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Factors Affecting Soil Populations of *Pythium ultimum* in the San Joaquin Valley of California

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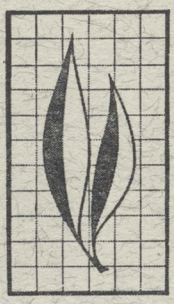

Pythium ultimum soil population levels were highest in the cooler seasons in the San Joaquin Valley, and always lowest during midsummer (August or early September). Crop residues, especially surface litter, supported population increases in the autumn (late September through November) in many fields, providing moisture was available and the substrate was suitable. A general seasonal pattern of *P. ultimum* fluctuations was evident in 7 of 10 field sites; populations were uniformly low in the remaining sites.

In the laboratory, *Pythium ultimum* did not develop or survive well on cotton leaves in soils held above 30 C for extended periods. High soil temperatures (30 to 37 C during July and August) apparently contribute to the uniformly low levels of *P. ultimum* encountered during the summer in field sites in the San Joaquin Valley. Soils that possessed uniformly low populations in the field during the course of this study did not support increases in *P. ultimum* when cotton leaves were incorporated under controlled conditions. *Pythium ultimum* developed well when cotton leaves were incorporated into soils which supported population increases under field conditions. It appears that certain soils are conducive to *P. ultimum* development, whereas others are suppressive. It is concluded that seasonal fluctuations in *P. ultimum* populations depend, in part, upon climatic conditions, the presence of organic residues, and soil factor(s).

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Factors Affecting Soil Populations of *Pythium ultimum* in the San Joaquin Valley of California[1]

INTRODUCTION

PYTHIUM ULTIMUM Trow is a widespread soil-borne pathogen in temperate climates, causing preemergence and postemergence damping-off and root necrosis in a great number of ornamental and crop plants. It is well adapted as a primary colonizer of organic residues, which prompted Garrett (1956) to classify *P. ultimum* as a "sugar fungus."

Sawada, Nitta, and Igarashi (1964) found *Pythium* was a principal colonizer of green manure in Japan where an association was apparent between *Pythium* colonization of organic residues and subsequent injury to oat seedlings. Watson (1971) recently found a similar relationship between *P. ultimum* colonization of crop residues and damage to lettuce in California.

The capacity to respond rapidly to nutrients from plant exudates or residues places *P. ultimum* in a strong competitive position as a saprophyte and as a seed pathogen (Singh, 1965; Stanghellini and Hancock, 1971*b*). Sporangia germinate within 1.5 h, and germ tubes extend at high rates after exposure to low nutrient levels (Stanghellini and Hancock, 1971*a*). These behavioral characteristics allow *P. ultimum* to avoid microbial antagonism during early stages of saprogenesis and pathogenesis.

While sporangia appear to be the principal functional inoculum of *P. ultimum* in California, thick-walled oospores may allow the fungus to survive adverse conditions for long periods (Lumsden and Ayers, 1975; Stanghellini and Hancock, 1971*a*). Under certain circumstances, dormant oospores may convert to thin-walled spores and function like sporangia (Ayers and Lumsden, 1975).

Little information is available on the influence of environmental factors on *P. ultimum* populations in the field. Robertson (1973) observed that populations of *Pythium* spp. tended to peak in New Zealand when soil temperatures were low and soil moisture was high. However, it was not determined how individual species were affected.

This study was undertaken as part of a comprehensive investigation of the biology of *Pythium ultimum* in California. The work reported here largely concerns population behavior in field sites located in the San Joaquin Valley. Where feasible, behavior of the fungus in the field was reexamined in the laboratory or greenhouse. Preliminary reports of portions of this work have been published (Hancock, 1973; 1974).

[1] Accepted for publication January 11, 1977.

MATERIALS AND METHODS

Pythium ultimum populations in field and experimental soils were estimated by the soil-drop method of Stanghellini and Hancock (1970) or by a wet-sieving procedure (A. R. Weinhold, unpublished). Random isolations of fungal hyphal tips from agar plates confirmed the specificity of these techniques for *P. ultimum*.

For rapid identification, isolated pythiaceous fungi were cultured on rolled oats agar (hot water extract of 50 g rolled oats and 20 g agar per liter) slanted on one side of each petri dish. The petri dishes contained water to a depth of ca. 5 mm. After the organisms grew into the water and fruited (in about 5 days at 21–24 C), sexual structures were viewed directly under low magnification (160–320×) with phase-contrast optics.

Field Sampling

Soil samples were taken at 2- or 4-week intervals from 10 selected field sites in the western portion of the San Joaquin Valley (Fig. 1). Sites were ca. 10 m in diameter in cultivated fields, and at least 15 m from the borders of the fields. Characteristics of the soil from each field site are presented in Table 1.

Thirty subsamples were taken randomly from the tillage layer (surface to 15 cm) of each field site, using a soil-tube sampler (2 cm inner diam), or trowel (when soil was too wet). The subsamples were bulked (1.5 to 3 kg), and transported to the laboratory in sealed plastic bags at ambient temperatures. Small soil subsamples (60 to 100 g) were used to determine moisture content. The data are presented on the basis of ovendry (110 C) soil. The remaining soil was air dried at room temperature (20 to 25 C) for 3 to 5 days, ground to pass a 2-mm screen, and mixed for 30 min with a Twin Shell Dry Blender (Patterson-Kelley Co., Inc., East Stroudsburg, Pa.).

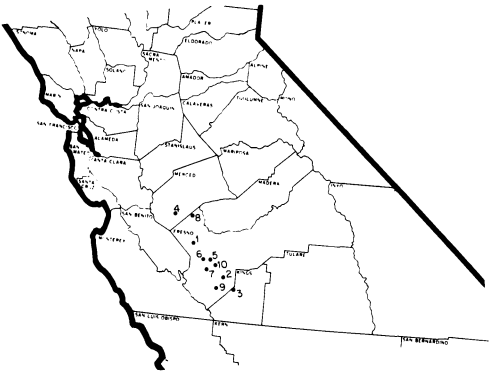


Fig. 1. Distribution of field sites sampled for *Pythium ultimum* in the San Joaquin Valley. The West Side Field Station (University of California), Five Points, Ca., is 1.5 kilometers from site No. 10.

