Selective Isolation of *Phytophthora capsici* from Soils

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ABSTRACT

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A selective medium and a dilution-plate technique were developed for the direct isolation of *Phytophthora capsici* from soil and from infected pepper plants, and for the quantitative estimation of the inoculum density of the pathogen in soil. The selective agar medium, a modification of Tsao's $P_{10}VP$ medium, contained Difco cornmeal agar (17 g/L) acidified to pH 3.8–4.0 with 3.5 ml of 1.0 N HCl added after autoclaving. The medium, designated as P_5VPP-BH, contained the following antimicrobial agents (μg/ml); pimaricin, 5; vancomycin, 200; pentachloronitrobenzene, 100; penicillin G, 100; benomyl, 2.5; and hymexazol, 20. Aliquots from concentrated soil dilutions (1 in 2, 1 in 4, 1 in 8, and 1 in 10) were placed on the surface of the P_5VPP-BH medium, incubated for 3–4 days at 20 C in the dark, washed with a cotton swab under running water, and examined with oblique fluorescent light when the agar surface was still wet. The average recovery of sporangia and zoospores on this medium from artificially infested soil was about 60 and 22%, respectively. Recovery of mycelial inoculum from soil on the P_5VPP-BH medium was satisfactory. Although *P. capsici* also could be recovered from soil with the most probable number technique, quantitation by this technique is limited to soils with $\geq$46 propagules per gram of soil. The number of propagules per gram of soil recovered from an artificially infested field plot declined rapidly from 6 days after infestation until no propagules could be recovered at about 40 days after infestation. Naturally infested soils from commercial pepper, squash, and eggplant fields in southern New Jersey averaged 0–24 propagules per gram of soil. Both compatability types of *P. capsici* were found in commercial fields and in several cases in the same field or on the same plant.

Additional key words: Capsicum annuum, selective medium, Phytophthora blight.

Phytophthora capsici Leonian, the soilborne fungus that causes root rot, crown rot, and blight of pepper (*Capsicum annuum* L.), is difficult to isolate by direct plating of infected plant tissues on agar media and even more difficult by soil dilution procedures. The study of survival of this pathogen in soil has been hindered by lack of adequate quantitative procedures for directly measuring its prevalence in soil.

Satisfactory methods for isolating *Phytophthora* spp. have been developed and the literature on methods has been adequately summarized (5,6,7), but there is nothing in these reviews on isolation of *P. capsici* from soil. Tsao and Ocaña (9) used a qualitative method for directly isolating *P. capsici* from heavily infested soils by planting "a mass of soil (3–5 mm in diameter)" on their pimaricin-vancomycin-pentachloronitrobenzene (PCNB) medium ($P_{10}VP$). Our preliminary attempts to isolate *P. capsici* from soil with the $P_{10}VP$ medium were unsuccessful because of heavy soil infestation with *Pythium* spp. and *Mortierella* spp., which grew more rapidly in the plates than did *P. capsici.*

Masago et al (2) and Tsao and Guy (8) suggested inclusion of the fungicide 3-hydroxy-5-methylisoxazole (hymexazol) in selective media to inhibit growth of *Pythium* spp. and *Mortierella* spp. Although hymexazol inhibited radial growth of *Pythium* spp. at 25 and 50 μg of active ingredient (a.i.) per milliliter, it permitted good growth of *P. capsici* (2).

The present study was undertaken to formulate hymexazol and other antimicrobial agents into selective media and to develop techniques for isolating *P. capsici* from soil and for estimating the inoculum density.

MATERIALS AND METHODS

Isolates used. Three isolates of *P. capsici* were used in this study. Isolate ATCC 15399 (compatability type A^2) was obtained from the American Type Culture Collection. Isolate P (type A^2) was isolated from peppers by S. A. Johnston and isolate S1 (type A^1) was isolated by G. C. Papavizas from infected squash from a field near Vineland, NJ. Sporangia, zoospores, and oospores, obtained by methods described elsewhere (4), were added to soil at concentrations indicated in each particular experiment. Mycelial fragments were obtained from 3-day-old culture mats grown in dilute V-8 juice (100 ml V-8 juice, 900 ml distilled water, 3 g $CaCO_3$, clarified by centrifugation at 13,000 *g*). Washed mats were comminuted in a blender for 30 sec and mixed thoroughly with soil at concentrations equivalent to 0.0, 1.1, 2.3, 3.4, and 4.8 mg dry wt per 50 g of oven-dry soil.

