Detection and Quantification of *Phytophthora capsici* in Soil

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ABSTRACT

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Several assay methods were compared for their efficacy in the detection and quantification of specific propagule types of *Phytophthora capsici* in soil. Zoospores, oospores, or sporangia and mycelial fragments were added to microwave-treated soil and nontreated field soil at densities from 1 to 1×10^4 propagules per g (ppg) of soil. Assay methods included standard soil dilution plating on selective medium without prior sample incubation, as well as dilution plating of soil, saturation water, or a pepper leaf disk bioassay after sample saturation, drainage, incubation for 5 days, and a 24-h resaturation period. Zoospore inoculum was detected at 10 ppg of soil or higher with standard soil dilution plating and the leaf disk bioassay, compared to >100 ppg of soil with dilution plating of soil or saturation water after sample incubation. Sporangial inoculum was detected at 1 ppg of soil with all assays when soil water matric potential was controlled during sample incubation. Oospores were detected at 1 ppg of soil with soil dilution and leaf disk assays after sample incubation. Maximum recovery rates were 10 and 100% of added zoospore and sporangial inoculum, respectively, with standard soil dilution plating (no incubation), and 30% of oospore inoculum with soil dilution plating after sample incubation. A constant soil water matric potential of −10 J/kg during the sample incubation period improved inoculum recovery with all assays, compared to incubation without controlled soil water matric potential. The sample incubation and saturation periods stimulated oospore germination and allowed detection and recovery of oospores added to soil. For most assay methods, recovery of all propagule types was lower in field soil than in microwave-treated soil; however, the lower limits of detection were comparable in both soils. Although no single assay method was suitable for the accurate detection and quantification of all propagule types of *P. capsici*, the leaf disk assay provided the best detection of all propagule types but was not sufficiently quantitative to estimate inoculum densities. Soil dilution plating without prior sample incubation was the best assay for the quantification of zoospores and sporangia, whereas soil dilution plating after sample saturation and incubation provided the best recovery of oospores.

Phytophthora capsici Leonian occurs worldwide and causes a root and crown rot as well as an aerial blight of leaves, fruit, and stems on bell pepper (*Capsicum annuum* L.), tomatoes, and cucurbits (2,17,24). The disease is polycyclic, in that multiple cycles of inoculum production and infection occur within a growing season, and dispersal of inoculum from previously infected plants plays a significant role in the development of epidemics (24,25, 26,27).

Unlike some *Phytophthora* spp., oospores of *P. capsici* play an active role in the infection process (2,3,4,13). They are the primary overwintering propagule and are believed to be the initial source of inoculum in the field (4). Other propagule types, including zoospores, sporangia, and mycelia, may be more important in the dispersal of inoculum and subsequent spread of infection later in the development of an epidemic. Because all forms of inoculum may be involved in infection as well as dispersal of the pathogen, accurate detection and quantification of all propagule types is essential for monitoring and assessing the potential for disease in a field.

Assay methods used to isolate, detect, and quantify *Phytophthora* spp. in soil have been reviewed (28). The most common assay method is soil dilution plating using a selective medium. Several effective media have been developed that inhibit most other soil microorganisms and permit the isolation of *Phytophthora* spp. (14,19,23,28). Unfortunately, oospores do not germinate readily on any agar medium and are not generally accounted for in soil assays (12,20,23). Therefore, oospores must germinate to form mycelia, sporangia, or zoospores before this form of inoculum can be detected and quantified. Assay methods that stimulate oospore germination may improve detection of the pathogen in soil. Various baiting assays, which use susceptible plant parts like fruit, seeds, leaves, or seedlings, often are used to detect and isolate *Phytophthora* spp. from soils (28). Baiting assays can detect lower levels of inoculum than dilution plating but tend to be less quantitative (10,28). Despite the widespread use of variations of soil dilution plating and baiting assays, it is not clear how efficient these methods are at detecting and quantifying all propagule types of *P. capsici* in soil.

The matric component of soil water potential (ψ_m) plays an important role in the production and germination of propagules of *P. capsici* (2,3,4,6,7,13). Sporangia of *P. capsici* are produced primarily at a ψ_m between −1 and −30 J/kg (1 J/kg = 10 mb) (1,7). Zoospores are generally released from sporangia at a ψ_m between saturation (0 J/kg) and −1 J/kg (18). Oospores of *P. capsici* require a period of soil saturation before germination will occur, but oospore germination is infrequent under conditions of constant saturation (2,13). Bowers and Mitchell (2) reported that repeated saturation periods were required before substantial numbers of oospores would germinate and cause disease. Hord and Ristaino (13), however, determined that oospores of *P. capsici* germinate predominantly by production of sporangia and that germination is initiated when oospores in soil are incubated at a ψ_m of −2.5 to −10.0 J/kg prior to a single saturation period. Germination of oospores of *P. capsici* generally is low, asynchronous, and increases with time of incubation even under optimal soil water conditions (12, 13). Incubation of soil samples for 5 days at a ψ_m of −10 J/kg prior to flooding for 24 h stimulated oospore germination, spor-

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angium production, and zoospore release (13). Inoculum detection techniques that incorporate such methodologies to stimulate oospore germination prior to soil assays may be important for the evaluation of initial inoculum density for this pathogen in the field.

