Effect of Soil Temperature and Soil-Water Matric Potential on the Survival of *Phytophthora capsici* in Natural Soil

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ABSTRACT

Bowers, J. H., Papavizas, G. C., and Johnston, S. A. 1990. Effect of soil temperature and soil-water matric potential on the survival of *Phytophthora capsici* in natural soil. Plant Dis. 74:771-777.

Field tests were established during the summer of 1982 and the winter of 1982–1983 to determine the ability of sporangia, zoospores, and oospores of *Phytophthora capsici* to survive at two depths, 4–6 and 20–22 cm. Average soil moisture and temperature were recorded daily. During the summer tests in a bioassay using soil sampled at 0 and 3 days, and 1, 2, 4, 6, 8, and 12 wk after initial infestation with sporangia of *P. capsici*, incidence of disease on pepper (*Capsicum annuum*) seedlings was 100% initially and declined until no disease was observed at 8 wk. As assayed in dilution plate tests, the number of propagules declined over time. In soil initially infested with zoospores, disease incidence in the bioassay declined until no disease was observed at 4 wk. Oospores, embedded in nylon mesh and placed in soil, were tested for viability with the vital stain tetrazolium bromide. Oospore viability decreased from 72.5% to 50–60% after 12 wk. Incidence of disease was low in a bioassay after 1 wk but increased to 100% after 12 wk. Depth in soil was not a significant factor for survival of any propagule ($P = 0.05$). In the overwintering test, oospore viability declined from 67% to 25–29% after 16 wk and to 8–9% after 27 wk. No disease was observed in the bioassay after 8 wk. In controlled environment tests designed to evaluate the effects of defined soil moisture and temperature levels on survival of the various propagules of *P. capsici*, all propagule types survived longer at -10^2 and -10^3 kPa and 15 and 25 C than at -10 and -30 kPa and -5 and 35 C. Low survival at -10 kPa and -5 and 5 C may explain the low survival observed in the overwintering field test where the weather was wet and cold. However, oospores were viable and were able to cause disease in a bioassay after 16 wk in most of the controlled moisture-temperature combinations. Thus, oospores may be the overwintering propagules of *P. capsici*.

Little work has been reported on the survival or ecology of *Phytophthora capsici* Leonian, the causal agent of Phytophthora blight of pepper (*Capsicum annuum* L.). *Phytophthora capsici* remained viable for 5 mo in moist soil when infested with mycelium (7,17) and could be isolated from naturally infested soil from commercial nurseries in The Netherlands after storage at 20 C for 13 mo but not after 21 mo (24). In Germany, *P. capsici* survived under outdoor conditions only in years with very mild winters, but the propagule(s) responsible for survival and the quantitative aspects of survival were not studied (10). Oospores remained infective for 6–10 mo in Mexico under field conditions but mycelium was not infective (16). The authors concluded that *P. capsici* survives from one year to the next by means of oospores. However, survival was not quantified, nor were environmental conditions monitored in the field.

In studies in Brazil, researchers attempted to determine the effects of moisture and depth in soil on survival of some propagules of *P. capsici* (5). Zoospores were not recovered after 60 days from any of three soil moistures as determined by a soil dilution plate method, and propagules of *P. capsici* did not survive in colonized tissue after 120 days in soil. In another study, oospores in soil remained viable from 210 to 240 days as determined by a bioassay (4). The soil moisture in the previous two studies was not monitored and tests were carried out only under greenhouse conditions without studying the effect of temperature and its interaction with soil moisture on survival.

The objective of this study was to quantify the survival ability of the various propagules of *P. capsici* in the field and in controlled environments in order to test the hypothesis that oospores are the main survival propagules. Soil temperature and soil moisture were monitored in the field and an attempt was made to relate the results from the field to those in controlled environments with the use of these environmental variables.

MATERIALS AND METHODS

Isolates. The isolate of *P. capsici* used in this study was obtained from an infected squash (*Cucurbita pepo* L.) plant in southern New Jersey in 1978 by G. C. Papavizas. This isolate, designated as Pc-S1, is of the A^1 compatibility type. Oospores were produced by crossing Pc-S1 with isolate ATCC 15399 (A^2 compatibility type) obtained from the American Type Culture Collection, Rockville, MD. Both isolates were maintained on Difco lima bean agar (LBA).

Soil. The soil used in all tests was a Matapeake fine sandy loam (2) from the Beltsville Agricultural Research Center, Beltsville, Maryland. The soil (pH 7.2) was analyzed by the University of Maryland Soil Testing Laboratory in College Park and was found to consist of 72% sand, 14% silt, and 14% clay, with an organic matter content of 0.8%. A soil-water characteristic curve was prepared with a ceramic pressure plate apparatus (Soilmoisture Equipment Corp., Santa Barbara, CA) to convert the percent of moisture to kPa of soil water matric potential (10^2 kPa = 1 bar).

Inoculum production. Sporangia were produced by growing isolate Pc-S1 on LBA for 2 wk under continuous, diffuse light at 25 C. The plates were flooded with 10 ml of sterile, distilled-demineralized water and the surface was wiped with a cotton swab to dislodge the sporangia. The suspension was filtered through a 200-μm nylon mesh to remove clumps of mycelium. Sporangia were counted in 1-μl drops and added to raw soil immediately. In field tests, 200 sporangia per gram of dry soil were added, while in growth chamber tests, 50 sporangia per gram of dry soil were used.

Journal Series Paper of the New Jersey Agricultural Experiment Station, Cook College, Rutgers University, New Brunswick. Portion of an M.S. thesis by the first author.