Soils. The soil used for artificial infestation experiments in the laboratory was a sandy loam (pH 6.4) from Beltsville. For artificial infestation in the field, we used a plot (13 × 20 m) of loamy sand at Beltsville (pH 5.8). The plot was infested with inoculum of isolates P1 and S1 grown together on autoclaved oats (oats:water 1:1, w/v). The 1-mo-old inoculum was spread on the surface of the soil (100 kg/ha) and rototilled to a depth of 8-10 cm on 12 June 1979. Peppers were transplanted in the plot on 13 June. Because more than 70% of the plants were attacked by *P. capsici*, the pepper plants were rototilled into the soil 35 days after transplanting. The plot was assayed 1, 6, 10, 20, 40, 80, and 100 days after infestation. Thus, sampling on the 40th day was done 4 days after the pepper plants were plowed under. Soil samples were taken for analysis from six areas between plants and about 10 cm from plants. Each sample was composed of 12 cores of soil placed in a plastic bag and mixed thoroughly.

Eight commercial fields from southern New Jersey were assayed periodically for *P. capsici*. The fields were assayed on 15 April, 14 May, 25 June, and 7 August 1979. Fields 1, 2, and 4 were planted to summer squash; field 5 to eggplants; and fields 3, 6, 7, and 8 to peppers. All plantings or transplantings were made by the growers after the 14 May sampling and before the 25 June sampling. The four pepper fields also were assayed on 30 October, approximately 1 wk after the disking of the residue from the pepper crop. All commercial fields were sampled in a manner similar to that used for the artificially infested field plot.

Effect of hymexazol on linear growth and sporangial production. Hymexazol (Tachigaren® 70% wettable powder, Sankyo Co., Yasu, Shiga-Ken, Japan) was suspended in water and added to three basal media at 0, 25, 50, and 100 μg a.i./ml of medium. The media used were $P_{10}VP$ medium (9); Mircetich's pimaricin-vancomycin medium (MPVM) (3); and the BNPRA medium devised by Masago et al (2). The BNPRA medium contains potato-dextrose agar, benomyl, PCNB, and the antibiotics nystatin, rifampicin, and ampicillin. Toxicity of hymexazol in the three media toward the linear growth and sporulation of *P. capsici* was studied in petri plates (15 ml agar per plate). Disks 5 mm in diameter of isolates P1 and S1 grown on hymexazol-free Difco lima bean agar (LBA) were transferred to the center of the plates and these were incubated at 25 C in the dark for 12 days. Linear growth was measured 4 days after inoculation and the amount of sporangial production was determined 12 days after inoculation. Sporangial production was assessed on the basis of an arbitrary scale of 0 = no sporangia to ++++ = more than 21 sporangia per microscopic field (×10 ocular and ×10 objective).

TABLE 1. Effect of hymexazol added to three isolation media on linear growth and sporulation of *Phytophthora capsici* isolates P1 and S1

Isolation medium	Hymexazol concn. in agar (μg/ml)	Colony radius at 4 days[u] (mm)		Sporangial production[v] of isolate S1 at 12 days
		P1	S1	
BNPRA[w]	0	2 f[x]	5 f	+
	25	1 f	4 f	0
	50	2 f	4 f	0
	100	2 f	4 f	0
MPVM[y]	0	20 bc	13 b	++
	25	18 cd	5 f	+
	50	16 de	5 f	+
	100	15 e	5 f	0
$P_{10}VP$[z]	0	24 a	22 a	++++
	25	22 ab	11 c	++++
	50	20 bc	9 d	++
	100	20 bc	7 e	++

[u] Four replications, two plates each.
[v] Rating system: 0 = no sporangia; + = <5 sporangia per microscopic field 2 mm in diameter; ++ = from six to 10 sporangia per field; +++ = from 11 to 20 sporangia per field; ++++ >21 sporangia per field.
[w] Potato-dextrose agar containing benomyl, nystatin, pentachloronitrobenzene (PCNB), rifampicin, and ampicillin (2).
[x] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[y] Mircetich's pimaricin-vancomycin medium (3).
[z] Pimaricin-vancomycin-PCNB medium (9).