Molecular detection techniques, such as species-specific probes (10,11), restriction fragment length polymorphisms (22), and polymerase chain reaction analysis (5,8,16), have great potential for improving our current detection abilities for *Phytophthora* spp. However, these methods have not yet been fully developed for direct quantification of inoculum in soil at the field level. In addition, in some cases the pathogen may still need to be isolated from the soil or amplified in number before it can be identified by molecular techniques (10). Thus, for large-scale field applications, dilution plating and baiting assays are still the most widely used methods, but there is a clear need for assay techniques that detect all forms of propagules and that are quantitative.

The objective of this study was to compare the relative efficacy and sensitivity of several variations on soil dilution plating and baiting assays in the detection and quantification of specific propagule types of *P. capsici* in soil. This information will be useful in evaluating assays for use in epidemiological studies on the temporal and spatial dynamics of disease of *P. capsici*. Preliminary results of portions of this work have been published previously (15).

MATERIALS AND METHODS

Inoculum preparation. Isolates of *P. capsici*, B1H-B14 (A1 mating type) and B2H-H4 (A2 mating type), were isolated from pepper plants in a commercial field in Sampson County, NC. Propagule types used included zoospores, oospores, and sporangia and hyphae. Sporangia and hyphae were included together as inoculum because they are closely associated in soil and share similar characteristics as propagules. All cultures were grown on V8 agar (20) at 24°C for 7 days prior to production of specific propagule types. For zoospore production, V8 agar cultures were cut into pieces, flooded with sterile distilled water, and incubated in the light at 24°C for 72 h. Zoospore release was induced by chilling cultures at 5°C for 1 h and then incubating at 24°C for 30 to 60 min. Zoospore suspensions were filtered through eight layers of cheesecloth to remove hyphal and sporangial debris. Zoospore concentrations were counted using a hemacytometer and added to soil in a water suspension. Sporangial inoculum was produced by adding colonized agar pieces to 500 cm^3 of vermiculite and 250 ml of V8 broth in autoclaved 1-liter glass mason jars and incubating in the dark at 24°C for 4 to 5 weeks. Inoculum, consisting of sporangia and hyphae, was quantified by plating on Masago's *Phytophthora*-selective medium (19), and appropriate amounts of the V8-vermiculite preparation were added to soil on a weight per volume basis to achieve the particular inoculum level desired.

Oospores were produced by placing V8 agar disks that were colonized by opposite mating types 5 to 6 cm apart on plates of clarified V8 agar (12). Plates were incubated at 24°C in the dark for 2 months. Oospores formed in a distinct band between the two mating types. Agar strips containing oospores were removed from five petri plates and homogenized in 240 ml of sterile distilled water for 90 s in a blender. Oospores were concentrated by three successive centrifugations at 1,935 × *g* for 10 min. Agar and mycelial fragments were removed with a pipette, and the oospore pellet was resuspended in sterile distilled water. Oospore concentration was estimated with a hemacytometer and adjusted to 10^5 oospores per ml. A 10-ml aliquot of the oospore suspension was treated with 200 mg of Novozym 234 (Novo BioLabs, Danbury, CT), an enzyme preparation with cellulase, chitinase, and protease activity, to digest sporangia and mycelial fragments and incubated on a shaker at 28°C for 24 h. Oospores were concentrated, and the enzyme was removed by three successive centrifugations as described above. Final oospore concentrations were determined with a hemacytometer, and oospores were added to soil in a water suspension.

All propagules were used immediately after preparation. Inoculum densities of 0, 1, 10, 100, and 1,000 propagules per g (ppg) of soil were used for each propagule type for each assay method tested. An additional inoculum level of 10,000 ppg of soil was used for zoospore and sporangial inoculum in initial tests. Inoculum of each propagule type was added to 0.5 kg of soil for each inoculum level and mixed thoroughly. The soil used in all tests was a sandy loam (59.0% sand, 30.3% silt, and 10.7% clay) taken from field plots in Clinton, NC. Air-dried soil was passed through a 2-mm mesh sieve and stored in plastic bags until used. Tests were conducted using microwave-treated and natural (nontreated) field soil. Water was added to soil prior to microwaving to reach a gravimetric soil water content of approximately 10%. Soil was microwaved for 2 min/kg of soil at high power (700 W), which killed all *Phytophthora* spp. and most other fungi (9), to allow an unobstructed assay of added inoculum. The field soil allowed assessment of the assay methods under conditions similar to those in field situations.

Pathogen assay methods. Four assay methods were conducted with soil amended with each of the inoculum levels for each propagule type in two separate series of experiments. Standard soil dilution plating was compared with techniques that stimulated oospore germination through the manipulation of soil water matric potential via saturation, drainage, incubation, and resaturation periods. In the first series of experiments, three assay methods were tested using microwave-treated soil with a soil water matric potential (ψ_m) not controlled during the sample incubation period. In subsequent tests, an additional soil assay method was included, soil water ψ_m was held constant during sample incubation, and assays were made using field soil as well as microwave-treated soil. The assay methods tested included standard soil dilution plating immediately after infestation, as well as soil dilution plating, dilution plating of saturation water, and a pepper leaf disk bioassay, all after sample saturation, drainage, incubation, and resaturation periods. All assays consisted of three replications of each inoculum density/propagule-type combination.

Preliminary tests were conducted to compare the recovery of propagules of *P. capsici* on two *Phytophthora*-selective media. Propagules in water suspensions, as well as soil infested with propagules, were plated on Masago's selective medium (19) amended with 20 µg of hymexazol and on PARPH medium per g (14). The media were evaluated for ease and reliability of identification and differentiation of colonies of *P. capsici*, as well as for the level of detection and quantification of added inoculum. The medium providing easier identification and better recovery, calculated as the percentage of added inoculum recovered by each medium, was used in all subsequent plating assays.