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Accepted for publication 20 March 1990.

Zoospores were produced by growing isolate Pc-S1 on LBA for 7–10 days and incubating sporangia in water for 5–15 min at room temperature. The zoospore suspension was then filtered through a 25-μm nylon mesh to remove the empty sporangia. Motile and encysted zoospores were counted in 10 1-μl drops. A concentration of 500 zoospores per gram of dry soil was used in both field and growth chamber experiments.

Oospores were produced by comminuting 2-wk-old cultures, one plate of each compatibility type growing on LBA, in 80–100 ml of sterile distilled-demineralized water in a sterile Sorvali omni-mixer (DuPont Instruments, Newton, CT). One ml of this mixture was added under sterile conditions to a petri plate containing approximately 18 ml of clarified V-8 juice broth. Cultures were incubated in the dark at 25 C for 2–4 wk. Clarified V-8 juice was prepared by adding 10 g of calcium carbonate to 1 L of V-8 juice, heating the mixture to 80 C, and centrifuging at 13,200 *g* for 10 min. One hundred ml of the supernatant plus 30 mg of cholesterol dissolved in 2 ml of 95% ethanol were added to 900 ml of distilled-demineralized water. After 2–4 wk, mycelial mats containing oospores were aseptically placed in the omni-mixer, which was one-half full of sterile, distilled-demineralized water. The mycelial mats were comminuted for 10 min at medium speed, and the resulting mixture was filtered through a 100-μm nylon mesh. Excess mycelium was scooped off the top of the mesh, placed back in the omni-mixer, recomminuted, and refiltered. The resulting filtrate was centrifuged for 2–5 min at 2,200 *g*. This was repeated until all of the filtrate was centrifuged and the oospores were concentrated in the pellet. The oospores were then resuspended and stored at 5 C until used, usually overnight.

For use in survival experiments, suction (11) was used to embed oospores on 25-μm nylon mesh (Nitex, Tetko, Inc. Elmsford, NY). The meshes were then placed in soil in accordance with the experiment.

Mycelium in tissue was prepared by inoculating healthy pepper plants (cv. California Wonder) grown in flats in a peat-vermiculite planting mix in the greenhouse (3). After 5–6 wk, the flats were placed in trays, the planting medium was saturated with water, and 1.0 ml of a sporangium suspension containing 1×10^4 sporangia was pipetted around the base of each plant. After 12–18 hr, the flats were removed from the trays and placed on a greenhouse bench. The mix was kept moist with routine watering. After 2 wk, the plants were harvested and the crown portion (5 cm above and below the soil line) was used for experiments. Oospores were produced in pepper tissue by the addition of sporangia from isolates Pc-S1 (A^1) and ATCC 15399 (A^2) to the saturated mix, which resulted in the formation of oospores in the crown portion. The plants were harvested and the crown portion was used for tests after 3 to 4 wk (6,13).

Although chlamydospores of *P. capsici* have been reported using a submerged culture method (23), all isolates producing chlamydospores, except those from eggplant, were isolated from nonsolanaceous hosts. All isolates of *P. capsici* in our collection do not produce chlamydospores with the submerged culture method (Bowers et al, *unpublished*).

Field tests. A field plot (18.3 m × 12.2 m) located at Beltsville, in which grass was grown in the field for the preceding 3 yr, was split in half lengthwise and 10 areas (five to a side) measuring 3.0 m × 1.5 m were rototilled to form subplots bounded by grass. Soil temperature and moisture were monitored at 4–6 and 20–22 cm depths with thermistor probes and gypsum blocks, respectively, attached to a CR21 micrologger (Campbell Scientific, Inc., Logan, UT). The ceramic pressure plate apparatus was used to calibrate the gypsum blocks.

Two field tests were performed during the summer of 1982 with sporangia and zoospores as inoculum commencing on 21 June and 13 July. The summer test with oospore inoculum commenced on 21 June. Overwintering tests with oospores, colonized tissue, and oospores in colonized tissue commenced on 30 October.

A destructive sampling technique was used in all field tests. The propagule type under study was added to or placed in moist soil (approximately −30 kPa) and then wrapped in a nylon bag to form a small bundle approximately 5 cm in diameter. Sporangia and zoospores were incorporated into soil at the densities mentioned previously. Four nylon meshes containing embedded oospores were placed into the soil in each bundle. Samples were placed in holes dug with a posthole digger in five randomly selected subplots. For each hole, which constituted a sampling unit, one bundle was placed at 4–6 cm and another at 20–22 cm depth (one propagule type per bundle per hole). At each sampling date, bundles from one sampling unit for each propagule type were retrieved from each of the five subplots and the appropriate assays were run (five replications per sample depth per sample time per propagule). Soil samples initially infested with sporangia and zoospores were sampled after 3 days and 1, 2, 4, 6, 8, and 12 wk during the summer of 1982. Soil samples containing nylon meshes with oospores were sampled after 0, 1, 2, 4, 8, and 12 wk during the summer 1982 test and after 0, 2, 4, 8, 16, and 27 wk during the winter 1982–1983 test. Colonized tissue and oospores in tissue were sampled only during the winter test and at the same time that the oospores in nylon mesh were sampled.