TABLE 2. Recovery of *Phytophthora capsici* from soil infested[a] with sporangia of isolates S1 and ATCC 15399 by the plate-dilution frequency technique as affected by benomyl, rose bengal, and hymexazol

Antimicrobial agent added to basal medium[b]	Concentration (μg/ml active)	Colonies/g of air-dry soil	
		S1	ATCC 15399
None (control)		60 a[c]	46 a
Rose bengal	40	84 a	46 a
Hymexazol	20	340 bc	220 b
Benomyl	2.5	224 b	170 ab
Rose bengal + hymexazol	40 + 20	96 a	46 a
Rose bengal + benomyl	40 + 2.5	90 a	46 a
Hymexazol + benomyl	20 + 2.5	440 c	380 c
Hymexazol + benomyl + rose bengal	20 + 2.5 + 40	76 a	46 a

[a] Five hundred sporangia were added per gram of air-dry soil. Dilutions of 1:4, 1:16, 1:64, 1:256, and 1:1,024 were used.
[b] The basal medium, a modification of the $P_{10}VP$ medium (9), contained Difco cornmeal agar (17 g/L) acidified to pH 3.8–4.0 with 3.5 ml 1.0 N HCl (added after autoclaving). The medium (P_5VPP-BH) contained also (μg/ml): pimaricin, 5; vancomycin, 200; pentachloronitrobenzene, 100; penicillin G, 100; benomyl, 2.5; and hymexazol, 20.
[c] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Isolation media. The $P_{10}VP$ medium (9) was used as the basal medium for our isolation and enumeration studies. The $P_{10}VP$ medium contains, per liter of water, 17 g Difco cornmeal agar, 10 mg pimaricin (Pimafucin® in a 2.5% sterile water suspension, Gist-Brocades N. V., Delft, The Netherlands), 200 mg vancomycin (Vancocin® 100%, Eli Lilly and Co., Indianapolis, IN 46206), and 100 mg PCNB (Terraclor® 75% wettable powder, Olin Corp., Agri Division, Little Rock, AR 72203). Because 10 mg/L of pimaricin were toxic to isolate P1 in preliminary tests and the medium allowed growth of many bacterial colonies, we modified the $P_{10}VP$ medium for our subsequent tests by reducing the concentration of pimaricin to 5 mg/L and adding 100 mg of penicillin G and 3.5 ml of 1.0 N HCl/L after autoclaving to acidify the medium to a pH of about 3.8–4.0. The modified basal medium is hereafter referred to as P_5VPP.

Rose bengal, hymexazol, and methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl, Benlate®, 50% wettable powder, E. I. duPont de Nemours and Company, Wilmington, DE 19898) were added to the P_5VPP medium at 40, 20, and 2.5 mg/L, respectively, singly, and in all possible combinations. The various media were then evaluated for isolation of isolates S1 and ATCC 15399 that had been added to soil as sporangia.

Isolation methods. Propagules were isolated from soil and their numbers were estimated by a plate-dilution frequency (most probable number) technique (1) and by the ordinary dilution-plate method. With the first technique, eight drops (0.05 ml each) from each of six dilutions of a fourfold dilution series (2 g of soil in 6 ml of water) were applied to each petri plate containing 15 ml of medium. Thus, six plates accommodated the six dilutions. The plates were incubated in the dark at 20 C for 4–5 days. The total number of droplets in the series from which *P. capsici* branched out were converted to the number of propagules per gram of air-dried soil by the use of a standard table (1). Corrections were made for soil moisture content and for the dilution.