In the first assay method, soil dilution plating was conducted after soil infestation without prior incubation of the sample. Forty grams of soil was added to 160 ml of 0.25% sterile water agar, stirred for 5 min, and 1-ml aliquots were plated on each of five plates of selective medium. At higher inoculum levels, additional 1:5 serial dilutions were made as needed. Plates were incubated in the dark at 24°C for 72 h, rinsed under running water to remove soil residue, and colonies of *P. capsici* were counted. Gravimetric soil water content of the soil samples was determined at the time of soil dilution and used to calculate inoculum density.

In all other assays, infested soil samples underwent a regime of soil saturation, incubation, and resaturation periods prior to estimation of inoculum density. In the first series of tests, in which ψ_m was not controlled during incubation periods, 40-g soil samples were placed in plastic cups with drainage holes, saturated briefly with distilled water, covered, and allowed to drain. After 5 days, drainage holes in the cups were plugged, and samples were resaturated with 60 ml of water for 24 h. This regime stimulates oospore germination (13). After the 24-h resaturation period, 1-ml aliquots of the saturation water, as well as 1:5 water dilutions, were plated on Masago's medium for the second assay method.

Plates were incubated, and colonies were counted as described above.

For the pepper leaf disk assay, five pepper leaf disks (0.5 cm diameter) were floated on the saturation water during the final resaturation period described above. After 24 h, the leaf disks were removed, surface-disinfested in 0.5% sodium hypochlorite for 1 min, rinsed in sterile distilled water, and plated on Masago's medium. Colonies of *P. capsici* were identified 72 h later, and the percentage of leaf disks colonized was determined.

For each inoculum density and propagule type tested, propagules also were plated directly onto Masago's medium to compare the optimum recovery on solid medium versus their recovery from soil by each assay method. Because oospores do not germinate directly on agar media, oospore germination also was tested in soil extract (12). Approximately 1×10^3 oospores were placed in 10 ml of soil extract, incubated for 12 days in the dark, and observed under a microscope to determine the number of germinating oospores out of 100 oospores in each of four quadrants of each petri dish. Each test was conducted at least twice for each propagule type and assay method.

In the second series of tests, soil water ψ_m was controlled, and several modifications in the sample incubation and assay procedures were made. In these tests, ψ_m was controlled using 350-ml Büchner tension funnels (6), and samples were incubated in a growth chamber at 24°C. Infested soil (100 cm^3) was placed in a tension funnel, saturated briefly by adjusting the ψ_m to 0 J/kg, and the ψ_m was adjusted to −10 J/kg. After 5 days at this constant ψ_m, soil was resaturated for 24 h. Dilution plating of the saturation water was conducted after 2 h of saturation in these tests, because greater numbers of zoospores were active at this time than after 24 h. An additional assay method, in which soil dilution plating was conducted after the saturation, incubation, and resaturation periods, also was included in these tests. After the 24-h resaturation period, ψ_m was returned to −10 J/kg for 2 to 4 h, and 40 g of soil from each funnel was plated as described previously. All other methods were as described above. All tests were conducted twice for each propagule and soil type for each assay method.

Statistical analyses were conducted using the general linear models procedures of the Statistical Analysis System, version 6.04 (SAS Institute, Cary, NC). Linear regression and analysis of covariance were conducted on log-transformed ($\log_{10}[x + 0.1]$) propagule counts and were used to characterize and distinguish inoculum recovery by the assay methods over all inoculum levels. Analysis of variance was conducted on media comparison and inoculum recovery data using a completely randomized design. Differences in inoculum recovery between field and microwave-treated soils over all inoculum levels were compared for each assay method by single degree of freedom planned contrasts. All tests for significance were conducted at $P \leq 0.05$.

RESULTS

Media comparison. Germination of zoospores was significantly greater on Masago's than on PARPH medium, as determined by the recovery of zoospores plated directly on the respective media (Table 1). Recovery of zoospore inoculum from soil was low on both media; however, recovery was greater on Masago's than on PARPH medium. Recovery of sporangial inoculum from microwave-treated soil by dilution plating also was significantly greater on Masago's than on PARPH medium (Table 1). Based on these and similar results in repeated tests, Masago's medium was used for all subsequent plating assays.

Pathogen detection and recovery with uncontrolled soil water ψ_m. Zoospore inoculum added to microwave-treated soil was detected at inoculum densities of 10 ppg and higher, and 7 to 10% of added inoculum was recovered at all inoculum densities when assayed by standard soil dilution plating without prior sample incubation (Fig. 1A). Direct plating of zoospores recovered an average of 62% of added inoculum over all inoculum densities. Zoospore inoculum was not detected at densities of 100 ppg of soil or lower by dilution plating of saturation water or pepper leaf disk assays. Sporangial inoculum added to microwave-treated soil was detected at densities of 1 ppg of soil or higher by standard soil dilution plating (Fig. 1B). Direct plating of sporangia and assay in soil by standard dilution plating recovered nearly 100% of added sporangial inoculum at all inoculum densities. Sporangial inoculum was detected at 10 ppg or higher by plating of saturation water, but recovery averaged only 7%. The pepper leaf disk assay also detected sporangial inoculum at 10 ppg or higher, and 100% colonization of leaf disks was observed at 1,000 ppg of soil. Recovery of both zoospore and sporangial inoculum by saturation water and leaf disk assays was significantly lower than by standard soil dilution plating according to analysis of covariance. Direct plating of oospores on Masago's medium resulted in <1% germination. Because of this low germination on media, oospores in soil could not be detected at inoculum densities of less than 100 ppg by any plating assay, and recovery was less than 1% (Fig. 1C). The leaf disk assay detected oospores at 10 ppg or higher with nearly 100% colonization of leaf disks at inoculum levels of 100 ppg or higher. All assay methods, with the exception of the leaf disk assay, demonstrated consistent recovery of inoculum at all inoculum levels for each propagule type, as demonstrated by the approximate linearity and constant slopes of the graphs in Figure 1A, B, and C.

