Relationship Between Inoculum Level of *Phytophthora capsici* and Mortality of Pepper

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ABSTRACT

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Relationships between the levels of oospore and zoospore inoculum of *Phytophthora capsici* and mortality of pepper (*Capsicum annuum*) were investigated. Oospore progeny from 20 pairings of pathogenic isolates of *P. capsici* varied in pathogenicity to pepper at an initial inoculum density of 25 oospores per gram of soil. Only oospore inoculum from specific pairings caused disease in growth room experiments. The highest mortality (30–75%) resulted from oospore inoculum produced from pairings involving certain isolates of A2 compatibility type. Oospore inoculum from other pairings caused little or no disease (0–5%). Further tests with oospores produced from one pairing resulted in an increase in disease with an increase in inoculum density. The ID_{50} was calculated to be 41 oospores per gram of soil. Inoculum efficiency, defined as the estimated number of infections per propagule and calculated as the slope of the regression line when the estimated number of infections (via the multiple infection transformation) was regressed over inoculum density, was 0.011. Low numbers of zoospores applied to the base of an expanding pepper leaf were capable of causing high levels of plant mortality; 75–95% plant mortality occurred with three zoospores per plant. When zoospores were added to free water above flooded soil, 75 and 95% of the plants exposed to 10 and 25 zoospores per plant, respectively, died in some experiments.

Phytophthora capsici Leonian is a heterothallic organism in which two compatibility types, designated A1 and A2, are needed for sexual reproduction. The sexual structure is the thick-walled oospore, which is believed to be the main survival propagule and primary source of inoculum in the field (7). Both compatibility types of *P. capsici* have been found in the same field and in the same diseased plant from the field (24,25,34, J. H. Bowers and D. J. Mitchell, *unpublished*). Asexual reproduction takes place by the formation of sporangia, which germinate indirectly to release zoospores. Zoospores are considered to be transitory inoculum and are the main dispersal and infection units of the pathogen (27,28,30,42). Chlamydospores, asexual spores found in other species of *Phytophthora*, are not formed in any of the isolates of *P. capsici* from Florida, New Jersey, and Brazil from a range of hosts nor have they been found in nature (24,25, J. H. Bowers and D. J. Mitchell, *unpublished*).

Phytophthora blight of pepper (*Capsicum annuum* L.), caused by *P. capsici*, is a potentially destructive disease in southern Florida, occurring as two distinct phases: a crown rot and an aerial blight (47). Initial disease in the field usually occurs as the crown rot phase resulting from infection by residual inoculum in soil, such as oospores, or transitory inoculum, primarily zoospores, infecting the roots or the stem at the soil line. Infection of the roots or crown results in a general wilting and development of a characteristic purplish black lesion (or canker) advancing up the main stem from the soil line. The advancing lesion eventually girdles the stem and kills the plant.

Aerial infection of pepper is thought to result from sporangia and zoospores produced on diseased plant parts during favorable environmental conditions; inoculum is dispersed in wind-blown rain or moving water over the soil surface during periods of heavy rainfall or excessive irrigation (40). This mode of dispersal has been demonstrated for leather rot of strawberry, caused by *P. cactorum* (19), and for Phytophthora blight of papaya, caused by *P. palmivora* (21). With *P. capsici* on pepper and tomato, all that is known is that free water is required for infection with zoospores and sporangia (28,40). Penetration of pepper leaves after inoculation with motile zoospores began in about 2 hr and was completed by 4 hr (23). In a study on the role of zoospores in the crown rot phase of the disease, 10^4 zoospores of *P. capsici* caused 100% plant mortality when inoculum was poured around a pepper plant (3).

Studies with oospores of heterothallic species of *Phytophthora* as the initial inoculum source are rare. Twenty oospores of *P. capsici* per plant caused wilt in pepper plants when the oospores were mixed in soil (2); however, the volume of soil per plant and, thus, the initial inoculum density and the level of disease incidence, were not reported. The time for symptom expression varied with oospore concentration, but the relationship was not quantified. No infection of tobacco roots was observed in a study with oospores of *P. parasitica* var. *nicotianae* over a range of initial inoculum densities (22).

The elucidation of the roles of various propagules in the initiation of infection and subsequent disease development, as well as the efficiencies of propagules in causing infection and disease, are important aspects of the relationship of inoculum to host infection. Our objectives were to evaluate the hypotheses that oospores of *P. capsici* are capable of causing disease in pepper seedlings and that low levels of oospore and zoospore inoculum can cause significant amounts of infection and mortality of pepper. Portions of this research have been presented previously (9).