With the ordinary dilution-plate method, dilutions of 1 in 2, 1 in 4, 1 in 8, 1 in 10, and 1 in 25 were made by suspending the equivalent of 1 g of air-dry soil in 1, 3, 7, 9, and 24 ml of sterile tap water, respectively, and shaking the suspensions by hand for 1 min. One milliliter aliquots were removed from the containers while the liquid was agitated by a magnetic stirrer and spread on the media (eight plates per replication). The plates were incubated in the dark at 20 C for 3–4 days and then washed with a cotton swab and a small stream of tap water to carefully remove all the soil from the agar surface. Colonies were best seen with an oblique fluorescent light while the agar surface was still wet. To identify the isolates, we transferred mycelia from colony edges to acidified cornmeal agar (3.5 ml of 1.0 N HCl/liter added after autoclaving) containing only pimaricin, vancomycin, and penicillin G. We also transferred colonies to LBA for sporangial production and identification. Unknown isolates were paired on clarified V-8 juice agar with isolates of known compatability type to determine the type of the unknown isolates.

Isolations from infected tissues were made by culturing washed segments (5 mm long) of infected pepper or squash plants on the P_5VPP-BH medium (see below) and then following the procedure of purification and identification of isolates described before.

Except where otherwise stated, five replications were used throughout and all the laboratory experiments were repeated three times. For the soil isolation experiments, 12 complete extractions (cores taken with a soil sampler) from a given area of a field constituted a replication.

RESULTS AND DISCUSSION

Tolerance of *P. capsici* to hymexazol. In contrast to the results of Masago et al (2), the BNPRA medium with or without hymexazol, inhibited growth and sporangial production of isolates P1 and S1 considerably (Table 1). Even after 12 days of growth, no sporangia developed on this medium containing hymexazol and very few developed on the hymexazol-free medium. The hymexazol was toxic to isolate S1, but not to isolate P1, in Mircetich's MPVM medium and in the $P_{10}VP$ medium; however, considerable numbers of sporangia developed in the $P_{10}VP$ medium at all concentrations of the fungicide used.

The discrepancies observed between our results and those of Masago et al (2) may be due to the differential sensitivities to hymexazol of the isolates used and to the composition of the media. This is supported by the fact that isolate S1 was more sensitive to hymexazol than P1, even in the $P_{10}VP$ medium, the best medium for linear growth and sporulation. Detrimental effects of the fungicide on production of sporangia as well as mycelial growth also should be taken into account if media and hymexazol concentrations are to be selected for isolation purposes. If sporangia are not produced on an isolation medium, identification of *P. capsici* may require transferring all colonies to a medium allowing sporulation. Therefore, we decided to use 20 μg/ml of hymexazol in the P_5VPP medium, a compromise concentration, for further tests.

Improvement of the P_5VPP medium for *P. capsici* isolation. In addition to hymexazol, we tested benomyl, rose bengal, and nystatin (Mycostatin® 4,690 units per milligram) at various concentrations for toxic effects on the linear growth and germination of sporangia and zoospores of *P. capsici*. Benomyl and nystatin were used by Masago et al (2) at 10 and 25 μg/ml, respectively, in their BNPRA medium. In our tests benomyl, nystatin, and rose bengal allowed the highest percent spore germination and the best linear growth at 2.5, 25, and 40 μg/ml, respectively. In further preliminary tests, however, nystatin inhibited colony development of *P. capsici* on dilution plates prepared from soil artificially infested with the pathogen even at 10 μg/ml. Nystatin, therefore, was not included in subsequent tests.

