Water Requirements for Sporangium Formation by *Phytophthora parasitica* in Relation to Bioassay in Soil

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ABSTRACT

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Formation of sporangia by *Phytophthora parasitica* from tomato was examined in unsterilized soil at constant matric potentials (ψ_m) between 0 and −300 mb (soil water content between saturation and field capacity) using three types of inocula: mycelium, chlamydospore-bearing mycelium, and infected tomato tissue. Maximum numbers of sporangia were produced between −25 and −300 mb from the first two inocula and between −50 and −300 mb with infected tomato tissue. Production of sporangia was reduced significantly at $\psi_m = -10$ mb and reduced to nearly nil at $\psi_m = 0$ with all inocula. Artificially and naturally infested soil samples, air-dried for 10–45 days and rewetted to either −300 or 0 mb for 4–5 days, were flooded and assayed by green tomato fruit baiting. Detection of *P. parasitica* was more efficient from dried soil samples that had been wetted to −300 mb before saturation than from those saturated immediately after collection. Soil samples collected during the summer months from two infested, dry-fallowed tomato fields usually failed to yield *P. parasitica* when flooded and assayed immediately after collection. The fungus was readily detected, however, when comparable soil samples were wetted to −300 mb for 4–5 days, then flooded and assayed. Soil samples collected during the growing season from 37 processing tomato fields in the Sacramento Valley were assayed by three baiting procedures, which (before flooding) involved the following treatments: no treatment (direct flooding), prewetting to −300 mb, and air-drying for 3 wk followed by wetting to −300 mb. The three procedures were about equally efficient for qualitative detection of the pathogen, but only the third resulted in significantly different bait infection levels among samples from fields with obviously diseased and apparently no diseased plants, respectively. Some fields with no obviously diseased plants were infested, but significantly higher inoculum levels were demonstrated in soil from fields with evident disease. Green tomato fruits facilitated the selective trapping of *Phytophthora* spp. pathogenic to tomato plants.

Soilborne *Phytophthora* spp. have interspecific and intraspecific differences in water requirements for sporangium formation in soil (5). Some species require saturated or flooded soil for significant sporangium production (16,20), whereas other species or isolates require matric potentials (ψ_m) of −25 to −300 mb and are inhibited in saturated soil ($\psi_m = 0$) (3,4,6,12,13). Behavior of different *Phytophthora* spp. or isolates in saturated soil has been attributed to inherent interspecific and intraspecific differences in aeration and/or moisture requirements for sporangium formation (5) as well as to different experimental conditions, particularly the types of inocula used (6).

As suggested by Duniway (3), the paucity of sporangium formation in saturated or flooded soil by certain *Phytophthora* spp. may adversely affect their isolation from soil by baiting techniques in which plant tissues, incubated in the water over flooded soil, are infected by zoospores (2,7,10,14,17, 18,21,25). If no or few sporangia are produced in flooded soil, the pathogen may not be isolated unless sporangia are already present (3).

In this study, the influence of soil moisture on the formation of sporangia by *P. parasitica*, the main causal agent of root and crown rot of processing tomato in California (9), was examined quantitatively using three types of inocula. Water requirements for sporangium formation, once determined, were used to improve the qualitative and quantitative bioassay of the pathogen in infested soil. A preliminary report of this work has been published (8).

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MATERIALS AND METHODS

Sporangium formation in soil at controlled matric potentials. The influence of soil moisture on the production of sporangia in soil was examined using three types of inocula: mycelial disks, chlamydospore-bearing mycelial mats, and infected tomato fruit tissue. An isolate of *P. parasitica* from tomato (isolate DM30-2, A2 mating type) was used in all experiments.

To obtain mycelial disks, *P. parasitica* was grown on lima bean agar for about 10 days at 20 C in the dark, where it produced abundant aerial mycelium but no sporangia or chlamydospores (3). Disks of agar (5 mm in diameter) with aerial mycelium were obtained with a cork borer, washed in sterile distilled water (SDW) three times for 10 min, coated with a thin layer of moist soil, placed in small envelopes of fiberglass window screen, and buried in soil at a depth of 5 mm (3).