Pathogen detection and recovery with controlled soil water ψ_m. Standard soil dilution plating without sample incubation detected zoospore inoculum at 10 ppg of soil, and recovery was 7.0% over all inoculum densities in microwave-treated soil (Fig. 2A). When soil was saturated and incubated at a ψ_m of −10 J/kg prior to resaturation, soil dilution plating did not consistently detect zoospore inoculum of 10 ppg or lower, and recovery was only 1% of added inoculum (Table 2). Dilution plating of saturation water did not detect zoospore inoculum of 100 ppg or lower and recovered only 0.2% of added inoculum. The pepper leaf disk assay detected zoospores at inoculum densities of 10 ppg or higher. Recovery of zoospores was significantly lower in nontreated field soil than in microwave-treated soil by soil dilution plating with or without sample incubation. Zoospores in field soil were detected at 100 ppg of soil by standard soil dilution assay but were not detected at 100 ppg or lower by dilution plating of soil or saturation water after incubation (Fig. 2B). Pepper leaf disk assay detected zoospores at 10 ppg in one test and at 100 ppg in a second test. Recovery of added zoospore inoculum in field soil was low (≤0.7%) for all assay methods (Table 2).

Sporangial inoculum was detected at densities as low as 1 ppg of soil by all assay methods in controlled ψ_m tests in microwave-

TABLE 1. Comparison of recovery rates of zoospore and sporangial inoculum of *Phytophthora capsici* directly or from soil by plating on two selective agar media at two densities

	Zoospore inoculum[y]			Sporangial inoculum[z]	
Medium	Density (ppg)	Direct plating (% recovery)	Soil plating (% recovery)	Density (ppg)	Soil plating (% recovery)
Masago's	1,000	65.6 a	7.2 a	100	122.0 a
PARPH	1,000	46.4 b	3.8 b	100	64.3 b
Masago's	5,000	67.5 a	6.1 a	300	102.9 a
PARPH	5,000	42.4 ab	4.4 ab	300	62.0 b

[y] Zoospores in a water suspension were adjusted to specific inoculum densities (propagules per g [ppg] of soil) and either plated directly on the selective media or added to microwave-treated soil and assayed by soil dilution plating. Values are shown as the percentage of added inoculum recovered by the assay. Means followed by the same letter within each column are not significantly different according to Fisher's LSD test.

[z] Inoculum density of sporangia and mycelial fragments in vermiculite-V8 culture medium was determined by dilution plating on nonselective medium and assayed by soil dilution plating.

treated soil (Fig. 3A). Incubation of soil samples at a constant ψ_m of −10 J/kg prior to resaturation resulted in recovery of greater numbers of propagules than were initially added to the soil at most inoculum densities when assayed by dilution plating of soil or saturation water after incubation. Recovery of sporangial inoculum was consistent at all inoculum densities for all three assay techniques and averaged 91.9, 97.3, and 384.6% of added inoculum for standard soil dilution plating, dilution plating of saturation water, and soil dilution plating after sample incubation assays, respectively (Table 2). The pepper leaf disk assay detected sporangial inoculum at densities of 1 ppg or higher, and 100% colonization of pepper leaf disks occurred at inoculum densities of 10 ppg or higher. Sporangial inoculum also was detected at densities of 1 ppg or higher in field soil by standard soil dilution plating, as well as by dilution plating of soil or saturation water after incubation (Fig. 3B). Recovery of added sporangial inoculum in field soil was significantly higher for the standard soil dilution assay than any other assay according to analysis of covariance. Recovery of sporangial inoculum was also significantly lower in field soil than in microwave-treated soil for all assay methods, except standard soil dilution plating (Table 2).

Oospore inoculum was detected at densities of 1 ppg or higher by pepper leaf disk or soil dilution assays after saturation and incubation at a constant ψ_m prior to resaturation (Fig. 4A). Recovery of added inoculum was highest by soil dilution assay after incubation and averaged 30% in microwave-treated soil (Table 2). In comparison, oospore germination under optimal conditions in soil extract was 33% after 12 days of incubation. Oospores were detected at 10 ppg by dilution plating of saturation water, and recovery was 14.3%. Oospore densities of 10 ppg or higher resulted in nearly 100% colonization of leaf disks in the leaf disk assay. Standard soil dilution plating did not detect oospore inoculum at densities of 100 ppg or lower. Oospore inoculum was detected in field soil at densities of 10 ppg of soil by soil dilution and leaf disk assays, at 100 ppg by saturation water assay, and at 1,000 ppg by standard soil dilution plating (Fig. 4B). Recovery of oospore inoculum by standard soil dilution and saturation water assays was significantly lower than by leaf disk and soil dilution after incubation assays in both microwave-treated and field soil according to analysis of covariance. Recovery of oospore inoculum by dilution plating of soil or saturation water after incubation was significantly lower in field soil than in microwave-treated soil