MATERIALS AND METHODS

Oospores of *P. capsici* do not germinate readily, and no efficient quantitative method exists to estimate the numbers of oospores in naturally infested soil. Therefore, oospores were produced in axenic culture and added to soil in defined numbers. Although oospores were produced in pepper tissue, sufficient quantities for the experiments reported herein were not obtained (7,8). Oospore inoculum was produced by placing four 1-cm^2 pieces of clarified V-8 juice (CV-8) agar (100 ml of clarified V-8 juice cleared by centrifuging V-8 juice amended with 15 g of $CaCO_3$ L^{-1}, 20 g of Difco agar [Difco Laboratories, Detroit, MI], and 900 ml of deionized water) with actively growing hyphae of two opposite

compatibility types in an Omni-mixer (Ivan Servall, Inc., Norwalk, CT) with 50 ml of clarified V-8 juice broth and homogenizing the suspension for 30 sec. One milliliter of the resultant suspension was added to 40 ml of clarified V-8 juice broth amended with cholesterol (Eastman Kodak Co., Rochester, NY) at 30 mg L^{-1} in a 250-ml Erlenmeyer flask and incubated in the dark at 25 C for 2 mo. In initial experiments, eight isolates of *P. capsici* (four A1 and four A2 compatibility types) from one farm in Delray Beach, FL, were paired in all possible combinations, and the oospores of 16 pairings were pooled for experiments. The isolates are listed in Table 1. (Isolate Cp-10 was not used in the pooled inoculum.) Isolates were originally selected based on positive pathogenicity tests with a low level of zoospore inoculum. Otherwise, only one pairing of two opposite compatibility types was used per experiment. Cultures were maintained on CV-8 agar and periodically transferred.

Oospores were harvested from mycelial mats in liquid culture. Six mycelial mats were combined in 55 ml of 10^{-3} M 2-(*N*-morpholino)-ethanesulfonic acid (MES) (Sigma Chemical Co., St. Louis, MO) in an Omni-mixer and homogenized for 30 sec. Buffer pH was adjusted to 6.1–6.2 with 0.1 N KOH. The resultant suspension of hyphae and oospores was diluted 1:1 (v/v) with a 1% solution of cellulase (EC 3.2.1.4, from *Penicillium funiculosum*) (Sigma Chemical Co., St. Louis, MO) in 10^{-3} M MES, which gave a final concentration of 0.5% cellulase, and the suspension was sonicated for 30 sec at 150 W (Braunsonic 1510, B. Braun Instruments, San Francisco, CA). After incubation for 18–24 hr in the dark at 25 C, the suspension was remixed in the Omni-mixer, sonicated again, and successively filtered through 150-μm and 75-μm nylon mesh with suction. Oospores were concentrated, and the cellulase and hyphal fragments were removed by successive centrifugations in 10^{-3} M MES for 2.5 min at approximately 1,500 rpm in a clinical centrifuge (International Equipment Co., Needham Heights, MA). Supernatant was drawn off with a Pasteur pipet. Centrifugation speed was reduced as the suspension became free of debris and until a reasonably pure suspension of oospores was obtained. Oospores were resuspended in 10^{-3} M MES, stored at 5 C overnight, and used the next day in experiments. Suspensions were plated on CV-8 agar and a selective medium (PARPH) containing 10 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifampicin, 100 mg of pentachloronitrobenzene, 50 mg of hymexazol, and 17 g of cornmeal agar (Difco) in 1 L of deionized water (22,32) to test for the presence of viable hyphal fragments and sporangia. Viable hyphal fragments or sporangia were not present in the inoculum for any experiment, and very few oospores (< 1%) germinated on either CV-8 or PARPH after 2–3 wk.

The relationship between the density of oospores in soil and infection was studied with the infested-soil-layer technique to allow uninjured roots to grow into an infested layer of soil (22). Microwaved field soil (110 g) from Delray Beach was infested with oospores at various densities, layered over 35 g of microwaved builder's sand, and covered with 125 g of microwaved uninfested soil in a 7.6-cm-diameter pot. The soil used in all experiments was a Myakka sand (sandy, siliceous, hyperthermic Aeric Haplaquod) (1) with an organic matter content of 0.95% and a pH of 6.6 in water. Raw field soil was air-dried, passed through a 2-mm mesh screen, and stored until used. Five kilograms of soil (at approximately 2% moisture) was microwaved for 7 min on high power (700 W) and stored for at least 1 wk before use (13).

Pepper seeds (cultivar Early Calwonder) were germinated in vermiculite and transplanted into the uninfested soil layer (one plant per pot) as the first true leaves were beginning to expand. Plants were maintained in a plant growth room at 25–27 C, with a 14-hr photoperiod (approximately 86 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation [PAR] at the level of the plants [LI-COR, Inc., Lincoln, NE]) and watered from below when the soil surface started to dry. Plants were not fertilized during the course of the experiments and were checked daily for the appearance of symptoms, which consisted of incipient wilt followed by constriction of the stem by a brown lesion extending up from the soil line. Experiments were performed four times with the bulked inoculum and seven times with a single pairing, with 20 plants per inoculum density. Each trial of an experiment did not contain all inoculum densities, but each inoculum density was repeated at least twice. A completely randomized design was used in all trials.