Chlamydospores were produced in 350-ml prescription bottles using the procedure described by Tsao (24). Chlamydospore-bearing mycelial mats were washed thoroughly with SDW, divided with forceps into small pieces about the same size as the 5-mm-diameter mycelial disks, and buried in soil as described before. Only mats in which no sporangia could be detected were used in these experiments.

Green tomato fruits (cultivar 145-B-7879) were washed in tap water, surface-disinfested in 0.5% NaOCl for 5 min, rinsed in SDW, and placed in glass petri plates containing a zoospore suspension of *P. parasitica*. After 72 hr, fruits with numerous brown lesions were removed from the plates and surface-disinfested as before. Infected (brown) areas of the fruit wall were used to prepare 5-mm-diameter disks, which were buried in soil as described for the mycelial disks. When the fruit disks were obtained, infection was superficial and the inner surface of the fruit wall was still green. No sporangia or other spore forms could be detected by microscopic examination of either brown or green fruit wall tissue.

Two soils were used: U.C. soil mix (1:1 mixture of fine sand and peat) and Yolo fine sandy loam (YFSL) collected from a field plot near Davis, CA. Both soils were sieved (1.6-mm openings) and used in the experiments without sterilization. The U.C. soil mix was used in all experiments, whereas the YFSL was used only as mentioned in the Results section. Soil moisture was controlled by using 9-cm-diameter Büchner funnel tension plates with hanging columns of water (3,4). All experiments were done at room temperature (22–25 C) and each treatment comprised five replicate funnels. After the incubation period, screen envelopes containing inocula were retrieved from soil and rinsed gently in water to eliminate the largest soil particles. Sporangia formed in soil by mycelial disks or by infected tomato tissues were estimated using the blending-centrifugation procedure described by Duniway (3). In experiments with chlamydospore-bearing mycelial mats, the inoculum was

examined with a microscope before blending, to determine the presence or absence of sporangia and whether the sporangia had originated from chlamydospores. Finally, the inoculum was processed as mentioned before and expressed as the number of sporangia per 100 chlamydospores in the original inoculum.

Matric potential and efficiency of soil bioassay. Influence of ψ_m on detection of *P. parasitica* in soil by green tomato fruit (GTF) baiting was examined using both artificially infested U.C. soil mix and naturally infested YFSL soil. Inoculum for artificial soil infestation was prepared by culturing *P. parasitica* on vermiculite-V-8 juice broth (V8JB) substrate in 1-L mason jars (9). After about 2 wk at 22–25 C, colonized vermiculite was added to steamed U.C. soil mix at 1:25 (v/v) and thoroughly mixed by hand. The YFSL soil was collected from a heavily infested tomato field plot near Davis, CA, 3 days after furrow irrigation. The two soils were spread in shallow layers on a greenhouse bench (20–28 C) and air-dried for 0, 10, 20, or 45 days to kill sporangia already present (12,13,19,22). After drying, four 350-ml subsamples were taken from each soil and placed on tension plates at ψ_m values of either 0 (saturation) or −300 mb (field capacity). After 4–5 days of incubation, each 350-ml soil sample was spread in a plastic box and flooded with water to about 1 cm from the soil surface. Green cherry tomatoes (cultivar Cherry Large), free of bruises, wounds, or infections, were washed in tap water, surface-disinfested in 0.5% NaOCl solution for 5 min, rinsed in SDW, and placed on the flooded soil (25 fruits per box) with their blossom ends touching the soil surface. Readings for symptom development were taken daily for 5 days and results were expressed as average percentage of infected fruit.

Bioassay of dry-fallowed soils. Soil samples were collected near Woodland, CA, during the summer from two dry-fallowed fields in which tomato crops in previous years had severe losses from Phytophthora root and crown rot. Repeated attempts to detect *P. parasitica* in these soils with the standard (direct-flooding) baiting technique either had failed completely (field A) or given erratic results (field B). To improve the detection of the pathogen, the following modification of the GTF baiting technique was tested: soil samples were placed on tension plates (350 ml of soil per plate) and adjusted to $\psi_m = -300$ mb to induce sporulation. After 4–5 days at 22–25 C, soil samples were flooded with water and assayed by GTF baiting as described. This procedure was compared with the standard (direct-flooding) bioassay, using 10 soil samples collected from 10 sites in each of the two fields. Percentage of infected fruit was scored as described before.