Benomyl, rose bengal, and hymexazol were finally selected for improving the P_5VPP medium for isolating *P. capsici* from soil with as little interference from bacteria, *Pythium* spp., *Mortierella* spp., and other undesired microorganisms as possible. Benomyl, rose bengal, and hymexazol were added to the medium singly, or in various combinations, and the media were used to isolate *P. capsici* from soil infested with 500 sporangia per gram of air-dry soil of isolate S1 or ATCC 15399 by the most probable number technique. Rose bengal used singly or in combination with benomyl, hymexazol, or with both benomyl and hymexazol did not improve the efficiency

TABLE 3. Recovery of *Phytopthora capsici* from soil infested with sporangia and zoospores of isolate S1 by the dilution-plate method on medium P_5VPP-BH[a]

Propagule added	Propagules added per gram of air-dry soil (no.)	Colonies per gram of air-dry soil (no.)[b]	Recovery (%)
Sporangia	5	3	60
	10	7	70
	20	10	50
	40	23	58
	80	53	66
	160	93	58
			Avg 60 ± 2.8
Zoospores	5	0	0
	10	5	50
	20	5	25
	40	7	18
	80	13	16
	160	32	20
			Avg 22 ± 6.2

[a] The basal medium, a modification of the $P_{10}VP$ medium (9), contained Difco cornmeal agar (17 g/L) acidified to pH 3.8-4.0 with 3.5 ml 1.0 N HCl (added after autoclaving). The medium (P_5VPP-BH) contained also (μg/ml): pimaricin, 5; vancomycin, 200; pentachloronitrobenzene, 100; penicillin G, 100; benomyl, 2.5; and hymexazol, 20.

[b] Dilutions of 1:8 were used for the three low concentrations of spores and 1:20 for the high concentrations. Average of three trials with 16 petri plates per replication.

[c] Confidence interval of the mean, $P = 0.05$.