TABLE 1

CHARACTERISTICS OF SURFACE SOILS AT THE SAMPLING SITES*

Site	Soil Type	pH	SP†	C‡	N‡
1	Panoche sandy clay loam	8.1	58.5	0.40	0.07
2	Oxalis clay	8.0	45.1	1.07	0.09
3	Lethent clay loam	7.9	57.0	1.47	0.10
4	Mocho clay	7.7	51.9	1.21	0.11
5	Oxalis clay	7.7	48.2	1.61	0.15
6	Panoche sandy clay loam	8.0	45.6	0.80	0.07
7	Panoche clay	8.1	60.0	1.21	0.11
8	Merced clay	7.3	68.4	2.28	0.14
9	Panoche sandy clay loam	7.9	46.8	1.07	0.07
10	Panoche clay loam	8.0	51.9	1.88	0.09

* Soil samples were taken from 0–15 cm.
† Water saturation percentage (SP).
‡ Carbon (C) and nitrogen (N) expressed on a percentage basis.

Pythium ultimum populations were unaffected by air drying for up to 5 days. However, populations declined in certain soils if they were air dried for longer than 5 days at temperatures that exceeded 25 C. Soil was stored in an air-dry condition in plastic bags at 10 C for 1 to 3 months without substantially affecting the populations of *P. ultimum*.

Quantitative soil assays for Pythium ultimum

Soil-Drop Method: Three replicates, 10 g each, of air-dry soil were taken from the mixed samples from each field site or experimental soil, and mixed with 250 ml water in a blender for 1 min. When *Pythium* populations were high, dilutions of 5 g soil per 250 ml water were used. One-ml samples of soil suspensions were plated on 2 percent water agar, and plates were incubated for 20 h at 22 to 24 C before being read (Stanghellini and Hancock, 1970). Sporangial populations were calculated from replicate counts and expressed on the basis of sporangia/g of air-dry soil. Statistical treatments of these tests are presented in Table 2.

Wet-Sieving Method: This procedure, developed by A. R. Weinhold (unpublished), consisted of wetting 3 to 50 g of air-dry soil (smaller quantities of soil are required when organic matter levels are high) and passing it through a 35-μm mesh screen with appropriate rinsing. The organic material retained was floated off the screen and rinsed onto coarse filter paper in a Buchner funnel. After further rinsing in the funnel, the filter paper was transferred to a petri plate, one percent melted agar was added, and the plate was gently swirled. Plates were held 24 h at 22 to 24 C before being read. Characteristic growth patterns allowed the identification of *P. ultimum*, distinguishing it from *Rhizoctonia solani* and other rapidly growing fungi. Each fragment of organic matter yielding *P. ultimum* was regarded as a "propagule," or unit of inoculum. *Pythium* populations are expressed as "propagules"/kg of air-dry soil. This method estimates *P. ultimum* levels in larger (>35 μm) organic substrates, a soil fraction that could be missed with the soil-drop technique.

TABLE 2

VARIABILITY ENCOUNTERED IN THE ESTIMATION OF *PYTHIUM ULTIMUM* POPULATIONS BY THE SOIL-DROP METHOD*

Population range (sporangia/g)	Number of samples	Sporangia per g		
		Mean	s	$s_{\bar{x}}$
25–75	22	40	44	26
75–150	18	116	88	51
150–225	8	192	70	41

* The standard deviation(s) and standard error ($s_{\bar{x}}$) were calculated on the basis of populations of *Pythium ultimum* in 3 subsamples of each bulked sample (30 field subsamples). Data represent means of the standard deviation and standard errors within each population range.

Soil temperatures at the West Side Field Station

Seasonal fluctuations of soil temperatures at the West Side Field Station (WSFS), Five Points, California, are presented for the period in which soil populations were measured (Fig. 2). These readings were taken at a 15-cm depth in exposed, nonirrigated soil, and are given as maximum and minimum temperatures.

Soil temperatures recorded at WSFS may be considered approximations of temperatures at the seven field sites within about a 25-km radius (Site nos. 2, 3, 5, 6, 7, 9, 10). Crop cover and irri-

gation reduce soil temperatures during summer months; hence, measurements at WSFS would not be accurate estimations of soil temperatures in cropped fields. But during the summer months they may be considered reasonable approximations of soil temperatures in dry, fallow fields.

Soil moisture measurements

Precipitation was measured at the WSFS weather station. These data were used in evaluating the influence of precipitation on *P. ultimum* populations during autumn months.

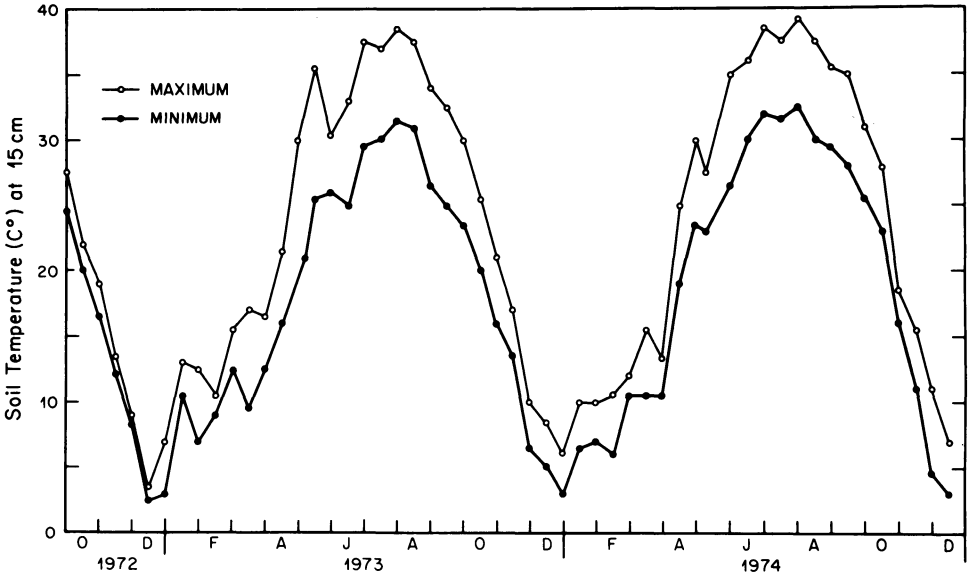


Fig. 2. Minimum and maximum soil temperatures recorded at the West Side Field Station during the period of study.

