Effect of the Matric Component of Soil Water Potential on Infection of Pepper Seedlings in Soil Infested with Oospores of *Phytophthora capsici*

M. J. Hord and J. B. Ristaino

Graduate research assistant and assistant professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27695.

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ABSTRACT

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Pepper seedlings (*Capsicum annuum* 'Keystone Resistant Giant') were grown in soil infested with 50 oospores of *Phytophthora capsici* per gram of soil. The matric component of soil water potential (ψ_m) was adjusted to 0, −2.5, −5.0, or −10.0 J/kg (1 J/kg = 10 millibars). Plants grown in soil at ψ_m values less than 0 J/kg were either not saturated or exposed to a single 2- or 24-h saturation period after 10 days. Plants were not infected without a period of soil saturation, but 33–83% of the plants grown at ψ_m values of −2.5, −5.0, or −10.0 J/kg before saturation showed symptoms of disease. Absence of disease in plants held in soil at constant ψ_m values of −2.5, −5.0, or −10.0 J/kg without a saturation period suggests that either oospore germination did not occur or oospores that germinated did not cause infection. In additional experiments, the incidence of foliar symptoms of disease increased over time after a single saturation period, and the rate of disease increase was similar after a saturation period of either 2 or 24 h. Incidence of disease 8 days after the saturation period was higher in plants grown for 10 days (65.3, 83.7, and 86.7%) than in plants grown for 5 days (27.8, 34.7, and 40.2%) at ψ_m values of −2.5, −5.0, or −10.0 J/kg before saturation. Incidence of foliar symptoms, the rate of disease increase, and final incidence of root infection were lower in plants grown in soil at a constant ψ_m of 0 J/kg than in plants grown in soil for 5 or 10 days at ψ_m values of −2.5, −5.0, or −10.0 J/kg before saturation. Less than 10% of the plants grown in soil maintained under constant saturation became infected, thus suggesting that continuous periods of soil saturation were not conducive to disease development. Total areas under the disease progress curve (AUDPC) increased with decreasing ψ_m and were higher in plants held at ψ_m values of −2.5, −5.0, or −10.0 J/kg for 10 days than 5 days before saturation. Leaf disks placed on free water in funnels during the saturation period became infected within 2 h, indicating that zoospores were present in the soil water. Germination of oospores in soil extract was predominantly by production of sporangia and increased from 8 to 47% between 5 and 10 days. Oospores of *P. capsici* may have germinated and formed sporangia in soil held at ψ_m values of −2.5, −5.0, or −10.0 J/kg, and zoospores released during brief periods of soil saturation probably acted as primary infective propagules.

Additional keywords: oomycetes, Phytophthora blight, Phytophthora root and crown rot, Solanaceae.

Phytophthora root and crown rot of pepper is a serious and economically important disease in many areas of the United States and throughout the world. The causal agent of the disease, the heterothallic fungus *Phytophthora capsici* Leonian, produces oospores that are considered primary survival structures in soil (1,4,19). Oospores of *P. capsici* occur in naturally infected pepper plants (17,19), and A1 and A2 mating types have been isolated from pepper and cucurbit fields in North Carolina and elsewhere (17,20). Oospores of *P. capsici* germinate predominantly by forming sporangia in distilled water, root extract, and soil extract (11). In addition, oospore germination in root and soil extracts is asynchronous and increases with time of incubation (11).