Quantitative bioassay of tomato field soils. Soil samples were collected from 37 processing tomato fields in the Sacramento Valley of California during the 1977 growing season. On the basis of presence or absence of evident aboveground *Phytophthora* symptoms, 18 fields were classed as infested and 19 as uninfested. From each field, one composite sample was taken from 20 sites underneath tomato vines. Each soil sample was assayed by three modifications of the GTF baiting technique, which (before flooding) involved the following soil treatments: no treatment (standard, direct-flooding procedure); prewetting to field capacity; and air-drying for 3 wk, then rewetting to field capacity. For each bioassay procedure and soil sample, three replicate 350-ml subsamples were used.

Percentage of bait infection was determined daily for 5 days as before. In addition, severity of symptoms on each fruit bait was assessed and rated on a scale from 0 (no evident infection) to 4 (submerged part of the fruit covered by coalescing brown spots). Disease severity ratings were then used to calculate a fruit infection index (FII) using the following formula: $FII = (0 \times N_0) + (1 \times N_1) + (2 \times N_2) + (3 \times N_3) + (4 \times N_4)$, where N_0, N_1, N_2, N_3, and N_4 are numbers of fruit baits (of 25) with the respective symptom severity rating. Thus, with this formula, the potential FII values range from 0 to 100.

Selectivity of the baiting technique. Pasteurized U.C. soil mix was artificially infested with 21 different isolates of *Phytophthora* or *Pythium* (Table 1) using inocula grown on vermiculite-V8JB. To test for pathogenicity of these isolates to tomato plants, infested soil was placed in 20-cm plastic pots to about 5 cm from the top. A layer of uninfested soil (1 cm) was added and 20 tomato seeds (cultivar 145-B-7879) were placed on the surface and covered with 1 cm of sand. For each isolate, there were five replicate pots and pathogenicity was assessed as the number of plants killed within 50 days. Controls consisted of five pots containing uninfested U.C. soil mix. Greenhouse temperatures ranged from 20 to 28 C.

Susceptibility of GTF baits to these isolates was determined using the soil bioassay method already described. For each isolate, there were five replicate 350-ml soil subsamples, each baited with 10 green tomatoes (cultivar 145-B-7879). Fruits of this cultivar were shown in a separate experiment to have susceptibility to *Phytophthora* infection similar to the cherry tomatoes used previously. Daily readings were taken for 5 days to determine incidence of fruit infection and type of fruit symptoms induced by each isolate. Fruit symptoms were classified into three types: 1) typical "buckeye" rot,

Table 1. Pathogenicity of *Phytophthora* and *Pythium* spp. to tomato plants in relation to the efficiency of their bioassay in soil by baiting with green cherry tomatoes[a]

Species	Isolate[b]	Percentage of dead plants[c]	Fruit bait infection[d]	
			Percentage	Type[e]
Phytophthora				
cactorum	20-1-9	0	0	0
cambivora	15-4-3	0	5	2
capsici	3-1-9	100	85	1
	DM 1538*	100	100	1
cinnamomi	18-4-5	2	3	1,2
citricola	16-2-5	2	20	2
citrophthora	19-1-3	0	0	0
cryptogea	18-2-1	0	13	1,2
drechsleri	13-1-1	0	7	2,3
erythroseptica	17-1-7	3	15	2,3
lateralis	10-1-3	0	0	0
megasperma	20-2-9	0	20	2,3
nicotianae	5-1-5	0	20	2
palmivora	2-2-9	0	0	0
parasitica	17-2-1	97	100	1
	DM 30-2*	100	100	1
phaseoli	10-1-5	0	10	2,3
syringae	14-4-9	0	0	0
Phytophthora sp.	DM 26A-10*	83	15	1
Pythium				
ultimum	DH-1*	15	0	0
aphanidermatum	DH-2*	12	0	0
Uninfested control	···	0	0	0

[a] Pathogenicity and bioassay tests were conducted in the greenhouse (20–28 C) using artificially infested U.C. soil mix.