Growth chamber tests. Control environment experiments were carried out in a factorial design at −5, 5, 15, 25, and 35 C (±2 C) and at −10, −30, -10^2, and -10^3 kPa soil-water matric potential. Sifted (3 mm), air-dried field soil was

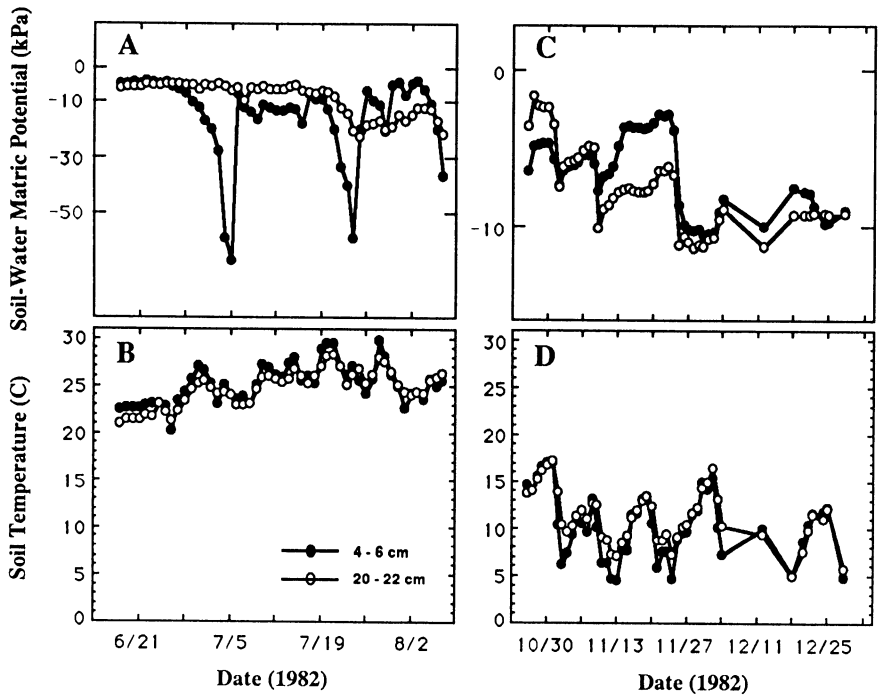

Fig. 1. (A) Soil-water matric potential (kPa) and **(B)** soil temperature (C) recorded in the field during the summer of 1982. **(C)** Soil-water matric potential and **(D)** soil temperature recorded in the field during the winter of 1982–1983 at depths of 4–6 and 20–22 cm using thermistor probes and gypsum blocks attached to a CR21 micrologger.

divided into 5-kg lots and brought up to the desired moisture level with tap water in specified amounts, which were derived from a standard curve. The soil was wrapped in double plastic bags and incubated at 23–26 C for a minimum of 2 wk and, for most experiments, more than a month before use in order to allow the natural population of microorganisms to stabilize. Propagules were added to or placed in soil from each moisture level. These four batches of soil, each at a different moisture level, were then divided further into each of the five temperatures. For each moisture-temperature combination, the soil was divided further into five lots. Five growth chambers were each set and maintained at a different temperature. Soil samples initially infested with sporangia and zoospores were sampled after 2, 4, 8, 12, 16, and 20 wk. Soil samples containing nylon meshes with oospores, colonized tissue, and oospores in tissue were sampled after 0, 4, 8, 12, 16, and 24 wk. Soil samples were also taken periodically and soil water content was determined gravimetrically for comparison with the soil-water characteristic curve, and water was added as needed to maintain the proper soil-water matric potential.

Assays. Sporangia and zoospores were assayed for viability with a dilution plate technique using the selective medium P_5VPP-BH (15) and with a bioassay. From the five bundles of soil retrieved from the field at each depth or from the growth chambers, 5 g of soil were used for a dilution plate assay, and the remaining soil (approximately 100 g) was placed in a 150-ml paper cup and a 4- to 6-wk-old pepper plant was transplanted to the soil (one plant per cup). The cup was placed on the greenhouse bench and watered from underneath to prevent splashing. Results of the dilution plate assay (five plates per dilution) were recorded in colony-forming units (cfu) per gram of dry soil. The results of the bioassay were recorded as the percentage of five plants showing characteristic symptoms after 2 and 4 wk.

Oospores were assayed for their ability to survive in soil with the use of a vital stain and a bioassay. Two of the four nylon meshes containing oospores retrieved from the field and growth chambers were treated with three drops of 0.1% tetrazolium bromide and incubated at 35 C for 24 hr (21). The meshes were then rinsed and observed microscopically. Viable oospores stained red to rose while nonviable oospores did not stain or stained black. The percentage of viable oospores were recorded (100 oospores counted per mesh).

A disease assay was also performed with oospores on a nylon mesh retrieved at the same time as those stained. An 8-cm pot was filled with No. 2 quartz sand and a small transplant hole was formed in the sand. One mesh was placed in the hole and a 4- to 6-wk-old pepper plant was transplanted on top of it. The pots were placed in saucers on a greenhouse bench and watered regularly. The percentage of five plants (one plant per retrieved sample) showing symptoms was recorded after 2 to 4 wk.

The ability of mycelium and oospores to survive in tissue was assessed with the bioassay method. Pots with sand were used as above and the crown portions of diseased pepper plants retrieved from soil were placed in the transplant hole (one bioassay plant per retrieved sample). The pots were placed in saucers on a greenhouse bench as before and the percentage of five plants (one plant per retrieved sample) showing symptoms was recorded after 4 wk.

Statistical analysis. Field tests were set up as a split-plot design with the individual subplots in the field representing the blocking factor and each subplot split into two depths. Propagule counts of sporangia and zoospores from each dilution plate were transformed as the square root of the sum of the colony-forming units per gram of soil plus 0.5 ($cfu/g = [cfu/g + 0.5]^{1/2}$; 20). The stain data for oospores were analyzed without transformation. An analysis of variance and the F test were run on the data for a split-plot design. Disease bioassay data for all propagules were not subjected to statistical analysis because of the low number of plants constituting a percentage (only one plant per replicate).

