable Fe and Mn. The linear regression slope of available P content of the 11 soils over percentage of lysed oospores is illustrated in Figure 4. Stepwise regression models were developed by analysis of means of the April and October soil sample collections of soil chemical and physical characteristics. In the model for October soil samples, high pH along with high levels of P and total N accounted for 84% of the variation in lysis of oospores. No other variable met the 0.15 level of significance for entry into the model.

TABLE 1. Chemical and physical characteristics among 11 loessial soils selected for study of oospores of *Pythium ultimum*[a]

Variable	Low	High	Mean
pH	4.4	6.5	5.3[b]
Organic matter (%)	0.53	1.73	1.36
Total nitrogen (%)	0.04	0.10	0.06
Carbon-nitrogen ratio	7.9	12.5	10.2
Available phosphorus (μg/g)	2	45	19
Exchangeable calcium (μg/g)	799	1,430	1,100
Exchangeable magnesium (μg/g)	63	294	139
Exchangeable potassium (μg/g)	111	285	194
Extractable iron (μg/g)	4,700	15,200	7,930
Extractable manganese (μg/g)	399	1,400	648
Mineral fraction			
Sand (%)	0.0	17.1	6.6
Silt (%)	65.0	84.8	75.7
Clay (%)	9.4	29.5	18.3

[a] Values are means from soil samples collected during April 1985 and October 1985 except for calcium, magnesium, potassium, iron, and manganese, which are from the April 1985 soil collection only.
[b] Mean pH was determined by calculating the mean H^+ ion concentration converted to pH.

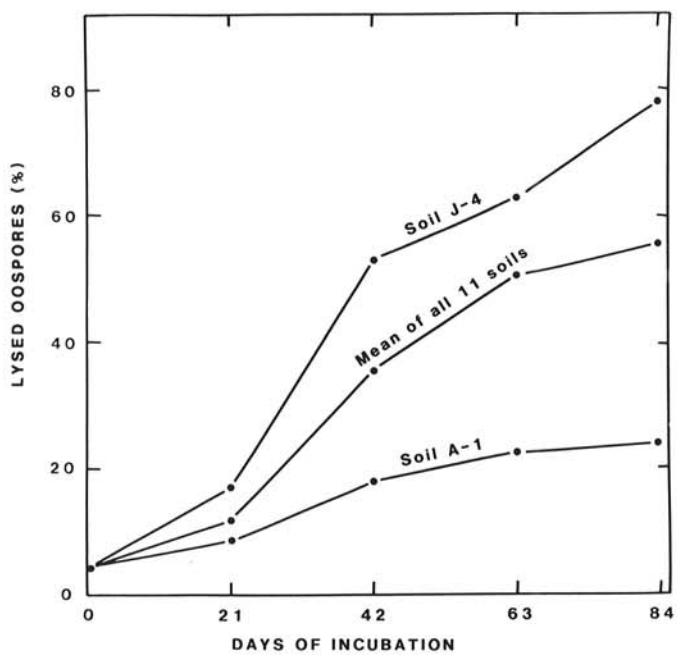

Fig. 3. Comparison of lysis of oospores of *Pythium ultimum* in agar films on glass slides in two of 11 soils, with means of all 11 soils collected from fields in western Tennessee. Data points are means for samples collected in April and October 1985.

Effects of altering pH and P on lysis. To determine if liming a strongly acid soil would affect lysis, $Ca(OH)_2$ was added to soil A-1 (pH 4.3). Oospores were buried in amended and nonamended soil. After 84 days the pH of the amended soil was 6.3 and 55% of the oospores were lysed. Only 33% were lysed in the nonamended soil. In additional experiments, amendments with $Ca(OH)_2$ and $Ca(H_2PO_4)_2 \cdot H_2O$ alone and in combination increased lysis (Table 3).