[b] Isolates followed by an asterisk originated from diseased tomato plants; all other isolates were provided by S. M. Mircetich, University of California, Davis.

[c] For each isolate, there were five replicate pots, each with 20 tomato seedlings (cultivar 145-B-7879). Records of killed plants were taken for 50 days.

[d] For each isolate, there were five replicated 350-ml soil samples, each assayed with 10 green tomato fruits (cultivar 145-B-7879). Infected fruit baits were recorded after 5 days of incubation.

[e] Fruit symptoms were classified into three types: 1 = typical "buckeye" rot, 2 = tiny superficial brown lesions that failed to expand, and 3 = watery soft rot (0 = no symptoms).

2) tiny superficial brown lesions that failed to expand, and 3) watery soft rot.

RESULTS

Sporangium formation in soil at controlled matric potentials. *P. parasitica* produced abundant sporangia within 1 day after mycelial disks were placed in U.C. soil mix. Maximal numbers were present from the second to the fifth days, and thereafter, sporangial numbers decreased; however, sporangia were still present after 25 days of incubation (Fig. 1).

Influence of ψ_m on production of sporangia in U.C. soil mix from all three inocula was similar (Fig. 2). When mycelial disks or chlamydospore-bearing mycelial mats were used as inocula, production of sporangia was maximal at all ψ_m values between −25 and −300 mb, whereas with infected tissues, the optimal ψ_m range was between −50 and −300 mb (Fig. 2). In nearly saturated soil ($\psi_m = -10$ mb), sporangium production was reduced significantly and no, or very few, sporangia were produced in completely saturated soil ($\psi_m = 0$) from all three inocula (Fig. 2).

Inocula of all three types were recovered from saturated soil and examined carefully by direct microscopic observation for small numbers of sporangia that might not have been detected by the blending-centrifugation procedure but none were found. Sporangia produced in soil by chlamydospore-bearing mycelial mats originated mostly from chlamydospores. The origin of some sporangia could not be determined, however, so it is possible that some sporangia formed from mycelia rather than from chlamydospores.

Matric potential and efficiency of soil bioassay. Exposure of GTF baits to artificially infested U.C. soil mix or to naturally infested tomato field soil that had not been air-dried resulted in 100% infection within 1 day of flooding. Results were similar whether soil samples were flooded directly or prewet to field capacity before flooding (Tables 2 and 3).

Air-drying reduced and/or delayed infection of bait fruit in both soils. This adverse effect of air-drying on the recovery of the pathogen, however, was alleviated by rewetting the soil to field capacity 4–5 days before flooding. Both the effect of air-drying and the alleviating

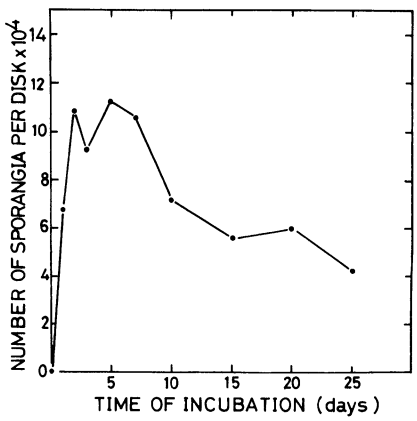

Fig. 1. Influence of time of incubation on production of sporangia by mycelial disks of *Phytophthora parasitica* in unsterilized U.C. soil mix at −100 mb matric potential. Inoculum was buried 5 mm below the soil surface and temperature of incubation was 22–25 C. Values are averages of five replicates.