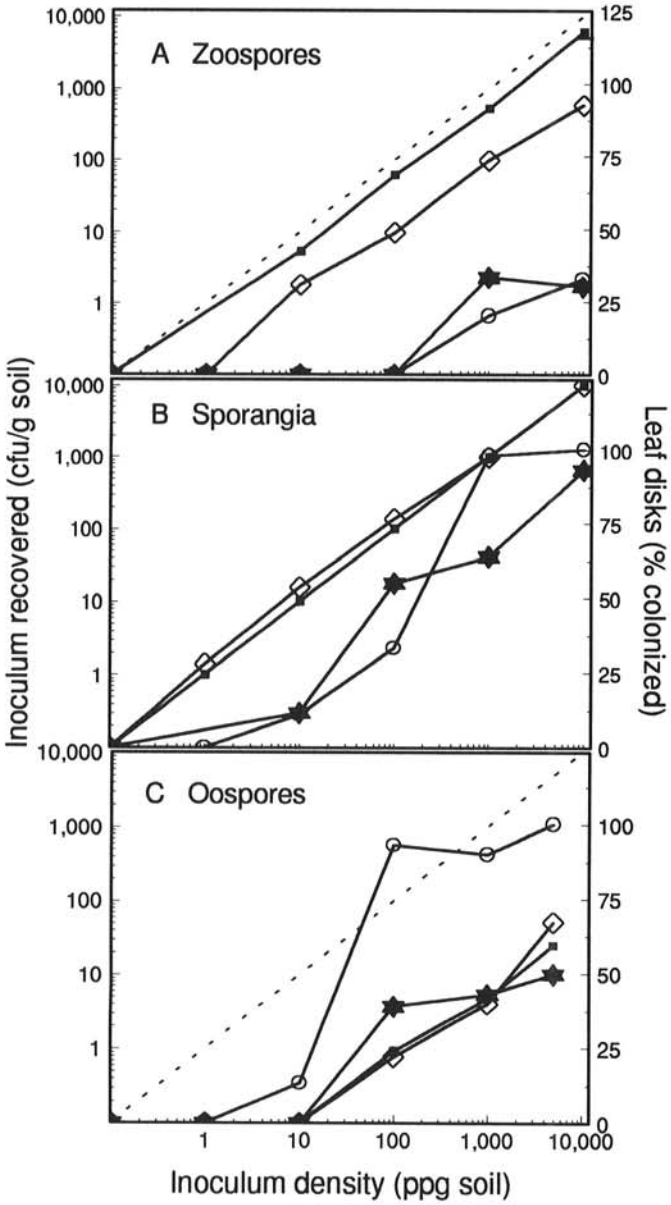

Fig. 1. Recovery of *Phytophthora capsici* added to microwave-treated soil as **A,** zoospores, **B,** sporangia and mycelia, and **C,** oospores by standard soil dilution plating without prior sample incubation (◊) or dilution plating of saturation water (∗) or a pepper leaf disk baiting assay (○) after sample saturation, drainage, incubation, and resaturation periods and with soil water matric potential not controlled during sample incubation. Propagules of each type also were plated directly on selective medium (■) for comparison. Dotted diagonal line (---) represents 100% recovery at all inoculum levels. Recovery by the pepper leaf disk assay is measured as the percentage of leaf disks colonized (right axis). All values are means of combined data from two similar experiments.

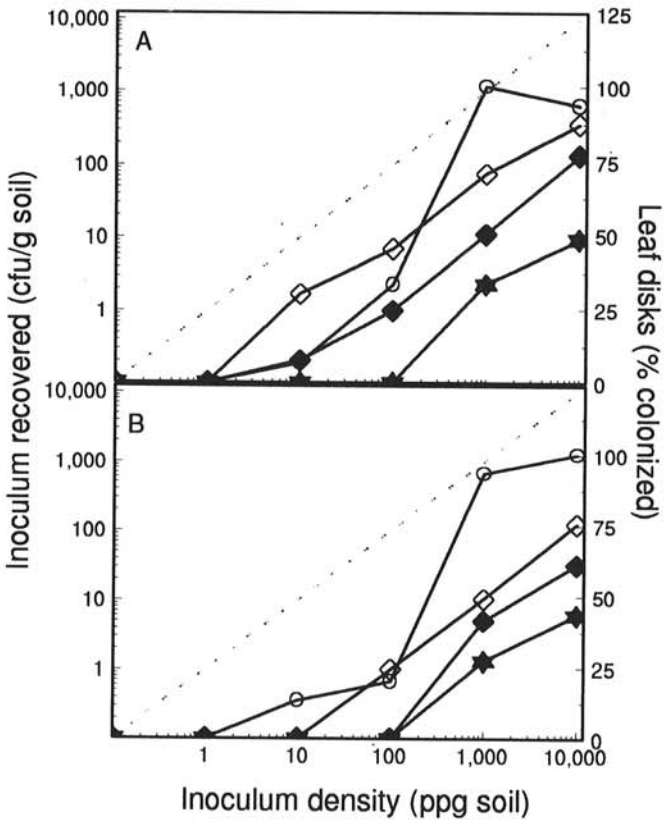

Fig. 2. Recovery of zoospores of *Phytophthora capsici* added to **A,** microwave-treated soil or **B,** nontreated field soil by standard soil dilution plating without prior sample incubation (◊) or soil dilution plating (♦), plating of saturation water (∗), or a pepper leaf disk baiting assay (○) after sample saturation, incubation at a constant soil water matric potential (ψ_m) of −10 J/kg, and resaturation. Dotted diagonal line (---) represents 100% recovery at all inoculum levels. Recovery by the pepper leaf disk assay is measured as the percentage of leaf disks colonized (right axis). All values are means of three replicates from a single representative experiment.