Plants were harvested as symptoms appeared and the plants were moribund. The entire root system and a small portion of the stem from each plant were washed free of soil in running water, dipped in 70% EtOH for 5–10 sec, rinsed twice in deionized water, and blotted dry on paper towels (22). Entire root systems were plated on PARPH and incubated at room temperature in the dark. The plates were observed for the characteristic growth of *P. capsici* from the stem and roots and checked microscopically. All symptomless plants were harvested at the conclusion of the experiments, typically 10–12 wk after transplanting, and the roots were plated on the selective medium. Only rarely was a plant infected without symptom expression at the conclusion of the experiments, thus, results are presented as the percentage of mortality.

Isolates of *P. capsici* were then transferred to CV-8 agar and tested for compatibility type. One or two isolation sites per root system were chosen based on the growth of the fungus into the medium. Compatibility type was determined by pairing each isolate with A1 and A2 compatibility type tester isolates on CV-8 agar amended with cholesterol at 30 mg L^{-1} and incubating the cultures at 25 C in the dark until the colonies overlapped. The tester isolates were Cp-9 (A1 compatibility type, isolated by G. C. Papavizas in 1978 from squash in Vineland, NJ) and Cp-10 (A2 compatibility type, ATCC 15399, originally isolated from bell pepper). Overlapped portions of the colonies were observed after 7 days for oospore formation. Isolates that formed oospores when combined with Cp-9 were designated A2, and those that formed oospores in combination with Cp-10 were designated A1.

Inoculum studies with zoospores of *P. capsici* were designed to represent zoospore dispersal and subsequent infection when water accumulates on the aerial portion of the plant and when water carries inoculum over the soil surface. Zoospores were

TABLE 1. Incidences of mortality in pepper seedlings after growth for 10 wk in soil infested with oospores from paired crosses of four A1 isolates and five A2 isolates of *Phytophthora capsici*[a,b]

Compatibility type		Percentage of mortality[c]	
A1	A2	Trial 1	Trial 2
Cp-6	Cp-7	40	55
Cp-6	Cp-10	75	70
Cp-6	Cp-11	0	0
Cp-6	Cp-13	0	0
Cp-6	Cp-15	0	5
Cp-8	Cp-7	55	30
Cp-8	Cp-10	50	70
Cp-8	Cp-11	5	0
Cp-8	Cp-13	5	0
Cp-8	Cp-15	0	0
Cp-12	Cp-7	30	10
Cp-12	Cp-10	40	55
Cp-12	Cp-11	0	0
Cp-12	Cp-13	0	0
Cp-12	Cp-15	0	0
Cp-14	Cp-7	40	35
Cp-14	Cp-10	65	50
Cp-14	Cp-11	0	0
Cp-14	Cp-13	0	0
Cp-14	Cp-15	0	0

[a] All isolates were selected based on positive pathogenicity tests on pepper and were isolated from one farm in Delray Beach, FL, except for Cp-10, which was obtained from the American Type Culture Collection. Isolate designations refer to the culture collection of *Phytophthora* spp. maintained by D. J. Mitchell.

[b] An initial inoculum density of 25 oospores per gram of soil was used for each cross.

[c] Mortality is expressed as the percentage of 20 pepper plants that died during the 8–10 wk of the experiments.

produced by a modified method used in previous work with species of *Phytophthora* (11,22,31,36). Isolate Cp-7 of *P. capsici* (A2 compatibility type), isolated from an infected pepper plant from the Whitworth Farm in Delray Beach, was used in all experiments. Four 1-cm^2 pieces of CV-8 agar with actively growing hyphae were placed in an Omni-mixer with 50 ml of CV-8 broth and homogenized for 30 sec. One milliliter of suspension was added to approximately 15 ml of CV-8 broth in a 15-cm-diameter petri dish and incubated at 27 C with a 12-hr photoperiod (approximately 42 μmol·m^{-2}·s^{-1} PAR at the level of the plates) for 5 days. Cultures were then rinsed four to five times with sterile 10^{-4} M MES, with the last two rinses at 15-min intervals. Buffer pH was adjusted to 6.1–6.2 with 0.1 N KOH. Cultures were incubated another 2 days in 10^{-4} M MES under the same conditions to produce sporangia. Zoospore release was induced by rinsing the cultures two to three times with 10^{-4} M MES, chilling at 5 C for 25 min, and allowing the cultures to return to room temperature. Abundant zoospores were observed after 25 min at room temperature. For use in experiments, the zoospore suspension from one petri dish was poured into a sterile beaker and diluted with 10^{-4} M MES until a concentration of approximately two to six zoospores per 2-μl droplet was reached. Encysted and motile zoospores were counted in 20 2-μl droplets, and the percentage of motile zoospores was recorded. Preliminary experiments indicated that encysted zoospores were also capable of causing disease; therefore, counts based on the total number of zoospores per droplet were used to calculate the volume of suspension needed for experiments. The zoospore suspension was stirred slowly with a small magnetic stir-bar during the time it took to count and perform the actual inoculations to distribute zoospores throughout the medium.