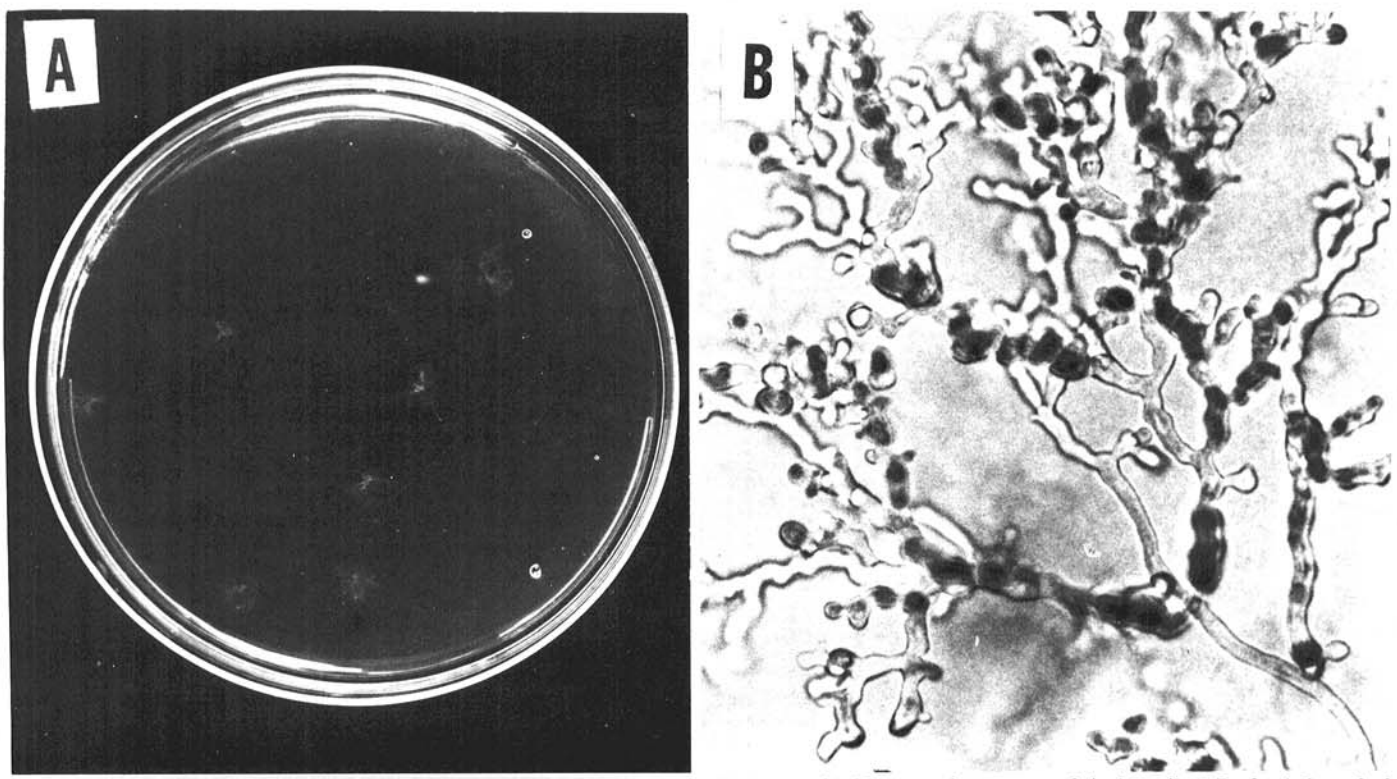

Fig. 1. A, Colonies of *Phytophthora capsici* on the P_5VPP-BH medium from soil infested with 25 sporangia per gram of air-dry soil. **B,** Profusely branched mycelium of *P. capsicia* on the P_5VPP-BH medium (×250).

of the P_5VPP medium in recovering *P. capsici* from soil (Table 2). In fact, rose bengal was actually toxic to *P. capsici* when combined with benomyl or hymexazol. Both benomyl and hymexazol at 2.5 and 20 μg/ml, respectively, improved the isolation medium. Benomyl + hymexazol was the best combination employed with both isolates. The hymexazol and benomyl were, therefore, selected as additional ingredients of the P_5VPP medium and the new medium containing these two fungicides will henceforth be designated as the P_5VPP-BH medium.

Two additional tests were performed with the most probable number technique. In the first experiment, sporangia of isolate S1 were added to soil at 0, 5, 10, 25, 50, and 100/g of air-dry soil; in the second experiment, they were added at 0, 6, 12, 24, 48, 96, and 192/g. In both tests, propagules could not be recovered by this method when added to soil at 50/g or less. The most probable number technique of Harris and Sommers (1) cannot detect accurately fewer than 46/g of air-dry soil. Because it is unlikely that naturally infested soils contain 50 propagules per gram of soil or more, the most probable number technique was dropped from our experiments. Further tests with the P_5VPP-BH medium were performed with the dilution-plate method.

Performance of the P_5VPP-BH medium with the dilution-plate method. The average percentage of sporangia of isolate S1 recovered on the P_5VPP medium with the dilution-plate method (dilution, 1 in 8) was 60%, with a range at the 95% confidence level of 57.2–62.8. (Table 3). The percentage recovery of zoospores from the artificially infested soil was much lower (22%), with a range of 28.2–15.8. Similar results were obtained with isolates P1 and ATCC 15399.

The P_5VPP-BH medium was superior to all other media and combinations tested not only because recovery on the medium was good, but also because the *P. capsici* colonies were distinct and easy to identify when wet agar surfaces were viewed with an oblique fluorescent light (Fig. 1A). The mycelium is branched closely and profusely with small swellings at the tips of lateral hyphae or even on the main branches (Fig. 1B). Many hyphae also developed repeated angles in an alternating fashion. The colonies begin to develop sporangia in 6–7 days.

Recovery of *P. capsici* (isolate S1) from mycelium (not bearing any kind of spores) that had been added to soil 2 wk or less before the assay, was excellent on the P_5VPP-BH medium (Table 4). There was a sizable drop in recovery between the second and fifth week. This experiment not only showed that the medium is satisfactory for isolating mycelia of *P. capsici*, but also that mycelial fragments can survive in soil to some extent, but not indefinitely (Table 4). *P. capsici* is not known to produce chlamydospores (5). Also, mycelia exposed to moist soil on nylon netting for several weeks did not develop sporangia or any other kind of propagules (G. C. Papavizas and J. H. Bowers, *unpublished*). Further experiments are needed to study survival of *P. capsici* propagules in soil.