(Table 2). Similar results were observed in subsequent repeated tests with each propagule type for all assay methods and both soil types, although there was some variation in minimum detection levels observed between tests (Table 2).

DISCUSSION

In comparing several techniques involving sample incubation, soil dilution plating, and baiting assays, no single technique was equally effective for the detection and recovery of all propagule types of *P. capsici*. Zoospores added to soil were detected at 10 ppg of soil or higher by standard soil dilution plating without prior sample incubation. Inoculum added as sporangia and hyphae was detected at densities as low as 1 ppg of soil by all assay methods. Oospore inoculum was detected at densities as low as 1 ppg of soil by leaf disk and soil dilution assays after sample incubation. Maximum recovery of zoospore and sporangial inoculum was 10 and 100% of added inoculum, respectively, by standard soil dilution assay, whereas oospore inoculum was recovered at 30% only by soil dilution after sample incubation.

Zoospores are ephemeral propagules that only survive for short periods in soil, generally from days to weeks, in the field (3,4,7). Zoospores are most vulnerable to changes in environmental conditions, particularly changes in matric potential (7,18). There was an apparent reduction in viability when zoospores were added to soil in these tests. Recovery was never higher than 10% for any method, yet germination of motile or encysted zoospores on media averaged 60 to 70%. Other studies have reported similarly low recovery of zoospores from soil, with ranges from 1 to 20% (4,7, 21,23). Low recovery of this type may be due primarily to poor survival of these propagules in soil and does not necessarily represent inefficient recovery of zoospores by the assay methods. Recovery of zoospores was even lower after sample saturation and incubation, indicating that zoospores were either lost during drainage or did not survive well during the sample saturation and incubation regime. This lower recovery was not due simply to a change from motile to nonmotile zoospores, because counts were equally low for soil dilution plating after saturation, and loss in motility would not affect counts from soil platings.

Sporangia and hyphae added to soil represent inoculum present as vegetative stages of the pathogen. These propagules survived well in the soil for moderate periods (4 to 8 weeks) (4,7) and were recovered readily by most of the assay methods assessed. Propagule numbers recovered by techniques that involved sample saturation and incubation were greater than the numbers of sporangia originally added to soil, indicating that propagule numbers increased with sample incubation. These methods were effective in stimulating zoospore release from sporangia and, thus, overestimated the inoculum present as sporangia in the soil. Dilution plating without sample incubation, however, provided nearly 100% recovery of sporangial inoculum. Papavizas et al. (23) reported recovery of 60% of added sporangial inoculum with a different selective medium.

Oospores in soil have traditionally been the most difficult propagules to detect and quantify because they do not germinate readily in culture or on agar media (2,3,4,23,27). Because oospores can persist for long periods in soil and represent the initial inoculum at the time of planting, they can be important propagules in disease development (3,4,7). Bowers and Mitchell (3) reported that oospore inoculum of *P. capsici* as low as 1 ppg of soil caused disease in 5 to 35% of pepper seedlings. In our tests, soil dilution plating techniques were not effective in detecting oospores unless the soil was first saturated and incubated as prescribed in our assay methodology. With these methods, oospores were detected at 1 ppg of soil compared to not less than 1,000 ppg of soil with the standard soil dilution assay. Sample saturation and incubation resulted in oospore germination, giving rise to sporangia and zoospore production, which then could be assayed by plating on selective media. Oospore germination was limited, however, since a maximum of 30% of the added inoculum was recovered by the soil dilution after incubation assay. This level of recovery was comparable to the level of oospore germination (33%) observed under optimal conditions in soil extract after 12 days.

Although inoculum added as oospores was detected best by the pepper leaf disk assay and dilution plating after sample saturation and incubation, both of these methods had disadvantages. The pepper leaf disk assay was very good at detecting all propagule types but was not sufficiently quantitative. This assay gave only a relative indication of the amount of inoculum based on the percentage of leaf disks colonized, and the number of leaf disks colonized was not very consistent from one test to another. Leaf disk assays can be made quantitative when incorporated with most probable number (MPN) assays (10,21,27). MPN assays, however, require separate, replicated leaf disk assays for a wide range

TABLE 2. Detection limits and percent recovery of specific propagule types of *Phytophthora capsici* by various assay methods in microwave-treated soil and field soil with soil water matric potential controlled during sample incubation

	Assay method[z]							
	Standard soil dilution		Soil dilution plating		Saturation water		Pepper leaf disk	
Inoculum type[y]	Detection (ppg)	Recovery (%)	Detection (ppg)	Recovery (%)	Detection (ppg)	Recovery (%)	Detection (ppg)	Recovery (%)
Microwave-treated soil								
Zoospore	10	7.0*	10–100	1.3*	1,000	0.2	10	...
Sporangium	1	91.9	1	384.6*	1	97.3*	1	...
Oospore	1,000	0.8	1–10	30.2*	10	14.3*	1-10	...
Field soil								
Zoospore	10–100	0.7	1,000	0.4	1,000	0.1	10-100	...
Sporangium	1–10	67.5	10	44.4	1-10	14.2	1	...
Oospore	100–1,000	1.2	1–10	14.5	10-100	3.0	10	...