Although oospores are believed to be the primary source of inoculum in the field, little is known about the influence of soil physical factors on infection of pepper in soil infested with oospores of *P. capsici*. We are aware of only one previous study on this subject (3). The matric component of soil water potential (ψ_m) plays an important role in the life cycle of *Phytophthora* spp. that cause root rots (6,7). Sporangia of *P. capsici* formed abundantly within 24 h on mycelial disks incubated in soil at −30.0 J/kg (1 J/kg = 10 millibars) but did not form in saturated soil within 24 h, unless mycelial disks were first incubated at ψ_m values of −20.0 or −30.0 J/kg (2). Likewise, maximum numbers of sporangia of *P. parasitica* were produced when mycelia and chlamydospores were incubated in soil at ψ_m values between −2.5 and −30.0 J/kg, but few sporangia were produced in soil at ψ_m values above −1.0 J/kg (12). Sporangia production by *P. cryptogea* on colonized leaf tissue occurred at −2.5 J/kg, whereas isolates of *P. cambivora*, *P. drechsleri*, and *P. megasperma* formed sporangia and released zoospores within 3–6 h in saturated soil (26). Release of zoospores from sporangia of *Phytophthora* species is extremely sensitive to small changes in ψ_m and occurs predominantly at ψ_m values between 0 and −1.0 J/kg (16). Zoospores were released from sporangia of *P. capsici* formed at −30.0 J/kg within 4 h of soil saturation, and zoospores were released within 1 h from sporangia of *P. cryptogea* formed previously at −2.5 J/kg (2,26). A saturation period of only 0.5 h was sufficient for zoospore release and infection of tobacco in soil naturally infested with inoculum of *P. parasitica* f. sp. *nicotianae* (23).

Many studies have considered the effects of ψ_m on the production and germination of sporangia of *Phytophthora* species, but little is known about the influence of ψ_m on oospore germination and disease initiation by oospores (3,15,25). Oospores of *P. megasperma* f. sp. *medicaginis* germinated and formed sporangia at ψ_m values of 0, −1.0, and −5.0 J/kg, whereas direct germination of oospores via germ tubes occurred at a ψ_m value of −10.0 J/kg (15). Oospores of *P. cactorum* germinated and formed sporangia in drier soils at ψ_m values between −20.0 and −500.0 J/kg (25). The mode of germination of oospores of *P. capsici* has not been determined in soil. However, pepper seedlings grown in soil infested with *P. capsici* at a density of 25 oospores per gram of soil were not infected after 37 days at a constant

ψ_m value of −12.5 J/kg. After a single 24-h saturation period, 20% of the plants were infected, and repeated floodings at 10-day intervals caused increases in plant mortality (3). Incidence of root rot of alfalfa in soil infested with 10 oospores of *P. m. medicaginis* per gram of dry soil decreased with decreasing ψ_m and was 78% in saturated soil but only 18% at a ψ_m value of −10.0 J/kg (15).

The objective of this study was to investigate the influence of a range of ψ_m values, the duration of a single saturation period, and the time period before saturation on the infection of pepper seedlings in soil infested with oospores of *P. capsici*. A preliminary report of a portion of this work (10) and a related study on the effects of physical and chemical factors on oospore germination in vitro also have been published (11).

MATERIALS AND METHODS

Preparation of inoculum. The two isolates of *P. capsici* used in this study were Sc2A (A1), isolated from pepper in Sampson County, NC (20), and ATCC 15399 (A2), obtained from D. Mitchell, University of Florida, Gainesville, and originally isolated from pepper in New Mexico. Cultures of *P. capsici* were maintained on V8 agar (200 ml of V8, 800 ml of distilled water, 2 g of $CaCO_3$, 15 g of agar) in petri dishes and transferred periodically. Oospores were produced by placing two V8 agar disks with mycelium of each isolate 2–3 cm apart on clarified V8 (CV-8) agar (V8 clarified by centrifugation at 4,349 *g* for 10 min before preparation of the medium consisting of 200 ml of CV-8 supernatant, 2 g of $CaCO_3$, 800 ml of distilled water, 15 g of agar). Cultures were incubated at 24 C for 2 mo in the dark.

Agar blocks containing oospores were removed from four or five cultures and comminuted in approximately 300 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 each. The supernatant was removed with a pipette, and the oospore pellet was resuspended in sterile distilled water. Concentration of oospores in the suspension was estimated with a hemacytometer and adjusted to 10^5 oospores per milliliter. We incubated 10 ml of oospore suspension with 200 mg of Novozym (Novo BioLabs, Danbury, CT), an enzyme preparation with cellulase, chitinase, and protease activity, to remove sporangia and mycelial fragments. The suspension was incubated on a shaker at 28 C for 24 h. Oospores were concentrated, and the enzyme was removed by at least three centrifugations as described above. Oospores were used immediately after preparation. Absence of viable sporangia and hyphal fragments was verified by placing 50 µl of the oospore suspension containing approximately 3×10^3 oospores on PARP medium (pimaricin, 10 ppm; ampicillin, 250 ppm; rifampicin, 10 ppm; pentachloronitrobenzene, 100 ppm; 17 g of Difco cornmeal agar, 1 L of distilled water) (14). Colonies were examined microscopically to determine point of origin.