DISCUSSION

Although there were significant differences among the soils in the quantities of thick-walled oospores remaining after 42 or 84 days, these differences were not significantly correlated with any of the soil variables studied. Quantities of thin-walled oospores and lysed oospores were correlated with certain of the variables, especially pH, C:N ratio, and available P content. Variables that were correlated significantly with lysis usually had a corresponding but opposite correlation with quantities of thin-walled oospores that were not lysed. Because relationships of physical and chemical

TABLE 2. Significant correlations (*r* values) between condition of oospores of *Pythium ultimum* and physical or chemical soil characteristics of 11 soils from western Tennessee[a]

		42 days of incubation[b]		84 days of incubation[b]	
Physical or chemical variable	Oospore condition (%)	April soil samples	October soil samples	April soil samples	October soil samples
Organic matter	Thin-walled	...	...	...	...
	Lysed	...	0.66*	...	0.63*
Carbon-nitrogen ratio	Thin-walled	...	−0.69*	−0.77**	−0.62*
	Lysed	...	0.72*	0.79*	0.62*
Phosphorus (μg/g of soil)	Thin-walled	−0.89**	−0.80**	−0.69*	−0.75**
	Lysed	0.88**	0.89**	0.67*	0.77**
pH	Thin-walled	...	...	−0.61*	−0.72*
	Lysed	...	0.63*	0.61*	0.73*
Clay (%)	Thin-walled	...	0.75**	...	0.71*
	Lysed	...	−0.75**	...	−0.69*

[a] Asterisks * and ** following correlation coefficients indicate statistically significant correlations, $P = 0.05$ and $P = 0.01$, respectively. A negative correlation is indicated by a minus (−) preceding the *r* value.
[b] Thin-walled oospores on agar sides placed in soil 42 or 84 days previously.

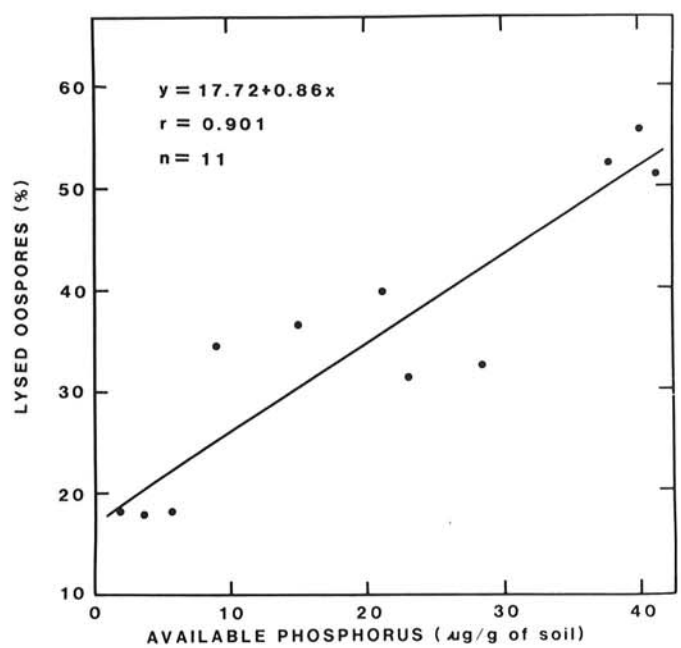

Fig. 4. Relationship of lysis of oospores of *Pythium ultimum* to available phosphorus concentrations in 11 soils from western Tennessee. Values are means after 42 days of incubation for soil samples collected in April and October 1985.

soil characteristics with thick-walled oospores were not established, their influence was on lysis of thin-walled oospores and not on rates of conversion from thick to thin walls.

In a previous study by Lumsden and Ayers (15), a definite and distinct relationship of rates of oospore-wall conversion with pH was established. Optimum pH for conversion was pH 7.0, and only 10% had converted after 8 wk at pH 4.5. In our study, 79, 85, 92, and 96% of oospores in soil A-1 (pH 4.3) had converted to thin walls after 21, 42, 63, and 84 days, respectively. The reason for this lack of agreement between the two studies is not known, but it may have been due to use of different isolates of *P. ultimum* or to differences in methodology. Lumsden and Ayers (15) applied oospores that had been dried up to 1 mo to the surface of water-saturated soil in closed petri dishes. We buried oospores freshly separated from mycelia in pots of soil that varied in moisture content.

According to the data in Table 2, available P, pH, and C:N ratio individually were strongly correlated with lysis of oospores. Total N was correlated positively with lysis, but not significantly ($P > 0.05$). In a stepwise regression analysis of means of the April and October soil collections, additive effects of the soil variables were emphasized. The C:N ratio was excluded from the models generated. The best 3-model system after 84 days of incubation consisted of a combination of pH, total N, and available P, which accounted for 84% of the variation.