[y] Microwave-treated soil (2 min/kg, 700 W) and nontreated field soil were used in parallel tests. Inoculum of each propagule type was added to soil at levels of 0, 1, 10, 100, and 1,000 propagules per g (ppg) of soil. Values followed by an asterisk indicate a significantly ($P < 0.05$) greater recovery for that particular propagule type and assay method in microwave-treated soil than in field soil based on planned orthogonal contrasts. Analyses were conducted on the $\log_{10}$ of actual recovery values rather than on percent recovery. All values are based on combined data from two repeated tests for each soil type, incubation method, and inoculum type.

[z] Assay methods included (from left to right in the table) standard soil dilution plating without prior sample incubation or soil dilution plating, plating of saturation water, or a pepper leaf disk assay after sample saturation, incubation, and resaturation periods. Soil water matric potential (ψ_m) was adjusted to −10 J/kg after a brief saturation period, and soil was incubated for 5 days, followed by a 24-h resaturation period. Pepper leaf disks were floated on the saturation water and assayed for colonization on selective media. 'Detection' represents the lowest inoculum level detected by that assay method, and 'recovery' represents the percentage of inoculum added that was recovered by the assay method over all inoculum levels. Detection limits listed as ranges represent differences in detection observed in repeated tests.

of 1:2 serial dilutions of the saturation water and are very labor, time, and resource intensive. For large-scale assays from field samples, combining MPN techniques with baiting assays, though effective, generally is not practical or efficient (21). Thus, baiting assays remain useful for detection of *P. capsici* in the field but are of limited value in quantifying the amount of inoculum present. The soil dilution after incubation assay, on the other hand, was effective at detecting and quantifying oospore inoculum but overestimated sporangial inoculum in microwave-treated soil. This technique also was not as effective as the standard soil dilution assay for quantifying zoospores. Thus, soil dilution plating after sample incubation may not be reliable for quantifying inoculum in soils in which sporangia or zoospores are the primary propagule types. However, this technique may be beneficial for detecting the pathogen in soils with very low inoculum densities.

The plating of saturation water after sample incubation showed some promise as an assay. It was slightly quicker and easier than dilution plating, because only small aliquots of water were plated rather than larger soil volumes. Recovery of zoospores was best when saturation water was sampled within 1 to 4 h after saturation, because zoospore numbers declined thereafter. Recovery of inoculum by this method was generally lower than by soil dilution plating, however, and also was more variable, possibly due to heterogeneous distribution of zoospores in the water, the occurrence of nonmotile zoospores, and complications with the timing of zoospore release.

Lower limits of detection, greater recovery of inoculum, and lower variability was observed for most assay methods when soil incubation periods were conducted at a constant ψ_m of −10 J/kg soil. When ψ_m was not controlled, the rate of drainage of samples could not be regulated adequately, and samples may have become too dry, thus inhibiting oospore germination and reducing viability of other propagules. As a result, although more cumbersome to implement, controlling ψ_m during sample incubation improved all assays requiring an incubation period prior to inoculum estimation.

Recovery of *P. capsici* inoculum was better on Masago's selective medium with 20 ppm of hymexazol than on PARPH medium. Colonies were easier to identify at earlier stages of growth on Masago's than on PARPH. In addition, colony counts were higher on Masago's than on PARPH. This is in contrast to observations by Papavizas et al. (23) who reported that *P. capsici* was inhibited on Masago's medium by nystatin. We have tested numerous isolates of *P. capsici* on Masago's and have not encountered this inhibition. In additional tests with these media, however, we determined that the 10 ppm of pimaricin in PARPH was inhibiting *Phytophthora*. When the pimaricin concentration was reduced to 5 ppm, colony counts on PARPH were comparable to those on Masago's medium. This effect has been documented, and the lower pimaricin concentration has been recommended by several researchers (20,23).

Detection and recovery of all inoculum types was more difficult in field soil than in microwave-treated soil for all assay methods. In the field soil used in these tests, numerous *Pythium* and *Mortierella* spp. were present, and their resemblance to and interference with the growth of *Phytophthora* spp. on agar plates made identification and quantification difficult. Even with the selective media tested, some *Pythium* spp. were not inhibited and

