Saprophytic Development of *Pythium ultimum* in Soil as a Function of Water Matric Potential and Temperature

R. Lifshitz and J. G. Hancock

Graduate research assistant and professor, respectively, Department of Plant Pathology, University of California, Berkeley 94720.
We gratefully acknowledge the advice of R. W. Schneider during the course of this study.
Accepted for publication 12 July 1982.

ABSTRACT

Lifshitz, R., and Hancock, J. G. 1983. Saprophytic development of *Pythium ultimum* in soil as a function of water matric potential and temperature. Phytopathology 73:257-261.

The population densities of *Pythium ultimum* increased markedly in natural soils amended, under laboratory conditions, with fragments (about 1 mm^2) of mature (green) dried cotton leaves. The population density increases during a 5-day incubation period were related directly to initial population densities of up to 100 propagules per gram of soil at 21 C. Population density increases were also directly related to soil water matric potentials between −5 and −0.25 bar at 16, 21, and 27 C. Population densities of *P. ultimum* were not significantly increased in water-saturated soils or when the matric potential was −10 bars or lower. In most of the natural soils, the optimal temperature for saprophytic development was lower than 27 C. When soils were previously sterilized, however, it was between 27 and 30 C, and this was similar to the range of temperatures at which maximum linear growth occurred in culture. Antagonistic microflora may play a key role in limiting the activities of *P. ultimum* in raw soil at temperatures between 21 and 30 C and thus may shift the optimum for growth and development to less than 27 C.

Pythium ultimum Trow is a widespread soilborne pathogen in temperate climates, where it may cause pre- and postemergence damping-off and root rot in many economically important plants. Population densities of the fungus are increased either after infection of juvenile plant tissues or by saprophytic utilization of fresh plant residues introduced into the soil (4). Stanghellini (13) suggested that exogenously dormant propagules (sporangia and ripened oospores) are the principal functional inocula of *P. ultimum*. In studying population dynamics of *P. ultimum* in cultivated field soils, Watson (16) and Hancock (6) both observed that major inocula increases were associated closely with the introduction of fresh crop residues into the soil following harvest. In this work, we studied the saprophytic development of *P. ultimum* in soil and how it is affected by water matric potential, temperature, and other soil microorganisms.

MATERIALS AND METHODS

Soil samples taken from the tillage layer (0–15 cm deep) were air-dried, ground, and sieved through a 1-mm^2 mesh screen. Soils from eight different sites in California were used: Panoche sandy loam (from West Side Field Station [WSFS], pH 7.8), Oxalis clay (from Boston, pH 7.7), Hesperia fine sandy loam (from Shafter, pH 7.2), Delano loamy sand (from Bidart, pH 7.0), Lethent clay loam (from Stone, pH 7.9), Oxalis clay (from Britz, pH 7.7), Zamora clay loam (from Davis, pH 6.9), and Lethent silty clay loam (from Boston No. 21, pH 7.8).

In adjusting water matric potential, soils were held within a ceramic plate extractor (Soil Moisture Equipment Co., Santa Barbara, CA 93105) under several pressures at 24 C for 15 hr.

Soil populations of microorganisms were determined according to Aaronson's plate dilution method (1). Soil extract agar (2) was used to estimate population densities of bacteria and actinomycetes. A rose bengal medium (11) was used to estimate populations of soilborne fungi. Soil-extract and rose-bengal-medium plates were incubated at 24 C for 4 or 14 days, respectively. Densities of exogenously dormant propagules of *P. ultimum* were measured by the soil-drop method (14).

Air-dried soil samples were sealed within polyethylene bags for sterilization treatments. The samples were then exposed to 6.0 Mrad of gamma radiation (unless mentioned otherwise) in a 3,000-Ci cobalt irradiator at the Lawrence Berkeley Laboratory, Berkeley, CA. This sterilized them completely (determined by lack of growth from soil crumbs in nutrient medium).