Soil water potential was measured with a psychrometric microvoltmeter (Wescor, Inc., Logan, Utah, Model MJ 55) and psychrometric probes (Wescor, Inc., Model P51) when water potentials were <-1 bar. Matric potentials (Ψm) were measured with tensiometers (Soil Moisture Equipment Co., Santa Barbara, Ca.) when water potentials were >-1 bar.

Maintenance of soil moisture during experimentation

In most tests, soil (ca. 250 g dry wt) was held in eight styrofoam cups (7 cm diam, 7.5 cm deep) set in rectangular (33 cm long, 23 cm wide, and 10 cm deep) plastic refrigerator containers. Each container was fitted with an air outlet and inlet through which water-saturated air was passed continually. Dampened cheesecloth was fitted into the container floor and lid, and was kept wet during the experiments. Psychrometric soil probes were buried (4 cm) in soil in cups in certain tests to monitor soil water potentials.

With the aerated refrigerator box procedure, soil moisure was maintained reasonably constant for short periods (2 to 3 weeks) at temperatures ranging from 16 to 33 C. With a sandy loam soil, starting moisture levels were adjusted to about 8 percent by wetting up air-dry soils. Moisture content normally dropped 1 to 2 percent during a 3-week test period.

In long-term experiments (3 to 10 weeks), soil moisture was maintained

with capillary columns similar to those described by Bateman (1961). Sandy loam test soils were held in styrofoam cups fitted with nylon net (2-mm mesh) bottoms. Cups were embedded in a moist sand: vermiculite (1:1 v/v) mixture 24 cm above the water level.

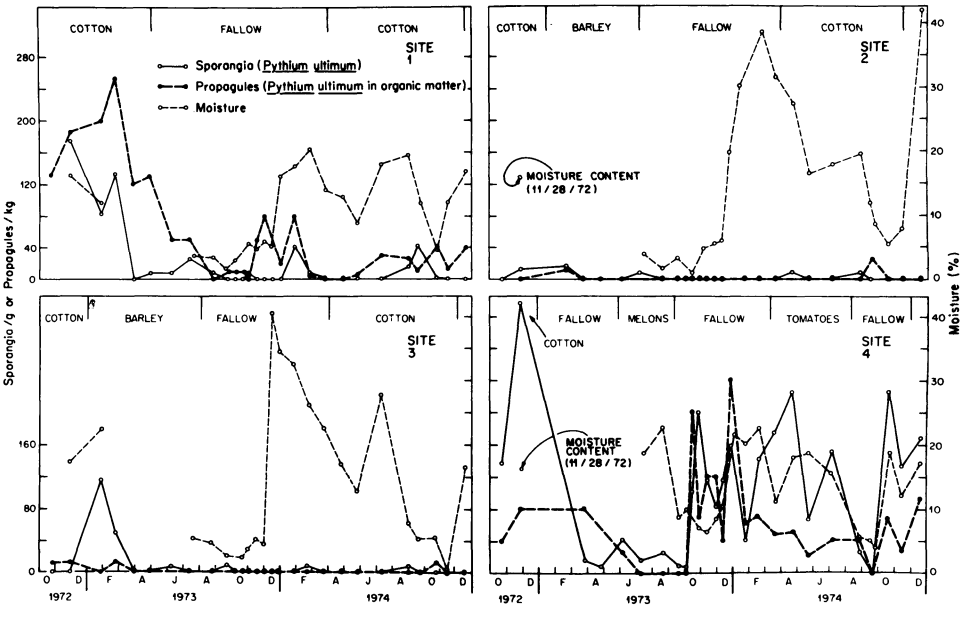
Pythium ultimum *colonization of cotton leaves: laboratory investigations*

In experimental studies on the production of sporangia in cotton leaves, 0.5 g of crushed leaves (greenhouse grown, air-dry; fragments ranged from 1 to 5 mm diam) was mixed thoroughly with 150 g of soil naturally infested with *P. ultimum*. In most of the work with leaf colonization, a Hanford sandy loam soil (55.4 percent sand, 30.8 percent silt, 13.8 percent clay; 0.25 percent organic matter; pH 7.2) was used. *Pythium ultimum* sporangial levels were normally less than 1 sporangium/g of soil at the beginning of experiments, and usually remained at such levels in untreated controls. In colonization studies, water potentials were initially adjusted to between –0.2 and –1.0 bar by gradually wetting up air-dry soil.