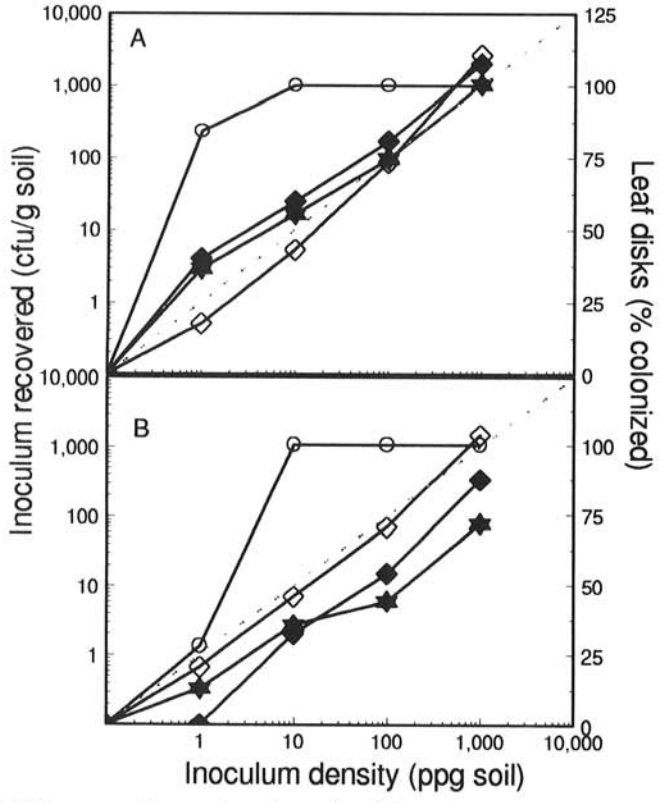

Fig. 3. Recovery of sporangia and mycelia of *Phytophthora capsici* added to **A,** microwave-treated soil or **B,** nontreated field soil by standard soil dilution plating without prior sample incubation (◊) or soil dilution plating (♦), plating of saturation water (✱), or a pepper leaf disk baiting assay (○) after sample saturation, incubation at a constant soil water matric potential (ψ_m) of −10 J/kg, and resaturation. Dotted diagonal line (---) represents 100% recovery at all inoculum levels. Recovery by the pepper leaf disk assay is measured as the percentage of leaf disks colonized (right axis). All values are means of three replicates from a single representative experiment.

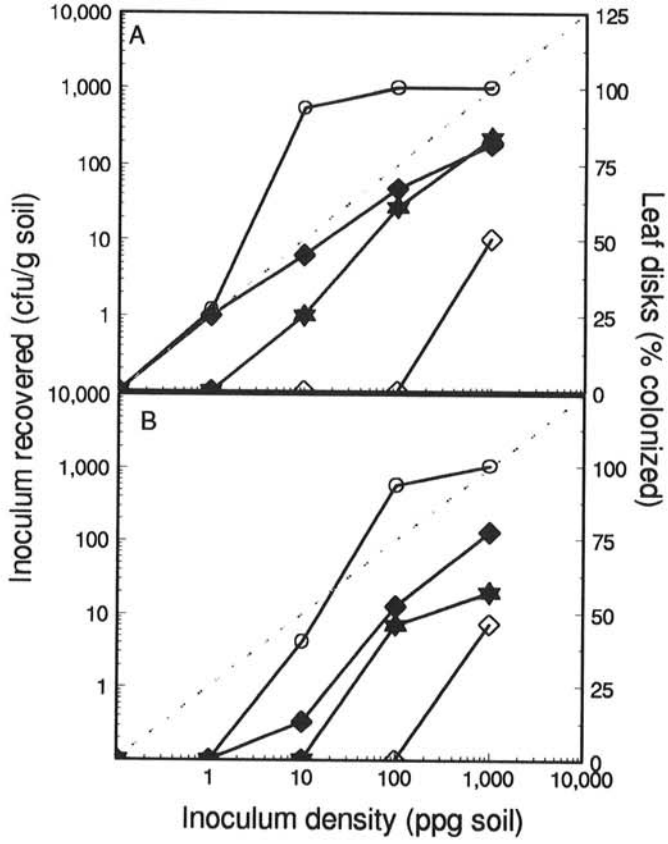

Fig. 4. Recovery of oospores of *Phytophthora capsici* added to **A,** microwave-treated soil or **B,** nontreated field soil by standard soil dilution plating without prior sample incubation (◊) or soil dilution plating (♦), plating of saturation water (✱), or a pepper leaf disk baiting assay (○) after sample saturation, incubation at a constant soil water matric potential (ψ_m) of −10 J/kg, and resaturation. Dotted diagonal line (---) represents 100% recovery at all inoculum levels. Recovery by the pepper leaf disk assay is measured as the percentage of leaf disks colonized (right axis). All values are means of three replicates from a single representative experiment.

produced colonies similar in morphology to *Phytophthora* spp. In addition, some colonies of *P. capsici* may have been restricted or overgrown by these fungi or other organisms present in the field soil, resulting in reduced counts and an underestimation of *P. capsici* inoculum density. However, even with the problems inherent with field soils, adequate quantification of inoculum was achieved. Detection levels generally were comparable in field soil and microwave-treated soil, although recovery levels of each inoculum type was lower in field soil than in microwave-treated soil for most assay methods.

Although no single assay technique was suitable for the accurate detection and quantification of all propagule types of *P. capsici*, our results indicated that the leaf disk baiting assay provided the most sensitive detection for all propagule types but, as expected, was not sufficiently quantitative to estimate population densities. Standard soil dilution plating without prior sample incubation was the best assay for the quantification of zoospore and sporangial inoculum, whereas soil dilution plating after sample saturation and incubation provided the best recovery of oospore inoculum. The best assay method to use depends on the objectives of the assay and the predominant propagule type likely to be present in the soil, which may change in the field during the course of the season or epidemic. Thus, the leaf disk or soil dilution after sample incubation assays may be necessary for the detection of initial inoculum in the form of oospores in the field, whereas standard soil dilution plating should give the best population estimates of active propagules during an epidemic and throughout the rest of the growing season. A combination of these assay methods may be necessary to accurately assess inoculum density in the field at different times. Conducting soil dilution plating assays both before and after sample saturation and incubation should provide the highest overall propagule estimates and will assure that all propagule types are represented.

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