In growth chamber tests, factorial experiments were set up with moisture, temperature, and time as the main factors. Because each growth chamber was maintained at a specified temperature and experiments were not replicated using different chambers set at the same temperature, each moisture-temperature combination was considered to be replicated in the same growth chamber. While moisture, temperature, and time were crossed, the replicates were not. For the purpose of analysis, the replicates were nested in moisture-temperature.

The disease bioassay data for all growth chamber experiments were not subjected to statistical analysis because of the low number of plants constituting a percentage that were available for each moisture-temperature combination at each assay date.

RESULTS

Field tests—summer. Soil-water matric potential and soil temperature recorded are presented in Fig. 1. In the first summer test with sporangia as the initial inoculum source, propagule numbers declined steadily from 5.3 to 0.0 cfu/g soil and from 3.4 to 0.2 cfu/g soil after 6 wk from the 4–6 and 20–22 cm depths, respectively (Table 1). No propagules were recovered at 8 wk (Table 1). Depth was not a significant factor ($P = 0.05$) in the decline of sporangia in the soil.

The disease assay also indicated a decline in the number of propagules with time (Table 1). No disease was detected in the assay plants after 6 wk in the field with inoculum placed at 4–6 cm, but 20% disease was present in the assay plants with inoculum placed at 20–22 cm. After 8 wk, the inoculum from 20–22 cm was still infective, even though no colonies

Table 1. Survival of propagules of *Phytophthora capsici* is soil in the field at two depths over time

		Summer 1982				Winter 1982–1983	
		Sporangia		Oospores		Oospores	
Week	Depth (cm)	cfu[a]	%D[b]	%V[c]	%D	%V	%D
0				72.5 ± 6.4	80	67.0 ± 12.7	100
1	4–6	5.3 ± 1.5	100	73.2 ± 1.6	20		
	20–22	3.4 ± 1.3	100	72.3 ± 1.5	20		
2	4–6	2.7 ± 1.2	80	59.2 ± 6.3	0	69.1 ± 4.2	100
	20–22	3.0 ± 1.0	100	63.2 ± 3.8	40	67.0 ± 2.7	100
4	4–6	0.9 ± 0.5	60	68.4 ± 6.9	80	66.2 ± 3.8	100
	20–22	1.4 ± 0.6	100	63.9 ± 11.4	20	67.3 ± 4.9	100
6	4–6	0.0	0				
	20–22	0.2 ± 0.3	20				
8	4–6	0.0	0	66.6 ± 6.4	100	58.6 ± 9.8	0
	20–22	0.0	40	60.1 ± 8.3	60	62.9 ± 5.5	20
12	4–6	0.0	0	60.3 ± 4.5	100		
	20–22	0.0	0	50.5 ± 6.1	100		
16	4–6					24.9 ± 9.2	0
	20–22					28.5 ± 10.6	0
27	4–6					9.2 ± 5.8	0
	20–22					7.7 ± 5.6	0

[a] Mean colony-forming units per gram of dry soil, ±95% confidence interval.
[b] Percentage of five plants with symptoms in the bioassay. D = disease.
[c] Mean percentage of oospores stained viable (V = viability) when treated with 0.1% tetrazolium bromide, ±95% confidence interval.

formed on the dilution plates. No disease was detected in further samplings. The second field test gave similar results.

In tests performed with zoospores as the initial inoculum source, assays detected low numbers of propagules of *P. capsici* after 2 wk in the field in the first test (0.4 cfu/g of soil) and after 3 wk in the second test. Subsequent samplings did not indicate the presence of *P. capsici* and, again, depth was not a significant factor ($P = 0.05$). Results similar to those of the dilution plate assays were observed in the disease assays.

Oospore survival showed only a slight decrease over the 12 wk of the summer test (Table 1). At time 0, 72.5% of the oospores were viable as indicated by staining, and, after 12 wk, 60.3% and 50.5% were viable at 4–6 cm and 20–22 cm, respectively. Depth was not a significant factor ($P = 0.05$).

In the disease assay, oospores were capable of causing 80% disease at time 0 (Table 1). After 1 wk in soil, the disease percentage dropped to 20% for both depths. After 8 wk in soil, percentages increased to 100% and 60% disease incidence for 4–6 and 20–22 cm, respectively. One hundred percent disease occurred from oospores buried at both depths for 12 wk.

Field tests—winter. Soil-water matric potential and soil temperature recorded are presented in Fig 1. Sporangia and zoospores were not included in the overwintering tests. During the winter test, the percentage of viable oospores decreased for 16 wk of exposure (Table 1). Sixty-seven percent of the oospores were viable at time 0, and after 16 wk in soil (19 February), 24.9% and 28.5% were viable at 4–6 cm and 20–22 cm, respectively. When sampled on 7 May after 27 wk, only 9.2% and 7.7% were viable at 4–6 cm and 20–22 cm, respectively. Data from the disease assay also showed a decline with time. Oospores in colonized tissue placed in the field did not survive longer than 8 wk during the winter. Disease incidence in the assay plants remained high (80–100%) at both depths at the 2- and 4-wk sampling, but then declined until no disease was detected in the assay plants at the 8, 16, and 27 wk sample times.