Pepper seeds were germinated in vermiculite and transplanted to microwaved field soil from Delray Beach in 5.7-cm-diameter pots when the first true leaves began to emerge. All plants were grown in an incubator at 25–27 C with a 14-hr photoperiod (approximately 42 μmol·m^{-2}·s^{-1} PAR at the level of the plants) and fertilized once with half-strength Hoagland's solution (20) 2–3 wk after transplanting. Plants were used in experiments when four to six true leaves had expanded.

The relationship between the number of zoospores and infection of the aerial portion of the plant was studied by placing pepper plants in a dew chamber (Percival Manufacturing Co., Boone, IA) at 18–20 C ambient temperature 1 day before inoculation. Plants were inoculated by placing microliter droplets containing the desired number of zoospores directly on the basal portion of a newly expanded leaf. Plants were returned to the dew chamber and incubated in the dark for 2 days and then returned to the incubator for 1 wk, after which symptom expression was visually assessed as the percentage of mortality. Preliminary experiments indicated that, under the conditions of the experiments, all infected plants developed disease symptoms and became moribund; conversely, *P. capsici* was not recovered from inoculated portions of symptomless plants that were placed on the selective medium. Plants were not plated in later experiments. One droplet was applied per plant, and 20 plants were used per inoculum level. Each trial of the experiment typically contained four inoculum levels, and seven trials were performed. Each inoculum level was repeated at least once. A completely randomized design was used in all trials.

The relationship between the number of zoospores and infection of the crown portion of the plant was studied by flooding the soil to a depth of approximately 1 cm above the soil surface and placing the desired number of zoospores in free water approximately 0.5–1.0 cm from the plant stem. This procedure simulated flooded conditions in the field. The flooded condition was maintained for 4 hr after inoculation, when the water was allowed to drain naturally. Wetted vermiculite was placed on the soil surface to impede drying, and the plants were returned to the incubator for 2 wk before disease was assessed. Initial experiments indicated that all infected plants developed symptoms. All symptomless plants were not infected, as evidenced by the absence of *Phytophthora* when they were plated on the selective medium. Thus, disease development was assessed visually after 2 wk and reported as the percentage of mortality. Each trial of the experiment contained five inoculum levels, and seven trials were performed. Each inoculum level was repeated in each trial. A completely randomized design was used in all trials.

RESULTS

In four trials to determine the relationship between the density of oospores in soil and disease incidence with a pooled suspension of oospores from 16 different pairings of isolates of *P. capsici*, mortality increased with an increase in the inoculum density. Mortality ranged from 5 to 35% at one oospore to 100% at 100 oospores per gram of soil (Fig. 1). The observed mortality at an inoculum density of 10 oospores per gram of soil ranged from 40 to 95% for the various trials.

In an attempt to reduce the observed experimental variation, we decided to use only one pairing of isolates in subsequent experiments. Oospores were produced with isolates Cp-6 and Cp-13, which originally were isolated from the same plant from a

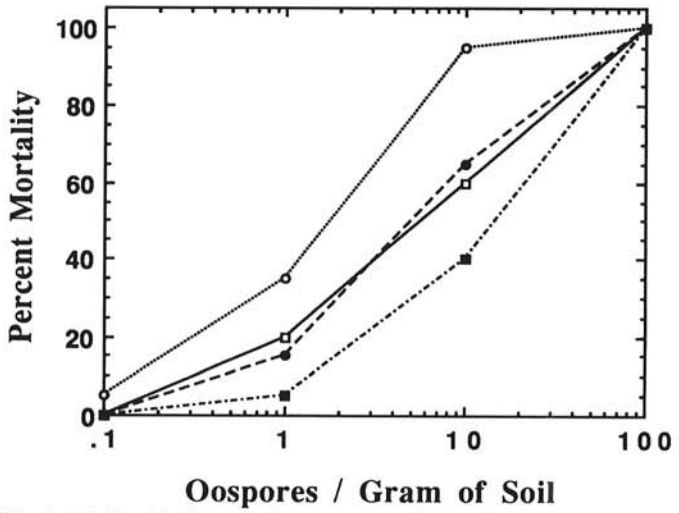

Fig. 1. Relationship between the percentage of mortality of pepper plants and the number of oospores of *Phytophthora capsici* per gram of soil for four trials of an experiment when oospores from 16 different pairings were pooled and used as inoculum; each point represents the percentage of 20 plants that died.

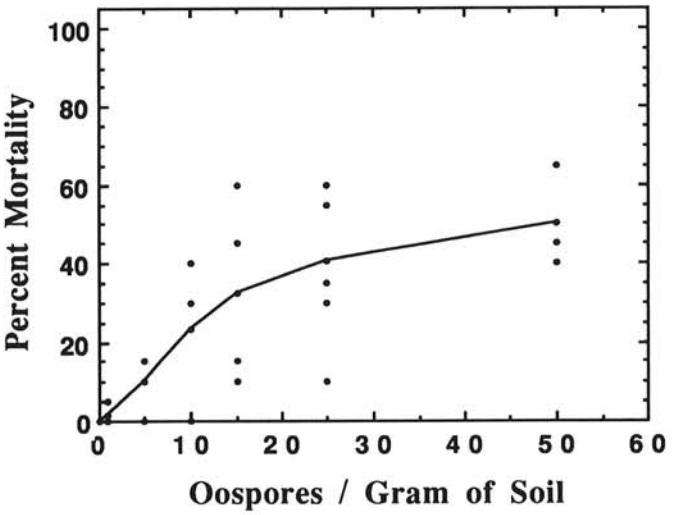

Fig. 2. Relationship between the percentage of mortality of pepper plants and the number of oospores of *Phytophthora capsici* per gram of soil when oospores from the pairing of isolates Cp-8 and Cp-7 were used as inoculum in seven trials; each point represents the percentage of 20 plants that died.