RESULTS

Populations of Pythium ultimum *in agricultural soils in the San Joaquin Valley*

Pythium ultimum populations were estimated in 10 sites (Fig. 1) in the San Joaquin Valley between October, 1972, and December, 1974. *Pythium ultimum* levels in organic matter, as determined by wet-sieving, generally paralleled populations determined by the soil-drop procedure (Figs. 3–5). Higher *P. ultimum* soil populations occurred during the autumn, winter, and spring seasons; lowest population levels invariably occurred during August or early September. Although the population fluctuations in the different field sites followed this seasonal trend, there were considerable differences in the levels of *P. ultimum* between sites; popula-



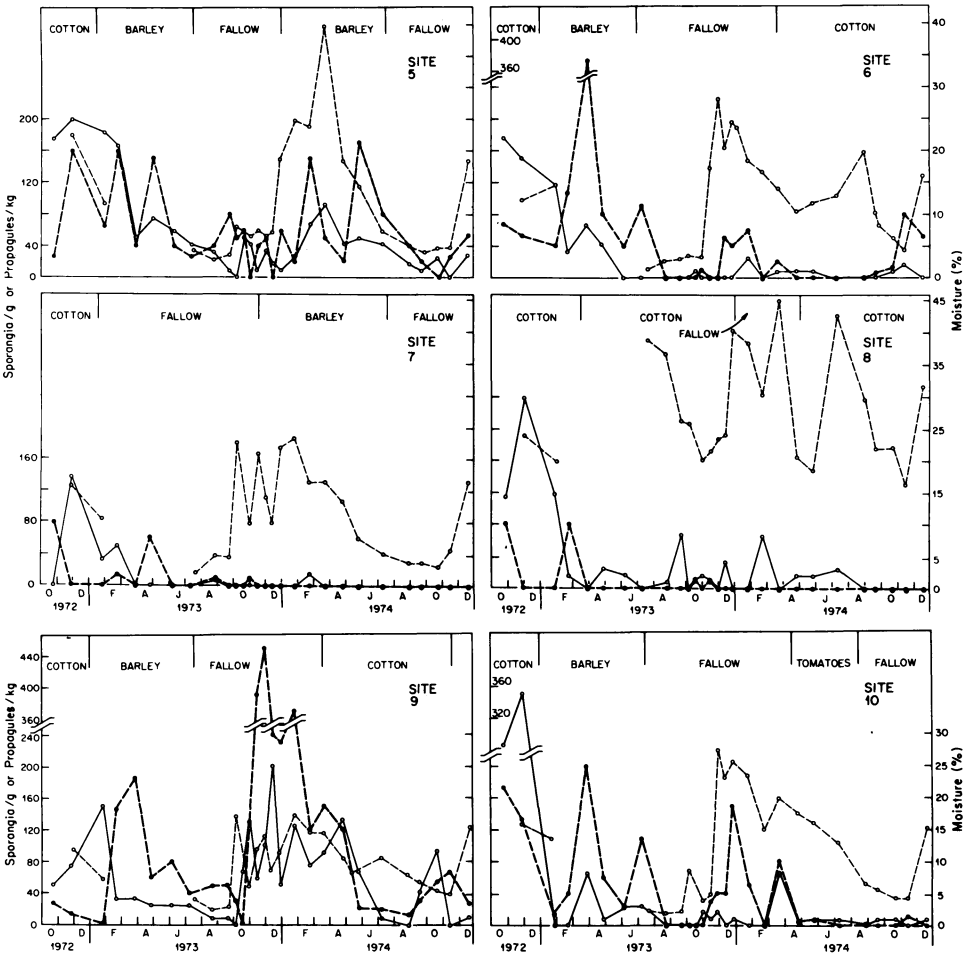


Fig. 3–5. Levels of *Pythium ultimum* in surface soils (0–15 cm) sampled in field sites at monthly intervals from October, 1972, through December, 1974. Moisture is expressed on a dry weight basis. The cropping sequences at each site are included.

tions were uniformly low in sites 2, 3, and 7 during the course of this work.

The population of *P. ultimum* substantially increased at most sites during late autumn and the winter months of 1972–73. Cotton was the crop in all field sites during 1972. Examination of *Pythium* hyphae growing on water agar from soil collected at this time revealed that many hyphae arose from cotton leaf fragments. In subsequent years, the upper 2 cm of soil and cotton leaf debris were sampled, and *P. ultimum* populations were compared with those in random samples from the top 15 cm. In all instances, *P. ultimum* levels were much higher (>4-fold) in the upper 2 cm of soil and leaf debris than in the random samples. Microscopic examination of *Pythium* detected with the wet-sieving method again revealed that leaf fragments were the source of hyphae. These observations indicated that the dead, dry, cotton leaves and leaf fragments on the soil surface were being colonized by *P. ultimum* (Fig. 6).

Because of agricultural rotation schemes, only site 8 was cropped with cotton in 1973. Therefore, three addi-

Fig. 6. Distribution of cotton leaves and petioles on the soil surface following harvest (October–November). Leaves in the furrow were crushed and pressed into the soil by the mechanical cotton harvester. *Pythium ultimum* can colonize these leaves following autumn rains.

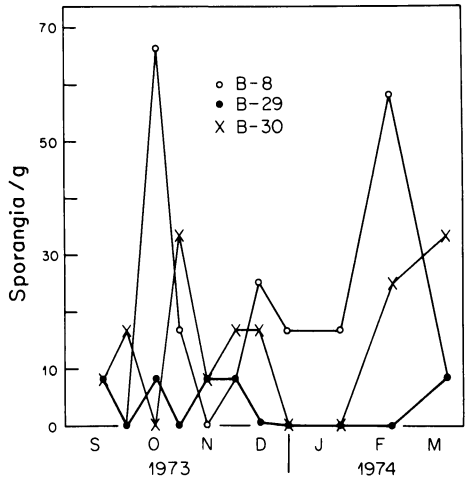


Fig. 7. Levels of *Pythium ultimum* in surface soils during the autumn and winter months, 1973–74, in 3 field sites planted in cotton during 1973.

tional sites planted to cotton were sampled during this season (Fig. 7). However, during October and November, 1973 and 1974, *P. ultimum* population increases did not appear to be closely associated with the cotton leaf fall that took place during these months. Rainfall was very low and infrequent during both October and November, 1973 and 1974 (Fig. 8).