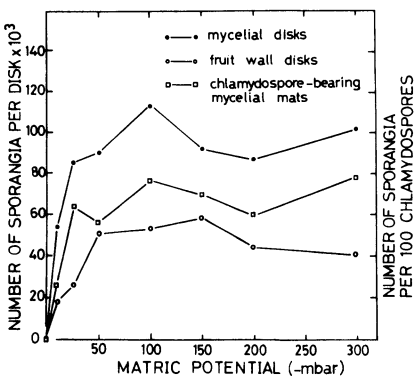

Fig. 2. Influence of matric potential on production of sporangia by *Phytophthora parasitica* in unsterilized U.C. soil mix. Inoculum consisted of mycelial disks (closed circles), disks from walls of infected green tomato fruits (open circles), and chlamydospore-bearing mycelial mats (squares). All inocula were buried 5 mm below the soil surface and sporangia per disk or sporangia corresponding to 100 chlamydospores (for chlamydospore-bearing mycelial mats) were determined after 3–5 days of incubation at 22–25 C. Values are averages of five replicates.

Table 2. Influence of different air-drying and rewetting treatments on detection of *Phytophthora parasitica* in artificially infested U.C. soil mix by baiting with green cherry tomatoes[a]

Air-drying[b] (days)	Rewetting treatment[c] (4–5 days)	Infected baits after indicated days of incubation (%)[d] 1	3	5
0	None	100	100	100
	Field capacity	100	100	100
	Saturation	100	100	100
10	None	0	0	49
	Field capacity	92	100	100
	Saturation	0	37	88
20	None	0	0	18
	Field capacity	63	100	100
	Saturation	0	0	25
30	None	0	0	9
	Field capacity	38	89	100
	Saturation	0	0	14
45	None	0	0	0
	Field capacity	0	35	74
	Saturation	0	0	0

[a] Inoculum for artificial soil infestation was grown on a vermiculite-V-8 juice broth substrate and added to steamed U.C. soil mix at 1:25 (v/v).
[b] For air-drying, soil was spread in shallow layers on a greenhouse bench at 20–28 C.
[c] Samples of air-dry soil (each 350 ml) were placed on Büchner funnel tension plates at 22–25 C and moisture was adjusted to either field capacity or saturation.
[d] Figures shown are averages of four replicates, each with 25 fruit baits.

Table 3. Influence of different air-drying and rewetting treatments on detection of *Phytophthora parasitica* in a naturally infested Yolo fine sandy loam soil by baiting with green cherry tomatoes[a]

Air-drying[b] (days)	Rewetting treatment[c] (4–5 days)	Infected fruit baits after indicated days of incubation (%)[d] 1	3	5
0	None	100	100	100
	Field capacity	100	100	100
	Saturation	100	100	100
10	None	0	93	100
	Field capacity	35	100	100
	Saturation	0	100	100
20	None	0	68	93
	Field capacity	0	100	100
	Saturation	0	77	85
45	None	0	15	35
	Field capacity	0	87	100
	Saturation	0	5	11

[a] Soil for this experiment was collected from a heavily infested tomato field plot 3 days after furrow irrigation.
[b] For air-drying, soil was spread in shallow layers on a greenhouse bench at 20–28 C.
[c] Soil moisture was adjusted to either field capacity or saturation using Büchner funnel tension plates at 22–25 C.
[d] Soil samples (each 350 ml) were flooded with water and assayed by incubating 25 green cherry tomato baits in each flooded soil sample for 5 days at 20–28 C. Figures shown are averages of four replicates.

effect of rewetting to field capacity before flooding were more pronounced with longer periods of air-drying (Tables 2 and 3). In artificially infested U.C. soil mix in particular, *P. parasitica* was not detected after 45 days of air-drying unless the soil was first wetted to field capacity before flooding (Table 2).

Bioassay of dry-fallowed naturally infested soils. Prewetting to field capacity greatly improved the detection of *P. parasitica* in soil samples from both of the dry-fallowed fields (Table 4). The pathogen was not detected in any of the 10 samples of field soil when the standard (direct-flooding) bioassay method was used but was readily detected in eight of 10 samples after a prewetting treatment (field capacity) before flooding. Bait infection in eight positive samples averaged 43% (Table 4). Similar results were obtained with field soil B, in which low levels of bait infection were observed in only three of 10 samples when no prewetting was applied, whereas all 10 samples resulted in relatively high levels of bait infection (84%) after prewetting to field capacity before flooding (Table 4). These results, showing that *P. parasitica* can survive in a dry-fallowed soil but in a form not detected by the standard bioassay procedure, were substantiated by heavy losses from Phytophthora root rot suffered by a tomato crop grown in field A the following year (N. Ioannou and R. G. Grogan, *unpublished*).