farm in Delray Beach. However, no disease occurred in two separate trials, and no infection was present when the roots were plated on the selective medium.

An experiment was then conducted to determine the relative virulence of oospores from individual pairings between isolates used for the pooled inoculum. Isolate Cp-10 was included in the tests as a non-Florida isolate. An inoculum density of 25 oospores per gram of soil was used to test oospores from each pairing of isolates. In two trials, only oospore inocula from certain pairings were able to cause disease in pepper seedlings (Table 1). The highest mortality was observed with oospore inoculum when each A1 compatibility type was paired with Cp-7 or Cp-10. Oospore inoculum from the other pairings produced little or no disease. The pairing of isolates Cp-8 and Cp-7 was selected to produce oospores for subsequent experiments.

A high degree of variation was observed in seven trials when the percentage of mortality was plotted over inoculum density, even though only oospores from one pairing were used as inoculum in the experiments (Fig. 2). For example, the percentage of mortality ranged from 10 to 60% with an inoculum density of 25 oospores per gram of soil. The overall amount of disease was lower than in the initial experiments with the pooled inoculum (Fig. 1). However, a substantial amount of disease occurred at low inoculum densities; the highest percentages of mortality with inoculum densities of 10 and 15 oospores per gram of soil were 40 and 60%, respectively.

Data on the percentage of mortality were transformed to account for multiple infections and expressed as $\ln(1/(1 - y))$, where y is the proportion of dead plants (46) (Fig. 3). Efficiency of inoculum was calculated as the estimated number of infections per propagule, or, simply, the slope of the regression when the estimated number of infections was regressed over the number of oospores per gram of soil. Thus, the efficiency of the oospore inoculum was 0.011. The ID_{50}, calculated from the regression equation, was 41 oospores per gram of soil. The variation in the data was expressed in a low r^2 value of 0.28 for the regression line ($P < 0.05$). When the transformed data were plotted on a $\log_{10}$-$\log_{10}$ basis (4,5), regression analysis yielded a slope of 0.63 ($P < 0.01$) (Fig. 4).

One hundred and sixty-four isolates of *P. capsici* were recovered from diseased plants in the experiment with the pooled oospore inoculum. The A1 compatibility type was recovered 75 times, and the A2 compatibility type was recovered 76 times. Twelve isolates did not form oospores with either tester isolate and were designated A0. One isolate, designated A+, formed oospores with both tester isolates. In the experiment with oospore inoculum from the pairing of Cp-8 and Cp-7, 215 isolates were recovered. One hundred and eleven isolates were A1 and 95 were A2. Two isolates were designated A0 and seven isolates as A+.

Low numbers of zoospores applied to the base of an expanding pepper leaf were capable of causing high levels of plant mortality (Fig. 5). Inoculation with three zoospores per plant resulted in 75% or greater mortality in four trials. Data transformed via the multiple infection transformation (excluding mortality values of 100%) and regressed over inoculum level yielded a slope of 0.05 that was not significantly different from zero ($P = 0.34$). Thus, a uniformly high level of disease occurred over all inoculum levels. An average of 78 ± 7% of the zoospores used in this experiment were motile.

The plot of the percentage of mortality over the number of zoospores per plant (Fig. 6) reveals the variability observed when zoospores were added to free water over a flooded soil. However, a high percentage of mortality also was observed with low numbers of zoospores. Ten and 25 zoospores per plant were able to cause 75 and 95% mortality, respectively, with the latter value repeated in different trials. Data transformed via the multiple infection transformation and regressed over inoculum level yielded a slope not significantly different from zero ($P = 0.87$); thus, the estimated number of infections did not increase with an increase in the number of zoospores. An average of 72 ± 11% of the zoospores used in this experiment were motile. A second 4-hr flooding period 2 wk after inoculation had no effect on further disease development (*data not shown*).