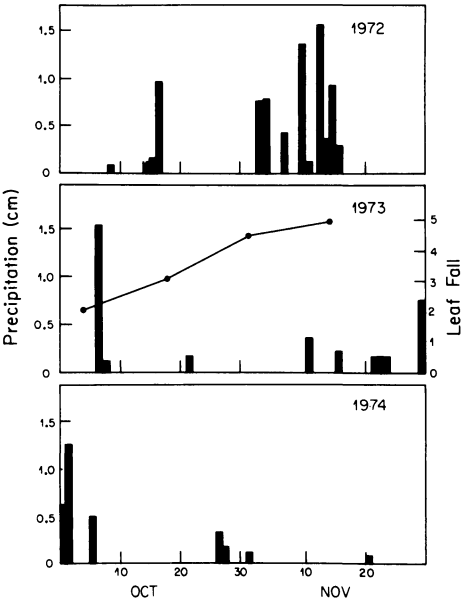


Fig. 8. Precipitation recorded at the West Side Field Station during October and November, 1972–74.

Pythium ultimum population increases in cotton leaf debris appeared to depend on available moisture during the autumn months. With the last irrigations occurring in early August, and harvest taking place from mid-October to early December, sufficient moisture for the colonization of fallen leaves by *P. ultimum* would depend on natural precipitation. During the autumn of 1972, there was substantial precipitation during early and mid-November, a period when defoliation was nearly complete (Fig. 8).

In sites 4 and 9, *P. ultimum* populations increased rapidly during the autumn months in 1973 when moisture was available after irrigation. These sites were cropped the previous spring (barley) or summer (melons), and organic residues were available for colonization. Yet quantitative changes in *Pythium* populations seemed to be characteristic of individual sites, with little association between population changes and cropping history. For example, with identical cropping sequences, sites 2, 3, 6, and 9 had distinctly different population patterns during this investigation.

There was no evidence that *P. ultimum* populations increased in the upper 15 cm of soils at field sites cropped during the summer months. Increases in *Pythium* "propagules" in organic matter did occur, however, during early spring, 1973, in those fields (sites 5, 6, 9, and 10) cropped in barley. Yet small population increases occurred during the same period in fallow fields (sites 1 and 7). Because barley cropping following a 12-month fallow period did not support an increase in *P. ultimum* in site 7 in 1974, the population increases in February and March, 1973, may have been a result of fragmentation and dispersal of decomposing cotton residues that had been colonized by *Pythium* the previous autumn. Nevertheless, the influence of winter barley plantings on *P. ultimum* soil populations is worthy of further investigation.

The autumn pattern of population increase may be a reflection of cultural patterns in commercial agriculture more than of limitations on growth and sporulation of *P. ultimum* during winter and spring months. In plot work on the Kearny Field Station (Parlier, California), the soil population of *Pythium* increased in the spring after herbaceous weeds were disced. However, consistent with results from the western side of the San Joaquin Valley, populations at the Kearny Field Station were lowest during the warm summer months.

Relationships among organic matter, soil temperature, and soil moisture appeared critical in the development of *P. ultimum* in the field. Why populations did not increase after cotton harvest in December of 1973 and 1974 is not clear, as cotton debris was available for colonization, and soil moisture was adequate (Fig. 3–5). Qualitative changes in cotton leaves may have affected their suitability for colonization. As shown in Fig. 9, cotton leaves collected from the soil surface of a cotton field during late November, 1973, did not support *P. ultimum* population increases as well as did those collected earlier. The nature of the change in suitability of these cotton leaves as substrates for *P. ultimum* is unknown.

Influence of soils from different field sites on Pythium ultimum *colonization of cotton leaves*

Under laboratory conditions, soils from different field sites differed in their capacity to support *P. ultimum*

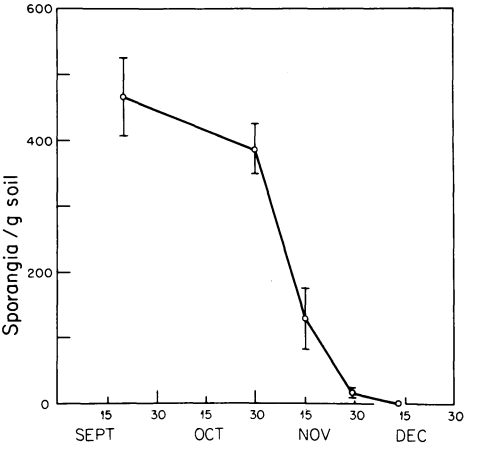


Fig. 9. Levels of sporangia of *Pythium ultimum* in Hanford sandy loam soil 10 days after mixing with cotton leaves. Leaves were collected on different dates from a cotton field during the autumn of 1973; collection dates are presented on the abscissa. Vertical bars represent the range of sporangial levels in duplicate soil containers.

TABLE 3

DEVELOPMENT OF SPORANGIA OF *PYTHIUM ULTIMUM* IN COTTON LEAVES IN SOILS FROM DIFFERENT FIELD SAMPLING SITES*

		Exp. 1†			Exp. 2		
		replicates			replicates		
Site No.	Control‡	a	b	% Moisture	a	b	% Moisture
		(sporangia/g)			(sporangia/g)		
2	<1	8	8	15.5	8	17	19.2
3	<1	<1	<1	26.8	<1	8	22.0
5	42	1175	658	28.4	–	–	–
6	<1	633	217	21.3	75	116	13.6
9	108	366	508	21.0	2283	1808	16.7

* Cotton leaves (0.5 g) were incorporated into soils (150 g), moistened, and held in a constant-moisture apparatus (aerated refrigerator containers) at 21–23 C for 7 days, and the soils then assayed for *Pythium ultimum*.

† Moisture was measured at the end of the experiment and is expressed on an over-dry-weight basis. Values are the mean of the 2 replicates.

‡ Sporangial populations in soils at time of incorporation of cotton leaves; *P. ultimum* populations did not change in unamended soils during the course of these experiments.

development in cotton leaves (Table 3). Soils from sites 2 and 3 did not support population increases, whereas sites 5 and 9 supported substantial increases. Population increases in soil from site 6 were intermediate compared to levels stimulated in other soils.

When cotton leaves were incorporated successively at weekly intervals, low *P. ultimum* population levels were still present in soils from sites 2 and 3 (Table 4). Populations in soils from site 6 were still several fold higher than those in soils from sites 2 and 3.