Populations of *Pythium* spp. have been shown to be affected by soil acidity. *Pythium* spp. were not isolated from soils with pH below 5.4 (2) but were isolated from Tennessee soils with pH levels as low as 4.3. However, a significant negative correlation was found with pH and isolation of *Pythium* spp. in Tennessee soils (12). In the present study, there was positive correlation between pH and lysis of oospores. Species variability at different pH levels could account for this difference in lysis and occurrence. Phosphorus availability is low in many soils with low pH. As P availability was increased (Table 3) by amending low pH soil with calcium phosphate, lysis occurred at an increased rate. When the pH was raised without increasing the availability of P, higher rates of lysis also occurred. This latter soil condition is probably rare in cultivated soils and did not occur in the soils examined by Johnson and Doyle (12). Oospores apparently are able to survive longer when placed in soils with both low pH and low available P. Long-term survival (more than 3 mo) of introduced oospores and pathogenic activity has not been investigated.

A significant negative correlation of soil clay content with lysis occurred in the samples collected in October. There was also a significant negative correlation of clay content with soil pH ($r = -0.76$). Because clay particles have larger surface areas with proportionally higher numbers of cation exchange sites than do silt or sand particles, soils with higher clay contents have the greater potential acidity. It is possible, therefore, that the clay association with lysis was dominated by the pH effect.

Relationships of plant diseases caused by *Pythium* spp. with soil pH and P levels have been reported, but the influence of these soil factors was attributed mostly to host growth response and not to survival of the pathogen (6). Wheat seedling disease caused by *P. graminicola* subramaniam was more severe in soils with pH levels of 6.5–7.1 than in more acid soils and in soils with deficiencies or excesses of P (13). Deficiencies of P were associated with severe Pythium root rots of sugarcane (5) and wheat (20). The improvement in growth of wheat from amendments of phosphorus fertilizer was due to the production of new roots, which lessened the chances for infection. Percentages of infected roots in P-amended soil were similar to those in nonamended soil (20).

The mechanism of lysis of oospores (not previously germinated) in soil is not known, but it is likely to be biological. Available P, total N, and pH have a profound influence on growth and activities of saprophytic soil microorganisms. After 84 days of incubation, 73% of the original oospores buried in soil A-1 were thin-walled and lysed. This soil had a reaction of pH 4.3 and contained 6 μg/g of available phosphorus. Conversely, only 15% of the oospores in soil J-4 were nonlysed and thin-walled. This soil had a reaction of pH 5.6 and contained 38 μg/g of available phosphorus. The levels of P and the pH at which lysis was highest are the most favorable for microbial growth, especially for bacteria and actinomycetes. It is assumed that thin-walled oospores, like thin-walled hyphae (14), are more susceptible to degradation by the greater quantities of antibiotics and enzymes produced under such favorable conditions.

TABLE 3. Lysis of oospores of *Pythium ultimum* in acid soil amended with calcium hydroxide and/or calcium phosphate

Soil	Amendment and rate of addition	Soil characteristics and oospore conditon after 84 days of incubation[y] pH	P (μg/g of soil)	Lysed oospores (%)[z]
A-1	none	4.3	4.8	26 a
A-1	$Ca(OH)_2$, 1.12 g/kg	6.1	3.2	47 b
A-1	$Ca(H_2PO_4)_2 \cdot H_2O$, 0.4 g/kg	4.3	48.8	51 b
A-1	$Ca(OH)_2$, 1.12 g/kg plus $Ca(H_2PO_4)_2 \cdot H_2O$, 0.2 g/kg	6.0	20.8	53 b
J-5	none	5.4	42.0	52 b

[y] Data values are means of two replicated experiments.
[z] Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

These same soils favorable for degradation of oospores could also be favorable for germination of oospores. From the data it was calculated that 4.1% of the lysed spores had germinated before lysis. In subsequent studies we have found that as many as 20% of lysed oospores buried in certain soils had previously germinated. Information on the survival and fate of such germ tubes from oospores would aid in a better understanding of the ecology of *P. ultimum* in soil.

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