DISCUSSION

Low numbers of oospores of *P. capsici* caused significant mortality of pepper plants in our study. In contrast, Kannwischer and Mitchell (22) reported that when oospores of the heterothallic fungus *P. p. nicotianae* were used as inoculum, no plants were infected after 50 days at initial inoculum densities of up to 100 oospores per gram of soil. Because the quantification of oospore numbers of *P. capsici* in the field is not yet possible because of the low numbers of oospores that germinate in vitro (33,35, 39,44), we are unable to correlate density of residual inoculum in the field and disease incidence. However, we have shown that low levels of oospore inoculum of *P. capsici* may affect disease incidence in the field. Low numbers of oospores formed by

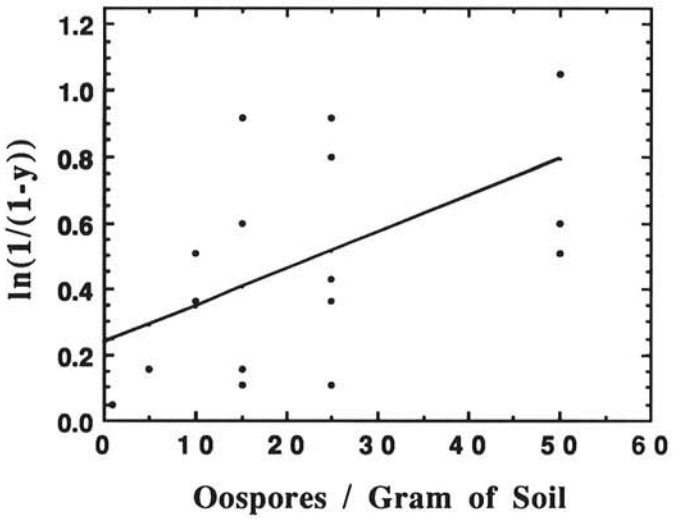

Fig. 3. Relationship between the multiple infection transformation [$\ln(1/(1 - y))$] of the proportion of mortality of pepper plants (y) and the number of oospores of *Phytophthora capsici* per gram of soil when oospores from the pairing of isolates Cp-8 and Cp-7 were used as inoculum; each point represents the transformed proportion of 20 plants that died. (The slope equals 0.011 [$r^2 = 0.28$, $P < 0.05$].)

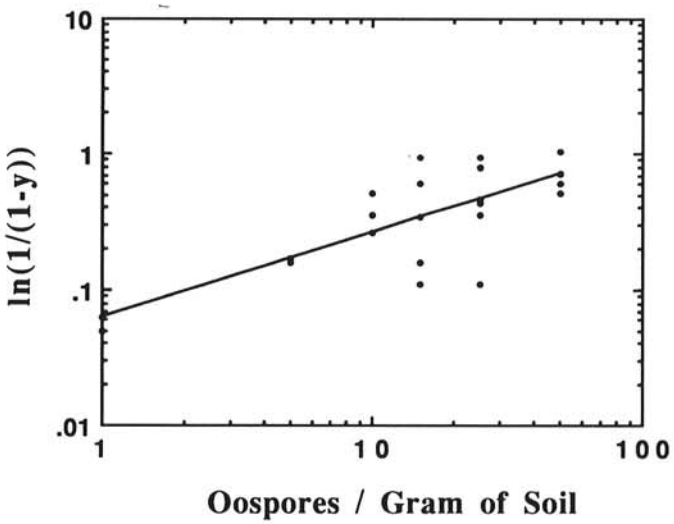

Fig. 4. Log_{10}-$\log_{10}$ plot of the relationship between the multiple infection transformation [$\ln(1/(1 - y))$] of the proportion of mortality of pepper plants (y) and the number of oospores of *Phytophthora capsici* per gram of soil when oospores from the pairing of isolates Cp-8 and Cp-7 were used as inoculum; each point represents the transformed proportion of 20 plants that died. (The slope equals 0.63 [$r^2 = 0.46$, $P < 0.01$].)

homothallic pythiacious fungi in other pathosystems have also been shown to cause a significant amount of disease (6,18,26,29). The calculated ID_{50} of 41 oospores of *P. capsici* per gram of soil determined in this study is within the range calculated for other host-pathogen combinations when oospores of *Phytophthora* and *Pythium* spp. were used as the initial inoculum (26,29,43).

Relationships of inoculum density to disease incidence often are reported as plots of $\log_{10}[\ln(1/(1 - y))]$ vs. $\log_{10}$[inoculum density] (4,5). In our study, the slope with oospores of *P. capsici* and pepper plants (Fig. 4) was 0.63, which is close to the slope of 0.67 that indicates a rhizoplane effect according to Baker (4,5). Similar slopes were calculated for oospores of *P. megasperma* var. *sojae* on soybean (26) and for oospores of *Pythium aphanidermatum*, *P. myriotylum*, and *P. polymastum* on various hosts (29). However, experimental evidence on the fate of oospores in soil and in the rhizosphere is lacking. Experimental information needs to be generated on the effects on oospore germination and survival of such factors as rhizosphere and rhizoplane microflora, chemical constituents (root exudates and lysates) and physical constraints (bulk density, soil water status, and aeration) in the soil environment in the presence and absence of a host root before the hypothesis of a rhizoplane effect can be supported.