TABLE 4

EFFECT OF MULTIPLE INCORPORATION OF COTTON LEAVES ON *PYTHIUM ULTIMUM* SPORANGIAL POPULATIONS IN SOILS FROM DIFFERENT FIELD SAMPLING SITES*

	Replicates		
Site No.	a	b	% Moisture
	(sporangia/g)		
2	25	25	32.1
3	17	142	36.5
6	708	692	20.9

* Leaves (0.5 g) were incorporated into soils (150 g) at 0, 7, and 14 days. Soils were incubated at 21–23 C, and assays made after 21 days. Soils contained less than 1 sporangium per g before leaves were added. Sporangial populations did not change in unamended soils.

Influence of water potential on development of Pythium ultimum

Soil moisture was apparently a limiting factor in development of *P. ultimum* in cotton leaves in the field. To determine the influence of soil moisture on this process, cotton leaves were incorporated into soils wetted to different levels. Water-saturated soils did not support *Pythium* development on cotton leaves. Yet, as indicated in Table 5, *P. ultimum* populations increased substantially at water potentials (Ψ) between –0.3 and –8 bars. *Pythium* populations did not increase at Ψ below –9 to –11 bars.

Tests were made on the effect of water potentials on *P. ultimum* in culture. A single isolate (67-1; ATCC 32939) was used throughout these studies. Germination or growth was measured on V-8 agar at 22–23 C. Sucrose, KCl, or $CaCl_2$ were used as the osmotica, and water potentials were measured psychrometrically. As shown in Table 6, sporangial germination was not greatly influenced

TABLE 5
INFLUENCE OF SOIL MOISTURE ON DEVELOPMENT OF SPORANGIA IN COTTON LEAVES*

Water Potential (– bars)	Sporangia/g		
	Exp. No. 1†	2‡	3‡
38.3	–	–	0
26.0	17	–	0
11.0	0	0	–
10.0	–	–	25
9.0	50	–	–
8.0	292	–	–
7.9	–	–	50
6.4	–	125	–
6.0	–	142	–
5.6	–	466	–
4.8	–	–	92
3.5	242	–	–
1.8	441	–	–
1.1	550	–	–
0.3	467	–	–
0.2	–	–	108
0.1	–	283	–

* Cotton leaves (0.5 g) were incorporated into a sandy loam soil (150 g), moistened, and held in a constant-moisture apparatus at 21–23 C for 7 days. A portion of the soil was used to determine moisture content, and a second portion was used for *Pythium ultimum* assays.
† Water potential was estimated from a moisture content-water potential standard curve.
‡ Water potential was measured directly with psychrometers at $\Psi < -1$ bar, or with tensiometers (matric potential) when $\Psi > -1$ bar.

TABLE 6
INFLUENCE OF WATER POTENTIAL ON SPORANGIAL GERMINATION IN CULTURE

Exp. 1*		Exp. 2†	
Ψ‡ (– bars)	germination§ (% control)	Ψ (– bars)	germination (% control)
1.8	100	7.3	98
9.5	76	16.8	88
15.0	67	27.3	74
22.0	40	42.0	4
28.0	18		
29.0	9		

* KCl used as the osmoticum.
† Sucrose used as the osmoticum.
‡ Water potentials were estimated psychrometrically.
§ Germination measured after 5 hours of incubation at 22 C on V-8 agar.

TABLE 7
INFLUENCE OF WATER POTENTIAL ON LINEAR GROWTH OF *PYTHIUM ULTIMUM* IN CULTURE*

KCl		$CaCl_2$	
Ψ† (– bars)	growth (% control)	Ψ (– bars)	growth (% control)
0	100	0	100
4.0	102	3.4	100
7.7	106	4.9	94
9.5	61	10.0	100
14.5	59	13.0	73
18.5	8	23.5	56
20.0	16	31.3	13
30.8	2		

* Growth measurements made in mm diameter after 2 days for KCl and 3 days for $CaCl_2$; growth was at 23 C on V-8 agar in 90-mm diameter petri dishes.
† Water potentials were estimated psychrometrically.

until Ψ were less than –20 to –30 bars. Linear growth, however, appeared more sensitive to reduced water potentials than was germination (Table 7). Growth was reduced significantly by Ψ below –15 bars, and greatly reduced below –20 bars.

Sporangial development in culture was not greatly affected by the range of Ψ found to limit *P. ultimum* development in soil. Using KCl as the osmoticum, Ψ below –25 to –30 bars were required before reductions in sporangial or oogonial formation could be detected on preformed mycelial mats. Complete inhibition of sporangial development was not noted until Ψ were below –35 bars.

TABLE 8
INFLUENCE OF SOIL TEMPERATURE ON DEVELOPMENT OF SPORANGIA IN COTTON LEAVES*

	Days after incorporation in soil			
	13 replicates		23 replicates	
Degrees, C	a	b	a	b
	(sporangia/g)			
2–4†	0	0	0	0
9–11	642	917	1317	1608
15–17	3800	2650	4216	3416
20–22	2067	1783	3100	1650
26–28	167	150	33	167
31–33	0	0	0	0

* Cotton leaves (0.5 g) were incorporated into a sandy loam soil (150 g), moistened, and held in a constant-moisture apparatus at matric potentials of –.2 to –.3 bars.
† In separate tests, soils incubated at 6–7 C yielded sporangial levels ranging from 260/g to less than 1/g (incubated 10 days).

Influence of temperature on the development of Pythium ultimum

Pythium ultimum population increases in cotton leaves in soil held at constant temperatures were greatest between 15 and 22 C (Table 8). Population levels were lower in soils held at 26 to 28 C, and no *Pythium* development was detected at 31 to 33 C. Although temperatures of 2 to 4 C suppressed increases in *Pythium* populations, levels did increase slowly at 9 to 11 C.