Oospores produced naturally in colonized tissue also did not survive the winter in the field as determined by the disease bioassay. One hundred percent disease incidence occurred in the assay plants 2 and 4 wk after burial and declined after that. After 8 wk, 20% disease occurred in the assay plants for 20–22 cm and no disease was evident from the 4–6 cm samples. No disease appeared in the assay plants, regardless of depth, at the 16 and 27 wk samples.

Growth chamber tests. When sporangia were used as the initial inoculum source, propagules of *P. capsici* were not recovered on dilution plates from soil incubated for 2 wk at −5 C, regardless of the moisture level, and at 5 C with soil-water matric potentials of −10 and −30 kPa (Fig. 2). Recovery at 2 wk was highest (3.25–9.5 cfu/g soil) from soil stored at 15 and 25 C with soil-water matric potentials of −30, -10^2 and -10^3 kPa. Recovery was lower (0.25–3.5 cfu/g soil) from soil stored at 35 C, regardless of the moisture level, and at 15 and 25 C at −10 kPa. After 8 wk, propagules of *P. capsici* were recovered only from soils stored at 15 and 25 C with soil-water matric potentials of −30, -10^2 and -10^3 kPa (1.3–4.3 cfu/g soil) and from soil stored at 25 C at −10 kPa (0.1 cfu/g soil). Propagules of *P. capsici* were not recovered on dilution plates in subsequent samples.

In a separate bioassay experiment, propagules of *P. capsici* were able to cause 100% disease when the soil was incubated for 2 wk at 5, 15, and 25 C over all moisture levels. No disease developed in the assay plants from soil incubated at −5 and 35 C. Disease incidence in the assay declined with subsequent samples, but after 8 wk, propagules from soil stored at 15 C were still able to cause 100% disease, with a lesser amount of disease from propagules in soil stored at 5 C. As in the field experiments, propagule recovery on dilution plates and disease development in the bioassay did not correlate well, in part because of the low number of plants constituting a percentage in each experiment.

When zoospores were added to soil as the initial inoculum source, propagules of *P. capsici* were not recovered on dilution plates from soil incubated for 2 wk at −5 C, regardless of the moisture level, nor at −10 kPa, regardless of the temperature. Recovery was highest at −30 kPa and 5 to 25 C (2.75–8.25 cfu/g soil) and at -10^2 kPa and 5 to 25 C (6.25–9.50 cfu/g soil). Recovery was less at -10^3 kPa over all temperatures (0.25–3.25 cfu/g soil). These trends continued, although at lower recovery levels, at the 4 and 8 wk sample dates. After 8 wk, propagules were recovered only at 15 C and −30 to -10^3 kPa (0.1–0.5 cfu/g soil) and at 25 C and -10^2 and -10^3 kPa (0.1 cfu/g soil). Propagules were not recovered at subsequent sample times.

The highest percentage of oospores stained with tetrazolium bromide after

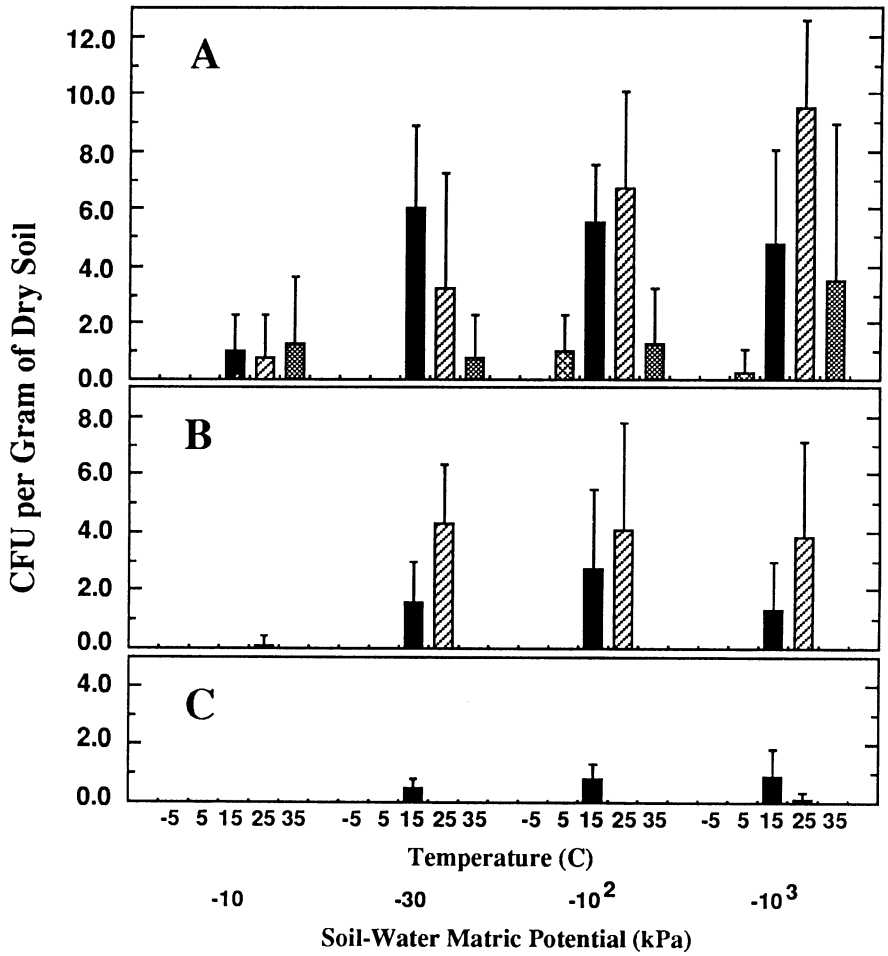

Fig. 2. The effect of soil-water matric potential and soil temperature on the survival of *Phytophthora capsici* when 50 sporangia per gram of dry soil were initially added to soil at **(A)** 2, **(B)** 8, and **(C)** 16 wk. Untransformed data reported as colony-forming units per gram of dry soil. Bars represent 95% confidence intervals.