For studies on the saprophytic development of *P. ultimum* in soil, cultures of the fungus (ATCC 32939) were grown in dishes containing rolled oat agar and water (6) at 22–24 C, under diurnal conditions, for 17 days. The mycelial mats were then removed and placed in Panoche sandy loam soil. This infested soil was saturated and then air-dried at 22–24 C for 48 hr. It was ground and sieved through a 1.0-mm^2 mesh screen, mixed thoroughly, and stored at 12 C. The propagule density was measured 24 hr before the inoculum was used in the experiments that measured saprophytic development. Densities were adjusted to 50 propagules per gram of air-dried soil by diluting (1:200, w/w) the infested soil with noninfested field soil and mixing thoroughly with a twin-shell blender (Patterson Kelley Co., East Stroudsburg, PA). Dry, mature green cotton leaves were crushed, sieved to final sizes ranging between 0.8 and 1.0 mm^2, and incorporated into soils (0.3 g of leaves in 100 g of air-dried soil). Moistened soils were placed within petri dishes (60 mm in diameter) and covered by transparent polyethylene (2 mil) to reduce evaporation. The incubation was at different temperatures for 1–5 days. The subsequent development of *P. ultimum* was measured in two ways: the soil-drop method (14) and a leaf colonization assay (F. N. Martin, *unpublished*) that yields the proportion of leaf fragments colonized by *P. ultimum*.

The population buildup assays summarize the overall saprophytic cycle beginning with germination of resting structures and proceeding through vegetative growth, colonization of substrate, and finally the formation of new propagules. The colonization assay mostly reflects the vegetative colonization phase that precedes reproduction.

RESULTS

Quantitative assessment of increases in population densities of *P. ultimum* in different soils. The relationship between initial and final population densities of *P. ultimum*, after a 5-day incubation period, was determined for cultivated field soils taken from six different sites. The initial population densities were adjusted to 0, 5, 25, 50, and 100 propagules per g of soil using the air-dried, artificially infested Panoche sandy loam. The water matric potential was adjusted to −1.2 bar and the temperature of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

incubation was 21 C. The population densities measured at the end of the experiment were positively correlated with the initial population densities in all of the soils used, but no significant differences were found among the linear regression slope values for different soils (7.91–13.12) (Table 1).

Population buildup at different water matric potential-temperature combinations. Four experiments were conducted in four different soil types; their moisture-release curves are shown in Fig. 1. In each experiment, soil samples (infested with *P. ultimum* and amended with leaf fragments) were held within petri dishes with soil water matric potentials adjusted to −0.25, −0.5, −1, −3, −5, −10, and −15 bars. Saturated soils were considered to possess a matric potential of 0 bar. The dishes were incubated at 16, 21, 27, and 32 C (±1 C) for 5 days before population densities of *P. ultimum* were measured. Each plate was treated as a replicate, and three replicates were used for each treatment.

Population densities at the end of incubation are shown in Table 2. At temperatures between 16 and 27 C, the highest population buildup of *P. ultimum* was at matric-potential levels of −0.25 and −0.5 bar. On the other hand, at all temperatures, no significant increases in population densities were found when soils were completely saturated or at matric potential levels of −10 and −15 bars. Temperatures that promoted the highest increases were 16 and 21 C regardless of water potentials. Buildup was generally lower at 27 C and lowest at 32 C. Among the different soil types, the increase of *P. ultimum* in the Oxalis clay soil (Boston) was greater than that in the other soil types whenever a detectable population buildup was observed.

Comparisons of saprophytic development of *P. ultimum* in sterilized and nonsterilized soil. Population increases in three different soils (WSFS, Davis, and Boston No. 21) were investigated over a temperature range of 12–33 C with a matric potential of −1 bar. Subsequent population densities after 5 days of incubation are shown in Fig. 2. The optimal temperatures for buildup of *P. ultimum* in sterilized soil reinfested with *P. ultimum* were always 27–30 C, but in nonsterilized soil the optima were at 18–21 C (WSFS and Davis) and 27–30 C (Boston No. 21). Although differences in inoculum increases over 5 days between nonsterile and sterile soil were small at 12–18 C, they greatly increased at the higher temperatures. In nonsterilized Davis and WSFS soil samples, an inverse relationship between buildup and temperature was observed between 21 and 30 C, whereas in sterilized soil, fungus buildup continued to increase. At 33 C, population increases over 5 days (above the initial density of 50 propagules per gram of soil) were not significant in either sterilized or nonsterilized soil.