Sporangial germination with isolate 67-1 was optimal between 20 and 30 C on V-8 agar. Germination was significantly reduced above 33 C and below 18 C.

Linear growth of *P. ultimum* isolates from the San Joaquin Valley was greatest from 23 to 30 C, with no growth at 36 C. Growth rates declined sharply below 20 C and were very low at 10 C. No growth was detected after 72 h at 5 C. A nearly identical growth pattern was evident in three *P. ultimum* isolates from decaying cotton seeds, as well as in three isolates obtained by direct isolation during population estimations by the soil-drop technique.

Sporangial formation was greatest on mycelia held between 20 and 30 C. Sporangia did not form at 36 C, and their formation was reduced below 20 C. Although it was delayed a few days, sporangial formation occurred at 3 C.

Survival of P. ultimum *in soil*

Levels of propagules in dry soil infested with sporangia from culture decreased more rapidly than did propagules formed naturally in cotton leaves (Table 9). However, regardless of the source of propagules, population levels declined rapidly at 32 C. In contrast, propagule populations were relatively stable at 16 C under dry conditions if the propagules had been formed in cotton leaves.

TABLE 9
INFLUENCE OF TEMPERATURE ON SURVIVAL OF *PYTHIUM* IN DRY SOIL*

	Sporangia per g			
	Culture†		Leaves†	
Weeks	16 C	32 C	16 C	32 C
0	1658 (4)‡		467 (6)	
4	504 (8.5)	46 (1.0)	271 (7.0)	237 (3.7)
10	266 (4.6)	12 (1.0)	420 (5.5)	79 (3.6)
14	420 (3.5)	0 (1.1)	399 (4.2)	0 (0.7)
22	96 (0.7)	0 (0.5)	179 (1.0)	0 (0.7)
34	96 (1.3)	0 (0.4)	288 (1.0)	0 (0.4)

* Soil type was Hanford sandy loam.
† Culture-grown mycelia and sporangia were incorporated into soil, or *Pythium* populations were raised by incorporating cotton leaves into moist soil (0.5 g dry leaves per 150 g soil) and incubating at 21 C for 1 week. *Pythium ultimum* populations were estimated 7 days after mycelia and sporangia and leaves were introduced into soil and thereafter at various times up to 34 weeks.
‡ Initial sporangia population. Percentage moisture is included in parentheses.

TABLE 10
INFLUENCE OF MOISTURE ON SURVIVAL OF *PYTHIUM ULTIMUM* IN SOIL*

	Dry		Moist	
Weeks	Sporangia/g	MC†	Sporangia/g	MC
0	129	13.0	129	13.0
2	283	0.3	233	14.0
4	299	0.2	254	12.0
7	258	0.2	178	12.0
10	220	0.4	120	14.0

* *Pythium ultimum* populations were raised by incorporating cotton leaves into Hanford sandy loam soils (12% moisture content) and incubating for 1 week at 21 C. Soils were held at 32 C during the experiment.
† MC = percentage moisture content.

With propagules formed in cotton leaves, population declines at 32 C were more apparent under moist soil conditions than under dry ones (Table 10). In one experiment, the half-life($t_{1/2}$) for propagule survival at 32 C was 95 days under dry conditions and 70 days under moist conditions.

Pathogenicity of Pythium ultimum *isolates retrieved directly from soil*

The pathogenicity of different *P. ultimum* isolates retrieved from agar plates during quantitative soil assays was tested and compared with that of isolates from naturally infected cotton seeds. Tests were done with untreated cotton seeds (Acala SJ-2) in a mixture of non-sterile clay soil and coarse sand (1:2 v/v). Inocula (mycelial mats with sporangia) were mixed with moistened soils 1 week prior to tests. Soils were mixed thoroughly after the 1-week incubation, and sporangial levels were estimated by the soil-drop technique. Final inoculum levels were adjusted to between 150 to 300 sporangia/g by dilutions with noninfested soil. Tests were run in the greenhouse at 23 ± 5 C.

The 10 *P. ultimum* soil isolates tested were all highly pathogenic, causing preemergence damping-off. There appeared to be little difference between isolates retrieved by the soil-drop or by the wet-sieving procedures, or between soil isolates and isolates taken directly from diseased cotton seeds collected in the San Joaquin Valley.

DISCUSSION

Seasonal population fluctuations noted in this study indicate that environmental factors have a strong influence on the behavior of *P. ultimum*. The low population levels in the late summer months appear to be related to

the high soil temperatures that range between 30 and 38 C during July and August in the San Joaquin Valley. Since *P. ultimum* does not reproduce in cotton leaves in soil above 30 C, and sporangia in cotton leaves do not survive well in soils held at 32 C, soil temperatures alone may account for the low levels of *P. ultimum* in soil samples collected during the late summer months.

Seasonal population changes encountered in this study could also reflect independent changes in the germinability of different survival structures. *Pythium ultimum* produces mycelia, sporangia, and oospores in organic residues (Watson, 1971). Mycelia may be produced in relative abundance in response to large supplies of nutrients (e.g., seed exudates) but are short lived (<24 h), and thus unlikely to contribute to propagule counts (Agnihotri and Vaartaja, 1967). Where increases of *Pythium* counts closely followed organic amendments (in the field or in the laboratory), it is likely that the propagules were largely sporangia. Oospore-ripening does not occur appreciably until 4 to 6 weeks after the spores are produced, even under optimal environmental conditions (Lumsden and Ayers, 1975).

Field data may provide a clue to the contributions of oospore-ripening to increases in population levels. For example, following cotton in 1972, sites 1 and 7 remained fallow during the following 12 months, thus avoiding the introduction of new organic matter during the next growing season. A small increase in *P. ultimum* occurred in site 1 during November and December, 1973. It is possible that this increase was a result of oospore-ripening. However, no increase in *Pythium* was noted in site 7 during the same period. Differences in results in this case could reflect differences in the original fungal populations in organic residues. In 1972, concentrations of sporangia were considerably higher in site 1 than in 7. Given this uncertainty, the contributions of oospore-ripening must still be regarded as unknown.