Although an average of 60% of sporangia were recovered (Table 3), an intermediate percentage, we should take into account the fact that sporangia of *P. capsici* are quite delicate and subject to lysis and destruction in soil (G. C. Papavizas, *unpublished*). Sporangia may release zoospores in less than 5 min in water (4). Because the isolation procedure involves several steps in water, several sporangia may release zoospores before they come into contact with the agar surface. The low recovery of zoospores may than account for the less-than-perfect recovery of sporangia from soil.

Recovery from artificially and naturally infested field soils. An average of 27 propagules per gram of air-dry soil were recovered on the P_5VPP-BH medium with the dilution-plate method (1 in 4, 1 in 8, and 1 in 10 dilutions) from a Beltsville field plot infested with isolates P1 and S1 1 day before assay (Fig. 2). Although the pairing of P1 with S1 results in oospore formation on the clarified V-8 juice agar (4), and these isolates were grown together for 1 mo on autoclaved oats, no colonies on the P_5VPP-BH medium appeared to originate from oospores. Most colonies originated from sporangia. No attempt was made to see whether oospores were developed on autoclaved oats.

The number of propagules per gram of air-dry soil recovered declined rapidly 6 days after infestation until none was recovered 40

TABLE 4. *Phytophthora capsici* isolate S1 recovery by the dilution-plate method on medium P_5VPP-BH[a] from soil infested with mycelial fragments

Dry wt of mycelium per 50 g of soil (mg)	Colonies per gram of air-dry soil (no.)			
	zero time	1 wk	2 wk	5 wk
0	0	0	0	0
1.1	100	97	110	14
2.3	270	240	290	36
3.4	410	400	440	61
4.8	490	495	460	90

[a] The basal medium, a modification of the P_{10}VP medium (9), contained Difco cornmeal agar (17 g/L) acidified to pH 3.8–4.0 with 3.5 ml 1.0 N HCl (added after autoclaving). The medium (P_5VPP-BH) contained also (μg/ml): pimaricin, 5; vancomycin, 200; pentachloronitrobenezene, 100; penicillin G, 100; benomyl, 2.5; and hymexazol, 20.

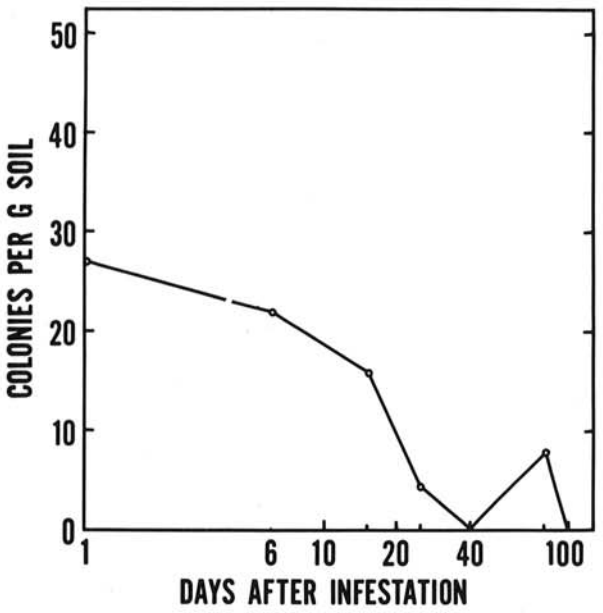

Fig. 2. Average number of viable propagules of *Phytophthora capsici* per gram of air-dry soil in an artificially infested field plot, as determined by the dilution-plate method on the P_5VPP-BH medium at intervals up to 100 days after infestation.