An experiment on leaf colonization at different temperatures was performed. It was similar to the one above but the soil (WSFS) was incubated for 3 days (sterile soil) and 1 or 3 days (nonsterilized soil). At the end of incubation, the fraction of fragments colonized by *P. ultimum* was determined by measuring the incidence of growth of *P. ultimum* from leaf fragments on water agar. Three

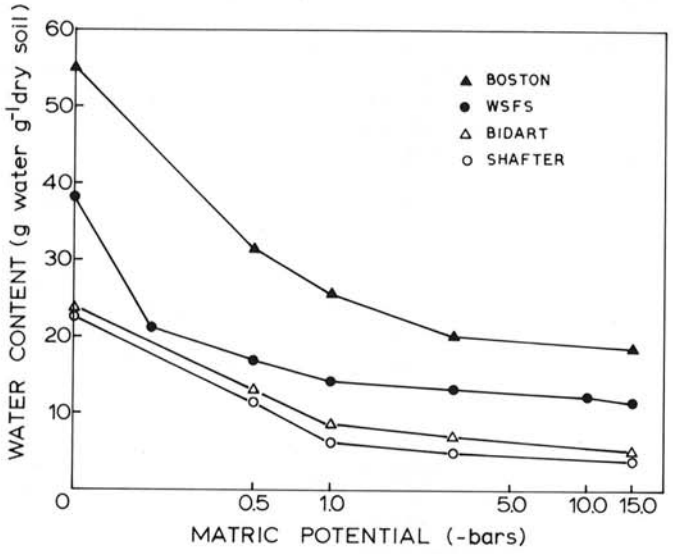

Fig. 1. Moisture-release curves of the soils: Panoche sandy loam (from West Side Field Station), Oxalis clay (from Boston), Hesparia fine sandy loam (from Shafter), and Delano loamy sand (from Bidart).

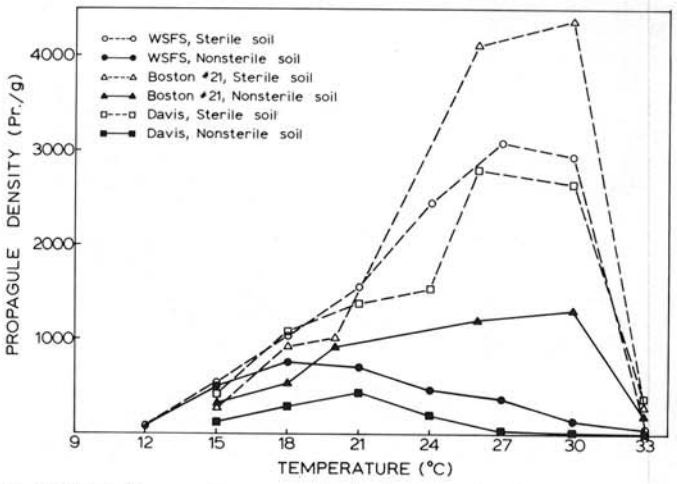

Fig. 2. Effect of temperature on population increase of *Pythium ultimum* in soil amended with cotton leaves. Density values are based on three replicates. The matric potential was adjusted to −1.0 ± 0.1 bar.

TABLE 1. Relationship between initial and final propagule densities of *Pythium ultimum* in field soils amended with cotton leaves after 5 days of incubation under laboratory conditions[a]

Soil site	Soil type	Final propagule densities based on initial population densities (propagules per gram)[b] of 0	5	25	50	100	Regression slope value	r^2
Boston	Oxalis clay	nd[c]	50	450	641	1,200	13.12	0.921
Britz	Oxalis clay	nd	25	492	891	1,367	12.78	0.966
West Side Field Station	Panoche sandy loam	nd	48	200	633	900	9.08	0.912
Shafter	Hesperia sandy loam	nd	64	460	342	920	8.96	0.888
Bidart	Delano loamy sand	nd	32	300	780	760	8.89	0.753
Stone	Lethent clay loam	nd	125	360	830	830	7.91	0.945

[a] Water potential was adjusted to −1.2 ± 0.1 bar and temperature was 21 ± 0.05 C.
[b] Initial population densities were adjusted by diluting the inoculum preparation with noninfested soil.
[c] nd = *P. ultimum* not detectable.