Quantitative bioassay of tomato field soils. The pathogen was detected with all three bioassay methods in soil samples from all 18 tomato fields that had evident Phytophthora root rot (Table 5). Twelve of the 19 apparently uninfested tomato fields were infested with *Phytopthora* when soil samples were assayed with or without prewetting to field capacity. The third method, involving air-drying of soil samples, failed to detect the pathogen in one of 12 samples.

Average FII values determined with either the standard or prewetting bioassay methods for fields with obviously diseased tomato plants were about equal to those determined for the apparently uninfested fields (Table 5). No differentiation could be made between the amounts of soil inoculum in the two groups of fields with either method. Such differentiation was accomplished when soil samples were processed with the third procedure, which involved a 3-wk air-drying treatment followed by rewetting to field capacity before flooding. Average FII values from this procedure were significantly higher for evidently diseased fields than for the apparently disease-free fields, especially on the second day of the assay.

Individual soil samples from different fields with diseased plants showed considerable diversity in their FII values, particularly after the second or third day of incubation.

Selectivity of the baiting technique. Of the 21 isolates tested, only two isolates of *P. parasitica*, two isolates of *P. capsici*, and one isolate of *Phytophthora* spp. were highly pathogenic to tomato plants (Table 1). Two species of *Phythium* (*P. ultimum* and *P. aphanidermatum*) and *Phytophthora cinnamomi, P. citricola,* and *P. erythroseptica* showed only low levels of pathogenicity and killed only a few plants (Table 1). *P. erythroseptica* also caused some stunting of inoculated plants.

Four isolates of *P. parasitica* and *P. capsici* also were trapped most effectively from flooded, infested soil by GTF baiting. Only the unidentified isolate DM26A-10 (*Phytophthora* sp.) was trapped poorly despite its severe pathogenicity to tomato plants (Table 1). This type of *Phytophthora* was isolated only once from a diseased tomato plant and its involvement in the etiology of Phytophthora root and crown rot of processing tomato in California is probably of minor importance. The two species of *Pythium*, also isolated from diseased tomato plants in the field, were not detected in soil by GTF baiting (Table 1). Various other *Phytophthora* spp., which were nonpathogenic or only slightly pathogenic to tomato plants, also were trapped at low levels by the GTF baits. Most of these species, however, produced fruit symptoms different from those produced by species pathogenic to tomato plants (Table 1).

Table 4. Influence of soil prewetting (field capacity) on detection of *Phytophthora parasitica* in two dry-fallowed, naturally infested field soils by baiting with green cherry tomatoes[a]

Soil wetting treatment[b]	Number of samples rated *Phytophthora*-positive[c]		Average percentage of infected fruit baits in positive samples	
	Soil A	Soil B	Soil A	Soil B
Direct flooding	0/10	3/10	0	4
Prewetting to field capacity	8/10	10/10	43	84

[a] Soil samples were collected during the summer from two dry-fallowed field plots in which tomato crops in the previous year had suffered severe losses from Phytophthora root rot. Ten composite samples were taken from 10 sites in each plot.
[b] From each soil sample, a 350-ml subsample was flooded and bioassayed without previous wetting (direct flooding); a second subsample was incubated on a tension plate at $\psi_m = -300$ mb for 4–5 days at 22–25 C (prewetting to field capacity), then flooded and assayed.
[c] Samples with one or more infected fruit baits (of 25) were rated as *Phytophthora*-positive.