Efficiency of oospore inoculum of *P. capsici* for infection of pepper plants was approximately 1.1% when defined as the ratio of the estimated number of infections to the number of oospores per gram of soil. English and Mitchell (12) reported inoculum efficiencies of 0.3–0.5% when efficiency was defined as the ratio of the observed number of infected tobacco roots per plant to the total number of chlamydospores of *P. p. nicotianae* within the volume of soil in which the plant was growing. They speculated that inoculum efficiency would increase with increasing time of plant growth in infested soil. In their experiments, tobacco plants were grown in infested soil for only 2 wk, whereas we grew pepper plants in infested soil for 10–12 wk. The longer exposure of the plants to the inoculum may have resulted in increased inoculum efficiency. Further, English and Mitchell (12) calculated inoculum efficiency based on the observed number of infected tobacco roots, whereas we estimated the numbers of infection indirectly via the multiple infection transformation from the proportion of dead pepper plants (46). Germination of oospores of *P. capsici* occurs at low frequencies (35,39,44); thus, estimates of infection efficiency may be much higher if based on oospore germination instead of initial inoculum density (45).

We recovered both compatibility types from one plant, and the assumption was made that plant death resulted in many instances from multiple infections from the initial oospore inoculum added to the soil. The probability of multiple infections, with an increased value of inoculum efficiency, supports the suggestion of English and Mitchell (12) that true values of inoculum efficiency would have been greater in their study if more than one infection had occurred per plant root. The probability of multiple infection would be enhanced further if either chlamydospores or oospores germinated and, as commonly assumed, formed germ sporangia, which then released zoospores that subsequently infected the plant.

Oospore progenies from different pairings of pathogenic isolates of *P. capsici* differed in their ability to cause disease in pepper seedlings (Table 1). All isolates of *P. capsici*, except isolate Cp-10, were isolated from diseased pepper plants from one farm in south Florida on the same day. Both compatibility types were isolated in equal numbers (*data not shown*). Genetic diversity was observed even though the isolates tested represent only a small proportion of the field population. Satour and Butler (39) and Polach and Webster (35) also observed that some progeny of pathogenic, paired isolates of *P. capsici* were nonpathogenic on pepper. Evidence of genetic recombination has been reported for pathogenicity and other phenotypic characteristics of several *Phytophthora* spp., based on analysis of single-oospore cultures (14,15,24,35,37,39). Single zoospore isolates from an individual oospore were of one genotype, but diverse genotypes were observed from oospores from a single cross (14,37,39). The genetic mechanism for the variation in oospore ability to cause disease has not been elucidated but may influence differential germination (35,37,39,44), hyphal viability (10), or oospore abortion (38,41).

The percentages of oospores that germinated and participated in the infection process are not known. It also is not known if symptoms resulted from a low number of initial infections from a few germinating oospores, with subsequent secondary spore production and infection, or from a high number of oospores germinating and participating in the initial infection of the plant. More research is needed to document the role of oospores in the infection process from the time they are added to soil until symptoms appear on the plant.

The roles of oospores of heterothallic species of *Phytophthora* other than *P. capsici* in nature is not known, and, mainly because of the low germination of oospores in vitro, genetic variation in these propagules has not been addressed to any extent. The role of oospores of a heterothallic *Phytophthora* spp. in disease was investigated in one study, but no infection was reported when oospores of *P. p. nicotianae* were used as inoculum (22). Only oospores from one pairing of isolates were tested, however. Based on our results, we suggest that lack of infection with oospores

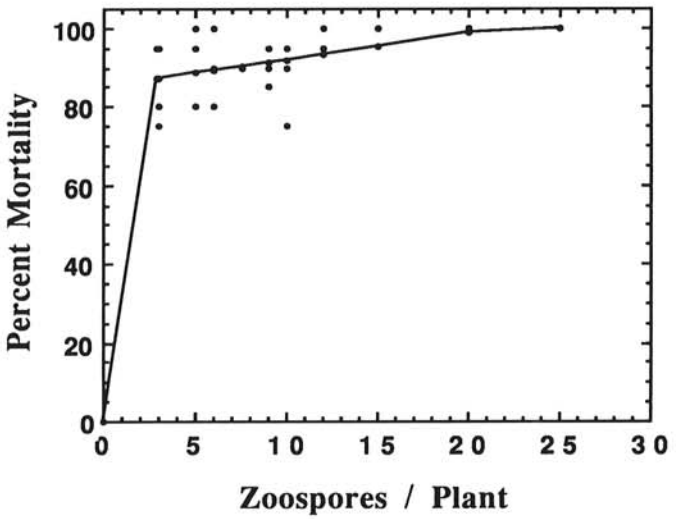

Fig. 5. Relationship between the percentage of mortality of pepper plants and the number of zoospores of *Phytophthora capsici* per plant when zoospores of isolate Cp-7 were placed in water on the basal portion of an expanding leaf in seven trials; each point represents the percentage of 20 plants that died.