TABLE 2. The effect of water matric potential and temperature on increases in population densities (propagules per gram) of *Pythium ultimum* in different soils amended with cotton leaves[a]

Site	Temperature (C)	Matric potential (bars) 0	−0.25	−0.5	−1	−3	−5	−10	−15
West Side Field Station	16	8 hi[b]	1,400 ab	1,000 bc	1,200 b	500 def	370 efgh	32 hi	25 hi
	21	42 ghi	1,200 b	700 cde	1,030 bc	1,200 b	490 defg	nd[c] i	25 hi
	27	25 hi	670 cde	230 efghi	400 defg	530 def	280 efghi	nd i	nd i
	32	8 hi	nd i	60 ghi	200 ghi	130 fghi	23 hi	nd i	nd i
Shafter	16	50 ghi	860 bcd	900 bc	800 cde	300 efghi	130 fghi	25 hi	92 ghi
	21	8 hi	900 bc	460 defg	300 efghi	60 ghi	200 ghi	92 ghi	100 ghi
	27	17 hi	670 cde	430 defg	360 efgh	130 fghi	130 fghi	nd i	nd i
	32	25 hi	42 hi	200 ghi	330 efgh	30 hi	nd i	nd i	nd i
Boston	16	32 hi	2,200 a	1,360 ab	1,600 ab	1,000 bc	460 defg	25 hi	167 fghi
	21	183 fghi	1,860 a	1,800 a	960 bc	660 cde	330 efgh	67 ghi	50 ghi
	27	50 ghi	1,900 a	1,400 ab	1,800 a	700 cde	92 ghi	nd i	nd i
	32	42 hi	58 ghi	160 fghi	400 defg	460 defg	25 hi	nd i	nd i
Bidart	16	42 hi	630 de	830 bcd	600 de	160 fghi	160 fghi	nd i	8 hi
	21	58 ghi	700 cde	430 defg	560 def	330 efgh	50 ghi	nd i	33 hi
	27	92 ghi	230 efghi	360 efgh	430 defg	260 efghi	8 hi	8 hi	nd i
	32	25 hi	nd i	30 hi	30 hi	nd i	25 hi	nd i	nd i

[a] The initial population density of *Pythium ultimum* was adjusted to 50 propagules per gram of soil.
[b] Values followed by different letters are significantly different at $P = 0.05$, according to Duncan's multiple range test.
[c] nd = *P. ultimum* not detectable.

TABLE 3. The effect of matric potential, temperature, and soil sterilization on population increases of *Pythium ultimum* in soil[a]

Sterilization[b]	Temperature	Matric potential (bar) −0.2	−0.5	−1.0
−	18	1,350 y[c]	960 y	633 xy
+	18	1,400 y	1,250 y	1,300 y
−	27	380 x	320 x	125 x
+	27	1,800 yz	2,450 z	2,880 z

[a] Initial population density of *Pythium ultimum* was adjusted to 50 propagules per gram of soil.
[b] Gamma irradiation of 4 Mrad.
[c] Values followed by different letters are significantly different at $P = 0.05$, according to Duncan's multiple range test.

replicates were used for each treatment. Maximal incidence of leaf colonization in nonsterilized soil after 3 days of incubation increased at 18 C (Fig. 3). At higher temperatures, incidences of colonization were lower. When nonsterilized soil was incubated for 1 day only, incidences of colonization were lower than those found after 3 days of incubation at the same temperatures, but the optimum was between 21 and 30 C. In sterilized soil, on the other hand, maximum colonization (almost 100% at 3 days) occurred between 21 and 30 C. No fragments were colonized by *P. ultimum* at 33 C in either sterilized or nonsterilized soil.

The influence of both temperature and matric potential on leaf colonization in sterilized soil was examined. Six combinations of matric potentials (−0.2, −0.5, or −1 bar) and temperatures (18 or 27 C) revealed a similar pattern of population changes, in which final population densities in sterilized soils were greater than those in unsterilized soil at 27 C (Table 3). However, no significant difference in the increase of population densities was found when incubation was at 18 C. The ratio of population densities between sterilized and nonsterilized soils tended to increase as the temperature increased and as the matric potential decreased (eg, 1.04 at 18 C and −0.2 bar and 23.4 at 27 C and −1 bar).