DISCUSSION

Our results on effects of ψ_m on sporangium formation by *P. parasitica* in soil (Fig. 2) are in close agreement with reported results for *P. cryptogea* (3,4), *P. cinnamomi* (6), *P. palmivora* (6), and *P. megasperma* (12,13). Despite slight differences in optimal or lower limiting ψ_m values, sporangium formation of all

Table 5. Qualitative and quantitative bioassay of *Phytophthora parasitica* in soil samples from processing tomato fields with evident disease or apparently no disease using three modifications of the green tomato fruit baiting technique

Bioassay procedure[a]	Disease presence in sampled fields[b]	Qualitative detection of *P. parasitica*[c]	Average FII values of positive samples on indicated day of incubation[d]				
			1	2	3	4	5
Direct flooding	Evident	18/18	0	54	83	88	95
	Not Evident	12/19	0	52	71	79	99
Prewetting to field capacity	Evident	18/18	0	62	78	90	95
	Not Evident	12/19	0	57	86	92	92
Air-drying plus rewetting to field capacity	Evident	18/18	0	35	68	82	91
	Not Evident	11/19	0	6*	32*	49*	55*

[a] Triplicate 350-ml soil subsamples were taken from each composite sample and assayed with each procedure, using 25 green cherry tomato baits per subsample.
[b] Based on presence of aboveground symptoms of Phytophthora root rot.
[c] Numerator indicates the number of *Phytophthora*-positive samples and denominator the total number of samples tested. Samples were rated positive if at last one fruit bait was infected.
[d] FII (fruit infection index) values were determined from the incidence and severity of fruit bait infections as described in the text. Figures shown are averages of all positive samples, each with three replicates. An asterisk indicates significant difference between figures of the same pair ($P = 0.05$, t test).

these species was partially or completely inhibited in saturated or flooded soil, probably because of their sensitivity to reduced aeration (3,4). In contrast, *P. cambivora* and *P. drechsleri* (20), as well as an isolate of *P. megasperma* from alfalfa (16), produced maximal numbers of sporangia in saturated or flooded soil. This difference in behavior of different species or isolates of the same species, eg, isolates of *P. megasperma* (12,13,16), has been attributed by Gisi et al (6) to the different inocula employed by various workers rather than to inherent interspecific and intraspecific differences in aeration and/or moisture requirements for sporangium formation.

Indeed, most studies indicating inhibition of sporangium formation in saturated soil used mycelial inocula (3,4,6,12,13), whereas those indicating maximal production of sporangia in saturated soil used colonized host tissues as inocula (16,20). Furthermore, *P. megasperma* responded with inhibition of sporangium production in flooded soil from mycelial inocula, whereas the same isolate produced abundant sporangia when oospores or colonized tissues were used (11). In contrast, however, our results showed that the type of inoculum had no effect on the behavior of *P. parasitica* in saturated soil. Thus, the reported diversity in the behavior of different *Phytophthora* spp. or isolates may reflect inherent differences rather than differences in experimental conditions, as suggested by Gisi et al (6). Bernhardt and Grogan (1) found that both *P. parasitica* and *P. capsici* from tomato could form sporangia in saturated soil if first incubated for 2 days at −200 to −300 mb ψ_m. Evidently, once sporangia are initiated under favorable soil-water conditions, their further development is not inhibited in saturated soil.

Controlled moisture experiments (Figs. 1 and 2) were done at temperatures (22–25 C) favoring production of sporangia rather than chlamydospores (22,24). Nevertheless, small numbers of chlamydospores were also formed, especially on colonized tissues buried in soil at −150 to −300 mb ψ_m. Under these conditions, the ratio of chlamydospores to sporangia was about 1:4. Fewer chlamydospores were formed on colonized tissues buried in soil at −25 to −100 mb ψ_m and practically none were produced in soil wetter than −10 mb ψ_m. Thus, water requirements for chlamydospore formation by *P. parasitica* are similar to, or slightly drier than, those for production of sporangia (Fig. 2). Similar results have been reported for *P. cinnamomi* and *P. cactorum* (5).

Chlamydospores of *P. parasitica* are known to germinate and form sporangia in most natural soils (22) but effect of soil water tension on germination of chlamydospores has not been determined before. Knowing water requirements for sporangium formation by germinating chlamydospores is particularly important because chlamydospores are the principal means of survival for soilborne *Phytophthora* spp., especially for heterothallic ones such as *P. parasitica* (26). In the case of Phytophthora root rot of tomato in California, chlamydospores may play an even more important role in the survival of the pathogen than in other heterothallic species because all isolates studied so far belong to a single mating type that precludes oospores as survival units (9).