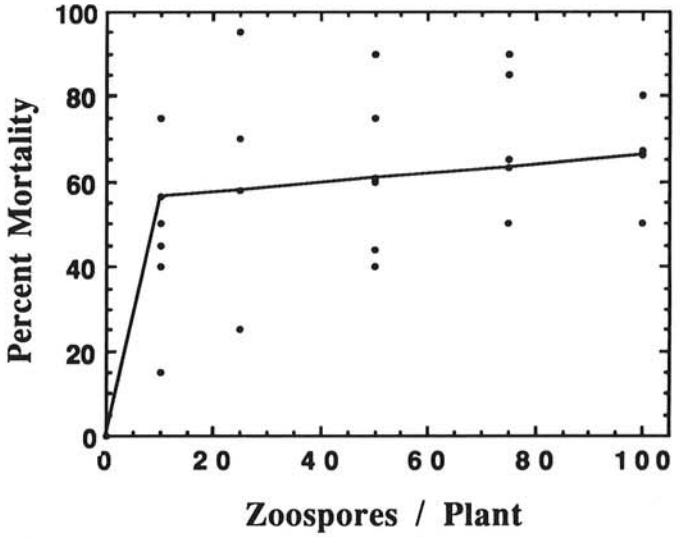

Fig. 6. Relationship between the percentage of mortality of pepper plants and the number of zoospores of *Phytophthora capsici* per plant when zoospores of isolate Cp-7 were placed in free water above a flooded soil in seven trials; each point represents the percentage of 20 plants that died.

from one pairing does not preclude infection from oospores from other pairings. Experiments designed to test the pathogenicity of oospore inoculum from several pairings within a population of isolates from one field and from various geographical regions may help define the role of oospores in the ecology of other heterothallic species of *Phytophthora* in nature. However, often only one compatibility type of many *Phytophthora* spp. occurs in a field or in an entire geographical region (48). The presence of both compatibility types of *P. capsici* in the same field may support the exceptional value of this fungus as a research model.

Both A1 and A2 compatibility types were recovered from diseased pepper plants in experiments with oospore inoculum. The ratio of A1 and A2 compatibility types was found to be 1:1 (75:76) when pooled oospore inoculum was used, and approximately 1:1 (111:95) when oospores from the cross of Cp-8 and Cp-7 were used. Ratios of 1:1 for compatibility type have also been reported for single-oospore isolates of *P. capsici* (35) and *P. drechsleri* (14). However, Satour and Butler (39) reported a 2:1 ratio of A1:A2 compatibility type in *P. capsici*, and Timmer et al (44) reported that the ratio between A1 and A2 compatibility types in *P. capsici* was almost always skewed in favor of the A1 compatibility type. In the field, *P. infestans* was reported to exist in equal numbers of A1 and A2 compatibility types in Mexico (17), and *P. capsici* was isolated in various ratios of A1:A2 compatibility types from pepper and squash fields in New Jersey (34). Other ratios are reported for other species of *Phytophthora* (15,37,41). Shaw (41) and Gallegly (16) discuss possible genetic explanations for the observed ratios reported in the literature.

Low numbers of zoospores of *P. capsici* caused high percentages of plant mortality when placed on expanding pepper leaves. All plants died at an inoculum level as low as five zoospores per plant. Further analysis was not attempted because of the high number of inoculum levels that contained data points of 100% mortality. We have shown, however, that zoospore inoculum is highly efficient given the appropriate conditions. Plants were maintained in the dew chamber for 48 hr after inoculation to optimize the conditions for infection. It is not known if an increase in inoculum occurred during incubation or if the observed mortality resulted directly from infection by the inoculum initially placed on the leaf and subsequent spread of hyphae in the plant tissue.

Many studies in the literature involving inoculation of aerial plant parts with *Phytophthora* spp. were conducted with very high levels of zoospore inoculum; more than 10^3 zoospores per milliliter have been employed, and the total amount of inoculum per plant was often excessively high (26,28,40). Sporangial suspensions have also been used, and these may increase the effective inoculum when indirect germination occurs and zoospores are released during the course of the experiments. A large number of simultaneous infections with a heavy inoculum load may break down host barriers that otherwise would remain intact. Thus, results may be influenced by high inoculum levels and may or may not be representative of the inoculum-infection relationship as it occurs in the field. We have shown that with low levels of zoospore inoculum applied directly to host tissue, under the appropriate conditions, the rapid disease progress often observed in the field can result from a few zoospores.

Low numbers of zoospores of *P. capsici* also caused high percentages of plant mortality when zoospores were placed in free water above flooded soil. Seventy-five and 95% of the plants exposed to 10 and 25 zoospores per plant, respectively, died in some experiments (Fig. 6). Although results were extremely variable, the regression of the estimated number of infections on the number of zoospores per plant yielded a nonsignificant slope, indicating no increase in the number of estimated infections per plant with an increase in the number of zoospores per plant. We believe that the variation was attributable to the use of biologically active soil; soil initially microwaved presumably was recolonized during the 3–4 wk of plant growth before the experiments were conducted. Zoospore attrition in a competitive environment was likely to occur and would be expected to vary from pot to pot and trial to trial. Thus, lower levels of infection and the percentage of mortality would be expected to occur at the same inoculum level with flooded plants than with directly inoculated plants.

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