Effect of selective gamma irradiation and temperature on incidence of leaf colonization and inoculum buildup. The effect of increasing radiation dosage on the survival of fungi, bacteria, actinomycetes, and *P. ultimum* population densities in WSFS soil was similar with each group of microorganisms (Fig. 4). When saprophytic development of *P. ultimum* was tested, incidence of

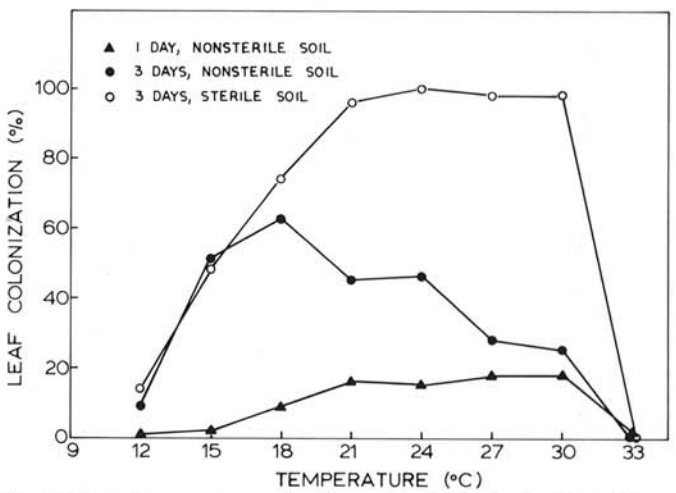

Fig. 3. Effect of temperature on incidence of leaf colonization by *Pythium ultimum* in soil. Percentages are based on three counts, 50 leaf fragments per count. The matric potential was adjusted to -1.0 ± 0.1 bar.

colonization and population density both increased dramatically in irradiated soil, and this effect was more pronounced at 27 C than at 18 C (Table 4).

DISCUSSION

Positive correlations between initial and final population densities of *P. ultimum* (0–100 propagules per gram of soil) were shown in several soil types amended with dried cotton leaves. Values of the linear regression slopes may reflect the conduciveness of a soil to fungal saprophytic development and may be useful in surveying and characterizing agricultural soils for pathogen-suppressive qualities and in testing the influence of physical and chemical treatments of soils on pathogen behavior. The conduciveness values of the different soil types, as measured in this work, further demonstrate the unusual saprophytic ability of *P. ultimum*.

Our study shows that *P. ultimum* could increase its population densities in soils at matric potentials as low as −5 bars, which is consistent with an earlier report that indicated population increases

would occur as low as −8 bars (6). Excepting water-saturated soils, populations of *P. ultimum* increased at 16, 21, and 27 C in direct relation to increases in the value of matric potential (Table 2). Unlike other pythiaceous species, *P. ultimum* does not produce zoospores readily; therefore, it has no apparent benefit from dispersal when soil moisture is high. However, the fungus may benefit from high water potentials for at least three other reasons: energetic, nutritional, and ecologic. High water potential literally means that little energy is needed for uptake of water from soil. Matric potential also affects the availability of soluble nutrients in the soil. However, there is apparently not always a simple relationship between soil water potential and diffusion of nutrients in soil. For example, Kerr (9) noted that pathogenic activity of *P. ultimum* was greater in loam soil than in sandy loam or sandy soil at similar matric potentials. He also found that pathogenic activity was greater in loam and sandy loam soil than in sandy soil at similar water content levels. His study indicated that the importance of soil moisture is in its influence on the amount of sugar exuded from seeds and that this determines disease incidence. Similarly, Sterne et al (15) observed greater pathogenic activity of *Phytophthora cinnamomi* in clay soil than in sandy soil at equal matric potentials. They also indicated that nutritional availability, rather than matric potential per se, limited pathogenic activity of the fungus in soils with low moisture levels. Tables 1 and 2 in our study show greater saprophytic activity of *P. ultimum* in clay soil than in sandy loam or sandy soils at similar matric potentials. This effect could result from differences in the availability of nutrients in these soils. High matric potential conditions could also provide an ecological advantage to *P. ultimum*. Griffin (5) suggested that *P. ultimum* benefits from high soil moisture because it is more tolerant of poor gas exchange than its antagonistic fungi. Kouyeas (10) showed that *Pythium* species were most active in colonizing plant tissue in soil at −0.4 to −1.0 bar, whereas other genera of soil fungi, such as *Penicillium, Aspergillus*, and *Trichoderma*, predominated at −3.8 bars.