4 wk in soil was observed with those stored at 5, 15, and 25 C over all moisture levels (43.3–64.8%; Fig. 3). Fewer oospores were viable when incubated in soil at −5 and 35 C (19.0–39.8%). After 12 wk, the percentage of viable oospores decreased in all moisture-temperature combinations, except for those stored in soil at 5, 15, and 25 C at -10^3 kPa (54.8–65.0%). After 16 wk, the number of viable oospores declined further with the highest percentage stained at 5, 15, and 25 C over all moisture levels (18.3–57.5%). Fewer oospores stained viable in soil stored at −5 and 35 C over all moisture levels (2–17%).

The results of the bioassay for oospores sampled at the same time as those stained was quite variable over temperature and moisture levels (Fig. 4). Disease incidence, which was relatively high in most moisture-temperature combinations at the 4-wk sample, generally declined at the 12-wk sample and then increased at the 16-wk sample.

Oospores that were produced in tissue were able to cause more disease in the bioassay after 4 wk in soil when incubated at 15 and 25 C (80–100%). Disease percentages were less at 5 and 35 C (20–60%) and very low at −5 C (0–20%). Disease incidence fluctuated in subsequent samplings; after 16 wk in soil, oospores in tissue were still able to cause disease in the bioassay and, thus, survive to the greatest extent when stored at 15 and 25 C with matric potentials of -10^2 and -10^3 kPa (80–100%). Oospores in tissue at other moisture-temperature combinations resulted in 0–20% disease in the bioassay.

DISCUSSION

Sporangia and zoospores of *P. capsici* were shown to have limited survival ability in soil. During the summer tests in soil initially infested with sporangia, *P. capsici* could not be detected after 6 wk in the field at a depth of 4–6 cm and after 8 wk at a depth of 20–22 cm in either the bioassay or the dilution plate assay. In growth chamber tests with soil initially infested with sporangia, *P. capsici* also could not be detected after incubation for 8 wk at soil temperatures similar to those present during the summer field test, regardless of the soil-water matric potential. Sporangia were also not detected after 2 wk at low temperatures, thus indicating that this propagule type is short-lived and not likely to be a factor in the overwintering of the fungus. The results of this study generally agree with reports in the literature on the ability of propagules of *Phytophthora* spp. to survive in soil (8,9,12,14,19,22,25).

In our tests with zoospores and sporangia, disease was present in the bioassay even though no propagules were recovered on dilution plates using a low dilution factor (1:1). Propagules of *P. capsici* seemed to survive at very low numbers in the soil for several weeks. The dilution plate technique apparently was not sensitive enough to detect these surviving propagules. However, the bioassay indicated that infective propagules were present. Only a few surviving propagules were capable of causing a significant amount of disease in the assay, even though no quantitative measure of inoculum density could be obtained. The dilution plate technique may be unacceptable with low numbers of propagules and may incorrectly estimate low inoculum densities of the pathogen in soil. A large number of samples may be needed to detect low numbers of propagules.

Oospores were shown to have a greater ability to survive in soil under the conditions of the field and growth chamber experiments. In the summer field test, oospores from culture were initially able to cause 75–80% disease, but when placed in soil for 1 and 2 wk were able to cause only 25–30% disease. The potential to cause disease in the assay plants was clearly reduced as a result of some form of inhibition because viability was maintained. Oospores placed at 5 cm in the field were able to overcome this inhibition and cause disease in the bioassay sooner than those placed at 20 cm. The fluctuating soil environment seemed to reverse the inhibitory effect. This happened sooner at 5 cm where the fluctuations were greater than at 20 cm where conditions were more constant. The oospores placed at 20 cm needed more time before they were able to