TABLE 5. Average number of viable propagules of *Phytophthora capsici* per gram of soil in naturally infested fields in southern New Jersey as determined by the dilution-plate method[a] on the P_5VPP-BH[b] medium and the occurrence and distribution of the two compatability types in the fields

Field no. and crop[c] planted in 1979	Colonies per gram of air-dry soil			Isolations made[d] (total no.)	Compatability types (abundance)	
	May 14	June 25	August 7		A^1	A^2
1 Squash	0	14	0	36	0	36
2 Squash	0	0	2	14	0	14
3 Pepper	0	11	7	13	4	9
4 Squash	0	4	12	16	16	0
5 Eggplant	0	0	0	0	0	0
6 Pepper	0	3	2	3	3	3
7 Pepper	0	24	5	18	11	7
8 Pepper	0	0	0	0	0	0

[a] Dilutions of 1:4 and 1:8 were used.
[b] The basal medium, a modification of the P_{10}VP medium (9), contained Difco cornmeal agar (17 g/L) acidified to pH 3.8–4.0 with 3.5 ml 1.0 N HCl (added after autoclaving). The medium (P_5VPP-BH) contained also (μg/ml): pimaricin, 5; vancomycin, 200; pentachloronitrobenzene, 100; penicillin G, 100; benomyl, 2.5; and hymexazol, 20.
[c] All three crops were planted after 1 June.
[d] Isolations were made from dilution plates and from infected plants.

days after infestation, or 4 days after plowing under the pepper plants (Fig. 2). About eight propagules per gram of soil were recovered 80 days after infestation (or 44 days after plants were plowed under) and none after 100 days.

The complete disappearance of *P. capsici* within about 30 days after infestation is not surprising because the soil samples for dilutions were obtained from between pepper rows (10 cm from the plants), an area that may not have been affected directly by the diseased, but undisturbed plants. There is nothing in the literature on the survival of oospores, sporangia, zoospores, or mycelia of *P. capsici* in soil. The recovery of *P. capsici* after peppers were disked into the soil may have resulted from mixing fresh sporangia or mycelia from the surviving pepper plants into the soil matrix. We were unable to find any oospores in infected pepper plants that were disked into soil. New studies are needed on the survival of *P. capsici* propagules, especially oospores, in soil. These studies, however, will require special techniques because oospores do not germinate readily on agar media (4).

Eight fields from southern New Jersey thought to be naturally infested with *P. capsici* were assayed periodically with the dilution-plate method on the P_5VPP-BH medium. No propagules were recovered from the commercial fields on 15 April and 14 May 1979, about 4 and 2 wk, respectively, before planting or transplanting plants susceptible to *P. capsici* (Table 5). The highest recovery was obtained on 25 June or 7 August, depending on the field assayed. The highest number of colonies obtained was 24/g of air-dry soil from field 7. Fields 5 and 8 had no measurable populations and blight did not appear on eggplant or pepper, respectively, up to the final assay time. The isolation data in Table 5 were obtained with soil samples collected from within plant rows. No *P. capsici* was recovered by the dilution-plate method from the four pepper fields assayed on 30 October, about 1 wk after disking the residue. These data indicate that the P_5VPP-BH medium is acceptable as an isolation medium from naturally infested soils and support our early contention that most commercial fields contain less than 50 propagules per gram of air-dry soil.

One hundred isolations were made from infected squash and pepper plants grown in the fields (Table 5) and directly from dilution plates prepared with soils from these fields. Thirty-four isolates belonged to the compatability type A^1 and 66 to type A^2. All isolates from squash in field 1 and from dilutions in field 2 were type A^2. Both mating types were isolated from fields 3, 6, and 7. All four isolates from plant segments in field 3 (type A^1) and five of the nine isolates from field 3 (type A^2) came from infected peppers; the other four of type A^2 came from soil collected within the rows. All seven isolates from plants in field 7 were of type A^2 and all isolates from soil were type A^1. Oospores also were found in infected plants from field 7, but the compatability type of the oospores could not be established. These results indicate that, at least in the pepper-growing areas of southern New Jersey, both compatability types of *P. capsici* can be found within single fields and also suggest that the pathogen may survive as oospores.

Our pathogenicity studies and microscopic observations verified that most of the 100 isolates from six fields in Table 5 belonged to *P. capsici*. All isolates from fields 2, 3, 4, 6, and 7 were highly pathogenic on peppers. Of the 36 isolates from squash from field 1, five were highly pathogenic, three were nonpathogenic and the remainder were moderately pathogenic on peppers. The morphological characteristics of sporangia and pedicels coincided with those established for *P. capsici* (5).

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