A clear disparity was seen between sterile and nonsterile soils in the relationship of soil temperature and saprophytic increases in inoculum densities of *P. ultimum*. We found that temperature-inoculum buildup profiles were similar after soils from three locations were sterilized and that increases in inoculum densities were always lower in nonsterilized than in sterilized portions of these soils. On the basis of other studies on soil microbes, these results were not unexpected (4). But, of particular interest was the finding that the temperature optima for inoculum buildup in nonsterile soils from two sites (WSFS and Davis) were regularly lower than in the sterile soils, whereas the nonsterile soil from a third site (Boston No. 21) had an optimum temperature similar to that for sterile soil. These results show that not only is the buildup of *P. ultimum* repressed in nonsterile soil but that a shift in the temperature optimum may occur. This indicates that soil factors changed by sterilization can significantly alter the behavior of *P. ultimum*.

Temperature optima for inoculum buildup of *P. ultimum* in sterile soils and one nonsterile soil were similar to those reported for linear growth rates and reproduction in culture (6,12). However, the lower temperature optima found in the two nonsterile soils were similar to those of a previous study, which found that saprophytic population increases in a nonsterile soil (Hanford sandy loam) were optimal at 15–17 C (6). Interest in these temperature relations was heightened because these latter results were consistent with the findings of field experimentation, which indicated that inoculum densities of *P. ultimum* increased mainly during the cooler months in the Central Valley of California.

We conclude that *P. ultimum* has the potential to grow and reproduce in organic matter in soil at warm temperatures but that this behavior is frequently modified in natural soil. Barton (3) showed a similar environmental disparity with *Pythium mamillatum*; maximal colonization of organic matter took place at 10 C, whereas optimal temperature for linear growth was 28 C. Barton (3) suggested that "the decreased colonization at the higher temperatures can be best explained on the basis of a 'microbiological factor.'" Such a "biological factor" could account for the results found when *P. ultimum* development was followed in gamma-irradiated soil. However, in this work, different dosages of radiation did not suggest which microbial group was responsible for the shift in temperature optima for *P. ultimum* development in natural soil. The optimal temperature for leaf colonization and population increases in treated soil (WSFS) was "shifted" toward higher temperatures (27–30 C) as the soil microflora was drastically reduced or totally eliminated. The "biological factor" was not effective against leaf colonization in natural soil during the first day of incubation; this "lag period" suggests that at least 24 hr is required before biological antagonism reaches its greatest activity.

P. ultimum is a successful pioneer colonist because of its ability to germinate and extend its germ tubes rapidly, thus avoiding competition. Species of *Pythium* are generally considered poor competitors in soil because their saprophytic activity is greatly restricted due to the activity of antagonistic microflora in soil (3,7). Katan and Lockwood (8) showed that when pentachloronitrobenzene (a fungicide that is not toxic to *Pythium* spp.) was introduced into soil, the saprophytic activity of *P. ultimum* was enhanced, apparently because of the inhibition of antagonistic fungi and actinomycetes. Our findings suggest that the activities of antagonistic microorganisms are the key factors limiting the saprophytic development of *P. ultimum* in natural soil at temperatures between 21 and 30 C and may also restrict the pathogenic activities of *P. ultimum* to more moderate or cooler temperatures.