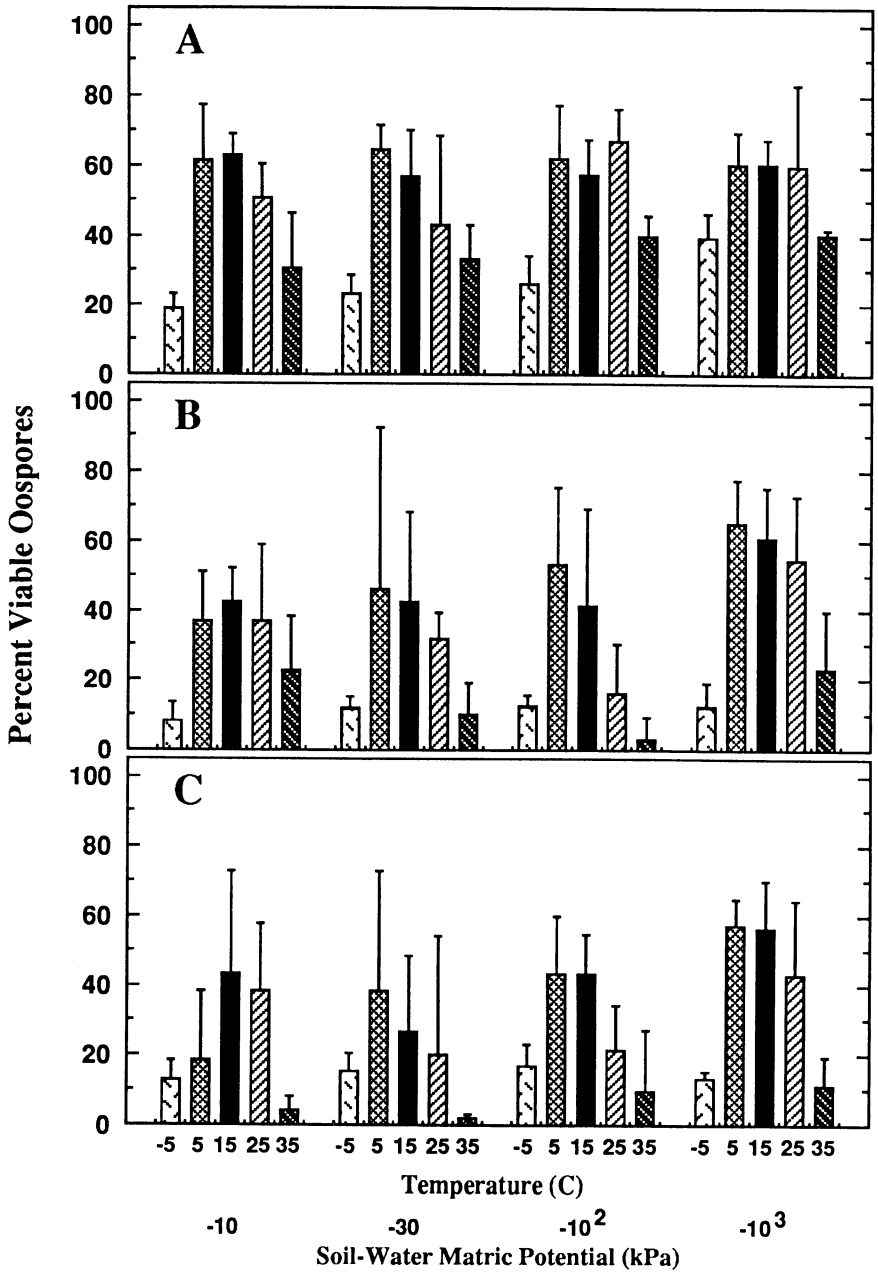

Fig. 3. The effect of soil-water matric potential and soil temperature on the survival of oospores of *Phytophthora capsici* at **(A)** 4, **(B)** 12, and **(C)** 16 wk. Data reported as the percentage of viable oospores as determined by the vital stain tetrazolium bromide. Bars represent 95% confidence intervals. At 0 time, 64.25 ± 3.53% of the oospores were viable.

germinate and cause disease in the assay, but they eventually reached the point where they were able to cause 100% disease in the assay. The initial exposure of the oospores to the soil environment appeared to inhibit germination, and thus disease, while time in the soil environment appeared to reverse the inhibition. The factors causing or inducing the inhibition might be different. In a constant state situation, the constitutive dormancy of the oospores may play an important role, while environmental factors may be more important in a natural, fluctuating system. The initial inhibition of disease in the bioassay followed by an increase in disease at subsequent sample times was also observed in growth chamber tests where the environmental variables were constant.

In the winter field test for oospores, the phenomenon just discussed did not occur. As before, the percentage of disease incidence in the assay plants was high in the beginning. However, after 8 wk, the incidence of disease had dropped to zero and did not increase, even after 27 wk. The main differences between the winter and summer field tests were the increased period of high soil moisture and the relatively cool soil temperature in the winter. During this period (30 October–26 December), the soil was very wet and did not freeze. The daily average soil moisture remained higher than −10 kPa for extended periods and the daily average soil temperature fluctuated between 5 and 15 C. In contrast, the soil moisture during the summer test was somewhat drier, and moderate to high soil temperatures prevailed.

The viability of oospores, as measured by staining with tetrazolium bromide, declined at various rates in all tests. This decline, however, did not relate to the results of the disease assay. The ability of the oospores to cause disease declined and then increased in the summer field test and declined rapidly in the winter test. The stain, however, indicated that oospore viability changed very little in the summer test and declined gradually in the winter test. In both instances, a significant percentage of oospores were stained (indicating viability), although they were not able to cause disease. For example, no disease was present in the bioassay of oospores from the 4–6 cm depth after 8 wk in the winter field test, although over 60% of the oospores were stained, indicating that they were viable.

Several factors could cause this disparity between the disease assay and the stain assay. Tetrazolium bromide takes up hydrogen ions given off during the enzymatic reactions of respiration. It is reduced and a compound called formazan is produced, which stains the cell red (1). Therefore, the reaction indicating that an oospore is respiring is a red staining of that oospore. The stain assay indicates the percentage of oospores respiring. The assay does not indicate that an oospore is capable of germination and, therefore, capable of causing disease. Sutherland and Cohen (21) reported that the ability of oospores to stain was not directly correlated with oospore germination, but they suggested that there may be an association between stainability and germinabitity. This has not yet been fully investigated.