Despite the development of several *Phytophthora*-selective media (15,23), baiting techniques are still widely used for both qualitative and quantitative bioassays of *Phytophthora* spp. in soil (2,7,10,14,17, 18,21,25). These methods usually employ susceptible seedlings, fruits, leaves, or other plant tissues that are incubated as baits in water over flooded soil. Under these conditions, zoospores are the most likely infective propagules, and thus, as suggested by Duniway (3), species that fail to produce sporangia in flooded soil are not likely to be detected unless sporangia are already present in the soil before flooding. In this study, *P. parasitica* survived long periods of air-drying in both artificially and naturally infested soils but in a form that was not detected by GTF baiting unless the air-dry soils were rewetted and incubated at field capacity for several days before flooding (Tables 2 and 3). Apparently, the air-drying treatments, especially the longest one, eliminated the sporangia initially present in the soil (12,13,19); thus, the pathogen could not be detected unless provided with soil moisture conditions favoring formation of new sporangia (Fig. 2). Wetting the air-dried soil to saturation did not improve the detection of the pathogen (Tables 2 and 3), apparently because no sporangia were formed (Fig. 2). Preflooding of soil samples did not affect the detection of *P. nicotianae* var. *nicotianae* (25) and *P. megasperma* (17).

Naturally infested field soils, depending on their water status at the time of sampling, may also require prewetting to nearly field capacity for reliable bioassay by baiting. Dry soils, such as those from the two dry-fallowed fields used in this study, may be erroneously rated *Phytophthora*-negative if flooded and bioassayed directly but can be shown to be heavily infested if assayed after prewetting to field capacity (Table 4). Supportive results have been reported by Hoy et al (7), who found that soil from a tomato field heavily infested with *P. parasitica* resulted, within 16 hr, in 100% infection of cherry tomato baits if collected 3 days after irrigation but produced only about 10% infection if collected 28 days after irrigation.

Evidently, numbers of viable sporangia of *P. parasitica* in soil can vary greatly, depending on soil moisture and other environmental conditions prevailing at the time of sampling. Because of this variability, levels of bait infection obtained in soil bioassays also are variable and usually do not represent the actual inoculum potential in soil. Furthermore, samples collected from infested fields during the growing season, when environmental conditions are more likely to favor sporangial survival, usually result in near 100% bait infection, although their inoculum levels may be relatively low. Indeed, in this study, soil samples from tomato fields with and without diseased plants gave similarly high FII values when assayed by either the direct-flooding or the prewetting procedure (Table 5). However, the two groups of soil samples resulted in significantly different FII values if they received a 3-wk air-drying treatment followed by rewetting to field capacity before flooding and bioassay (Table 5).

Other investigators have also used baiting techniques to estimate quantitatively the inoculum potential of *Phytophthora* spp. in soil, mainly by bioassaying soil samples serially diluted with uninfested soil to determine the highest dilution at which infection occurred (14,17,21). Regardless of the approach, even with techniques that involve direct soil plating on selective media (15,18,23), results of quantitative assays will be more meaningful if the influence of soil water is taken into account. Inasmuch as production of sporangia by *P. parasitica* (Fig. 2), as well as by several other *Phytophthora* spp. (3,4,6,12,13), can occur over a fairly wide range of soil ψ_m values, for routine soil bioassays it usually is not necessary to control soil moisture precisely using tension plates. Simpler and more convenient methods such as watering soil samples in pots with drain holes can be as effective as tension plates.

Selective media for *Phytophthora* spp. (23) usually are not suitable for soil bioassays because they also allow the growth of *Pythium* spp. Recently, however, a selective medium has been developed that allows recovery of *Phytophthora* from soil without interference from associated *Pythium* spp. (15). Tomato fruit baits completely exclude *Pythium* spp. and also are selective for *Phytophthora* spp. pathogenic to tomato plants (Table 1), provided only green and unwounded fruits are used. For quantitative soil bioassays, this characteristic is important because it enables direct interpretation of the results without in vitro isolation of the pathogen. Thus, despite considerable progress in the development of *Phytophthora*-selective media, baiting techniques will still be useful for both qualitative and quantitative soil bioassays.

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