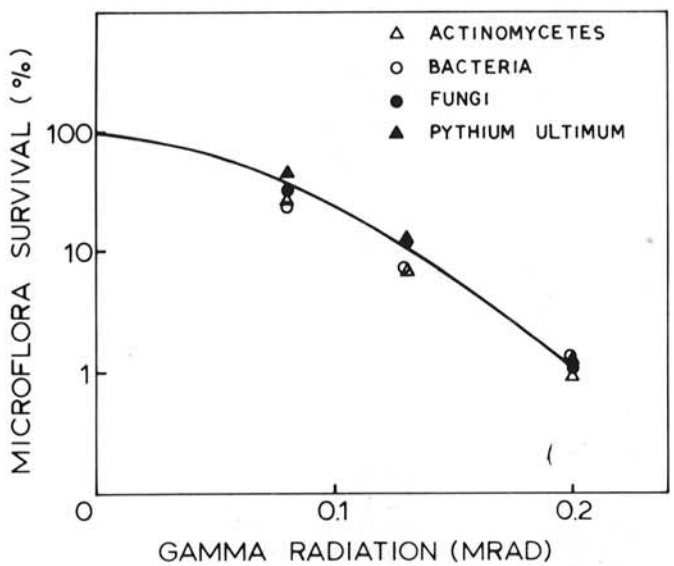

Fig. 4. Microflora survival in gamma-irradiated soil (100% = 2.5×10^4 colony-forming units (cfu) of fungi, 1.2×10^4 cfu of *Pythium ultimum*, 8.2×10^6 cfu of bacteria, and 1.4×10^6 cfu of Actinomycetes per gram of soil).

TABLE 4. The effect of gamma irradiation and temperature on the incidence of leaf colonization and population increase of *Pythium ultimum* in reinfested soil[a]

	Temperature (C)			
	18		27	
Gamma irradiation (krad)	Colonization (%)	Population (propagules per gram)	Colonization (%)	Population (propagules per gram)
0	52 v[b]	880 y	12 v	300 x
35	94 w	1,080 y	78.5 w	960 y
100	100 w	1,220 y	96 w	2,500 z
400	96 w	1,400 y	100 w	3,150 z

[a] Initial population density of *Pythium ultimum* was adjusted to 50 propagules per gram of soil (Panoche sandy loam from West Side Field Station) and water matric potential was adjusted to -0.5 ± 0.1 bar.

[b] Values followed by different letters are significantly different at $P = 0.05$, according to Duncan's multiple range test.

LITERATURE CITED

1. Aaronson, S., ed. 1970. Experimental Microbial Ecology. Academic Press, New York. 236 pp.
2. Allen, O. N. 1957. Experiments in Soil Bacteriology, 3rd ed. Burgess Publishing Co., Minneapolis, MN. 117 pp.
3. Barton, R. 1961. Saprophytic activity of *Pythium mamillatum* in soils. II. Factors restricting *P. mamillatum* to pioneer colonization of substrates. Trans. Br. Mycol. Soc. 44:105-118.
4. Garrett, S. D. 1956. Biology of Root-Infecting Fungi. Cambridge Univ. Press, New York. 293 pp.
5. Griffin, D. M. 1963. Soil physical factors and the ecology of fungi. II. Behavior of *Pythium ultimum* at small water suctions. Trans. Br. Mycol. Soc. 46:368-372.
6. Hancock, J. G. 1977. Factors affecting soil populations of *Pythium ultimum* in the San Joaquin Valley of California. Hilgardia 45:107-122.
7. Hendrix, F. F., Jr., and Campbell, W. A. 1973. Pythiums as plant pathogens. Annu. Rev. Phytopathol. 11:77-98.
8. Katan, J., and Lockwood, J. L. 1970. Effect of pentachloronitrobenzene on colonization of alfalfa residues by fungi and streptomycetes in soil. Phytopathology 60:1578-1582.
9. Kerr, A. 1964. The influence of soil moisture on infection of peas by *Pythium ultimum*. Aust. J. Biol. Sci. 17:676-685.
10. Kouyeas, V. 1964. An approach to the study of moisture relations of soil fungi. Plant Soil 20:351-363.
11. Martin, J. P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215-232.
12. Middleton, J. T. 1942. The taxonomy, host range and geographic distribution of the genus *Pythium*. Mem. Torrey Bot. Club 20:1-171.
13. Stanghellini, M. E. 1974. Spore germination, growth and survival of *Pythium* in soil. Proc. Am. Phytopathol. Soc. 1:200-223.
14. Stanghellini, M. E., and Hancock, J. G. 1970. A quantitative method for the isolation of *Pythium ultimum* from soil. Phytopathology 60:551-552.
15. Sterne, R. E., Zentmyer, G. A., and Kaufmann, M. R. 1977. The influence of matric potential, soil texture, and soil amendment on root disease caused by *Phytophthora cinnamomi*. Phytopathology 67:1495-1500.
16. Watson, A. G. 1971. The effect of decomposing green crop residues on lettuce injury in the Salinas Valley. Ph.D. thesis. Univ. Calif., Berkeley. 273 pp.