In contrast to the behavior of *P. ultimum* in the San Joaquin Valley, no general decline in soil populations occurs in the Salinas Valley during the summer. In fact, Watson (1971) noted marked increases in the soil population of *P. ultimum* during July. Soil populations in the Salinas area occasionally exceeded 3,000 propagules per g, levels approximately 10-fold greater than the highest levels encountered in the San Joaquin Valley over a 5-year period.

The Salinas Valley is considerably cooler than the San Joaquin Valley during midsummer, which may explain some of the differences in results between the two regions (Table 11). The use of cover crops as green manure in Watson's study area may also have contributed to the differences. However, rises in propagule levels in midsummer

TABLE 11

SOIL TEMPERATURES IN THE SALINAS AND SAN JOAQUIN VALLEYS IN 1974

	Salinas*		San Joaquin†	
Month	Minimum C	Maximum C	Minimum C	Maximum C
March	8	14	9	17
April	10	18	15	22
May	14	21	22	28
June	16	25	30	35
July	20	25	32	37
August	20	25	30	37

* Temperatures recorded at 17 cm at the U.S. Department of Agriculture Experimental Station, Salinas, California, by D. Danesh.

† Temperatures recorded at 15 cm at the University of California West Side Field Station, Five Points, California.

were not associated with an immediate incorporation of organic substrates into soils (Watson, 1971).

With the incorporation of a green cover crop (i.e., barley) into agricultural soils in March in the Salinas Valley, Watson (1971) found a sudden rise of *P. ultimum* population levels. He suggested that the subsequent summer peak reflected a conversion of fungal biomass, formed in the spring, to "isolatable propagules." Since the mycelia of *P. ultimum* is ephemeral in soil, this explanation seems unlikely. However, oospore-ripening could explain Watson's (1973) results (Ayers and Lumsden, 1975; Lumsden and Ayers, 1975).

Differences in *P. ultimum* seasonal population patterns between the San Joaquin and Salinas valleys can be ascribed to differences both in climate and agricultural practices. Temperatures are not favorable for *P. ultimum* in the San Joaquin Valley during the summer months. Moreover, cover crops are not commonly used in commercial agriculture in the west side of the San Joaquin Valley; hence, the incorporation of organic matter into soils does not occur in the early spring when *Pythium* development could occur. Incorporation of crop residues into soil in the autumn supports population increases, providing moisture is available and the substrate is suitable. Because these environmental conditions do not always coincide, *P. ultimum* populations may remain low over several seasons in many agricultural soils in the San Joaquin Valley.

Soil temperatures recorded at the West Side Field Station ranged from 10 to 28 C from mid-October to mid-November, a range favorable to *P. ultimum* development. The limiting factor during this period in cotton fields may be moisture. When it rained in early November in 1972, population levels of *P. ultimum* increased at most field sites. In subsequent years it was drier during this period, and a general increase in the *P. ultimum* population did not occur.

In conflict with the population trends noted throughout this study, population levels increased in one cotton field in late summer where significant "natural" defoliation had taken place, and moisture was available. *Pythium ultimum* populations increased at site 1 in September, 1974, where defoliation was induced by verticillium wilt in late August, and the field had received a late irrigation. This result indicates that more attention should be directed toward the microclimate at the soil surface during leaf colonization.

Pythium populations declined sharply in most fields during the winter of 1973, with sporangial counts reaching moderate to low levels by March and April. A relatively rapid decline in soil populations of *P. ultimum* also occurred in subsequent years after soil populations had increased in response to cotton defoliation. This decline could not be linked with agricultural practices (e.g., discing).

Although these results are not definitive, *P. ultimum* propagule levels remained higher for a longer period after barley than after cotton. The relationship between the type of organic substrate and longevity of *P. ultimum* is unknown. Organic substrates may aid in the survival of associated propagules, as found in comparisons between the longevity of culture-produced propagules and those formed in cotton leaves.

Pythium development in cotton leaves in soil was insignificant below a Ψ of ca. –10 bars. However, in pure culture, sporangial germination and formation were not influenced greatly by Ψ values until they dropped below –20 bars. The sensitivity of hyphal growth to moisture stress may negatively affect the competitive ability of *P. ultimum* in nature.

Although results cited by Cook and Papendick (1972) indicate otherwise, considerable caution should be exer-

cised when comparing culture studies with field behavior. In culture, sporangial germination and formation and hyphal growth were optimal between 22 and 30 C; in soil, *P. ultimum* population increases in cotton leaves were the greatest between 15 and 22 C. It appears that optimal environmental conditions for *P. ultimum* are different in nature than in culture. Increased microbial competition at high temperatures, and interactions with other physical soil factors, may contribute to the differences in behavior of *P. ultimum* in soil and in culture.

One of the more interesting aspects of this study was the observation that *P. ultimum* populations remained low in certain field sites while fluctuating between high and low levels in others. Contrasts were most striking, for example, when comparing sites 2 and 3 with sites 4 and 9. The different seasonal fluctuation patterns appeared to be characteristic of the different sites. Yet, the cause of these specific site patterns is not obvious. *Pythium ultimum* was present in all sites and, with only a few exceptions, cropping sequences and cultural practices were generally similar.

There was no significant relationship apparent between soil types and patterns in *Pythium* population fluctuations in the different field sites. However, population changes appear to be controlled by soil factor(s). Laboratory tests revealed that *P. ultimum* developed well when cotton leaves were incorporated into soils which were "highly responsive" (i.e., sites 4 and 9) under field conditions, but developed poorly in soils that were characteristically "unresponsive" (i.e., sites 2 and 3) in the field.

The relationship between "suppressive" and "nonsuppressive" soils, reported for several soil-borne diseases (Toussoun, 1975), and the results in the present study, need clarification. Implications for biological control are evident, and the basis for differences among soils in their ability to support *P. ultimum* is worthy of future investigations.

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