Our field results seem to indicate that *P. capsici* survives in the field from one season to the next in very low numbers of viable oospores. Both compatibility types of *P. capsici* have been isolated from the same field and from the same plant in production fields in southern New Jersey, and oospores have been observed but not isolated in diseased pepper stems (Bowers et al, *unpublished*). The field conditions, however, were not typical. The overwintering season was very wet and the soil did not freeze for more than a few millimeters on the surface for short periods of time. On the other hand, if the soil had frozen, propagule survival might have increased. The activities of antagonistic microorganisms would have been slowed considerably, free soil water would have been reduced, and the constitutive dormancy or inhibition of oospores might have been broken. Oospore survival has been reported to be low with very wet soil conditions as a result of

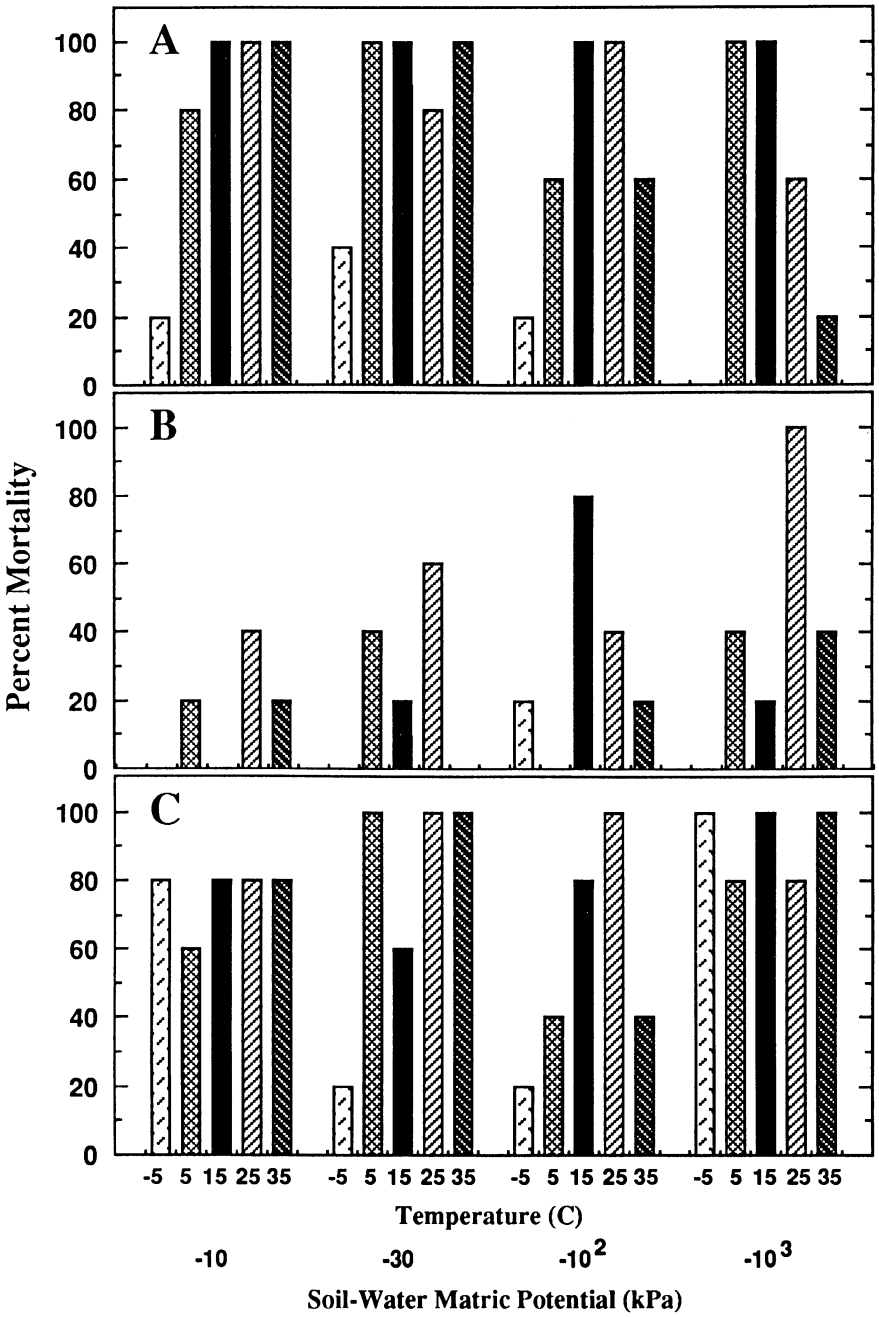

Fig. 4. The effect of soil-water matric potential and soil temperature on the survival of oospores of *Phytophthora capsici* at **(A)** 4, **(B)** 12, and **(C)** 16 wk. Data reported as percent of five plants diseased in the bioassay. At 0 time, 100% disease occurred in the bioassay.

extensive parasitism by a variety of soil organisms (18). Parasitism of oospores was observed in our studies but was not investigated further.

The growth chamber tests suggested that this hypothesis may be correct. The disease assay for oospores showed an increase in the disease incidence when oospores were stored in soil at −5 C for 16 wk. Oospores in tissue also showed some ability to cause disease in the assay after 16 wk in frozen soil, especially at the drier moisture levels. An exposure to freezing temperatures in the soil for a certain amount of time may enhance the ability of oospores to germinate and cause disease in the presence of a susceptible host. Under normal field conditions in some years, the soil usually freezes to a considerable depth and the disease is usually present in commercial fields the next season.

In another respect, the vital stain tetrazolium bromide may reflect real survivability. In the winter field test, the vital stain assay showed a natural decline in oospore viability with time until only a small percentage of oospores was stained on 7 May (27 wk). This time period corresponds to the period when the transplanting of peppers to the field begins in commercial operations in southern New Jersey. It is theoretically possible that the few oospores still respiring would be capable of causing disease in the field and, thus, starting an epidemic under favorable conditions. This hypothesis still needs to be tested under conditions of defined inoculum and correlated with oospore germination studies.

ACKNOWLEDGMENT

This research was conducted while the first author was employed by the Biocontrol of Plant Diseases Lab. We thank the U.S. Department of Agriculture for use of materials and facilities at the Beltsville Agricultural Research Center.

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