Soil infestation and preparation of plant material. Soil (Gilead loamy sand: 87% sand, 8% silt, 5% clay, pH 5.6) was air-dried and passed through a 10-mm sieve. Soil was saturated, then a soil moisture release curve was determined for soil at ψ_m values between 0 and −30.0 J/kg with Büchner tension funnels (5). The vertical distance from the top of the porous plate to the water level in a reservoir was used as the reference distance for determining column heights and adjusting ψ_m in the tension funnels (5). Gravimetric water content at each ψ_m value was measured by drying soils at 105 C. Approximately 70% of the soil water was released from soil with ψ_m values between 0 and −10.0 J/kg (Fig. 1).

Four kilograms of soil was moistened with 200 ml of water, placed in polyethylene bags, and microwaved for 7.5 min at least 1 mo before being used (8). Uninfested, microwaved soil was tested in the greenhouse, and disease did not occur. Therefore, uninfested controls were not used in the experiments described. Soil for each of four blocks was infested with oospores suspended in distilled water, and an inoculum density of 50 oospores per gram of soil was obtained. Gravimetric water contents ranged from 4 to 8% immediately after soil infestation. Soil was mixed thoroughly by hand to distribute oospores throughout the soil volume. Soil dilutions were not conducted on infested soils, because oospores do not germinate readily on selective media.

The viability of oospore suspensions was evaluated by incubating oospores in 3 ml of filter-sterilized soil extract in 10 glass petri dishes (60 mm wide × 15 mm deep) at 24 C in the dark (11). Oospore germination on the bottom of the dish was observed after 5 and 10 days. Oospores that germinated by producing either a germ tube or sporangia were counted. The percentage of oospores that germinated was calculated based on microscopic observations of 100 oospores per dish.

Pepper seedlings (*Capsicum annum* L.) of cultivar Keystone Resistant Giant were grown in Metro Mix 220 (W. R. Grace & Co., Cambridge, MA) in a greenhouse for 2 wk, until the first pair of true leaves began to emerge. We trimmed roots to 1–2 cm to provide uniform size and transplanted them into 100 g of infested soil that was packed to a depth of 3 cm at a bulk density of 1.03 g/cm^3 in 150-ml Büchner tension funnels. The top surface of the tension funnels was covered with plastic wrap that reduced evaporation, and funnels were maintained in a growth chamber at 24 C with a 14-h photoperiod (cool white fluorescent light, 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Seedlings were planted on the same day that soil was infested with oospores.

Effects of ψ_m and duration of a single saturation period on disease. We conducted experiments to evaluate the influence of the duration of a single period of soil saturation on infection of pepper over a range of ψ_m values. Two seedlings were transplanted into soil in each tension funnel. The experimental design was a randomized complete block with a factorial arrangement of treatments. The two factors were ψ_m and duration of soil saturation. Each treatment was replicated six times. We adjusted soil to saturation by using the top of the soil as the reference point, whereas we used the vertical distance from the top of the porous plate to the water level in a reservoir as a reference point to determine column heights at other ψ_m values. Soil in one treatment was maintained at constant saturation, whereas soils in the other treatments were either adjusted to constant ψ_m values of −2.5, −5.0, or −10.0 J/kg for 15 days without a saturation period or were adjusted to ψ_m values of −2.5, −5.0, or −10.0 J/kg and then saturated for 2 or 24 h after 10 days. Roots had colonized the entire soil volume before saturation. Funnels were returned to the original ψ_m values after the saturation period by changing the height of the funnels above the reservoir as described previously. The ψ_m value of soil was not altered in treatments maintained at constant saturation. Experiments were repeated, and data from one experiment are shown.

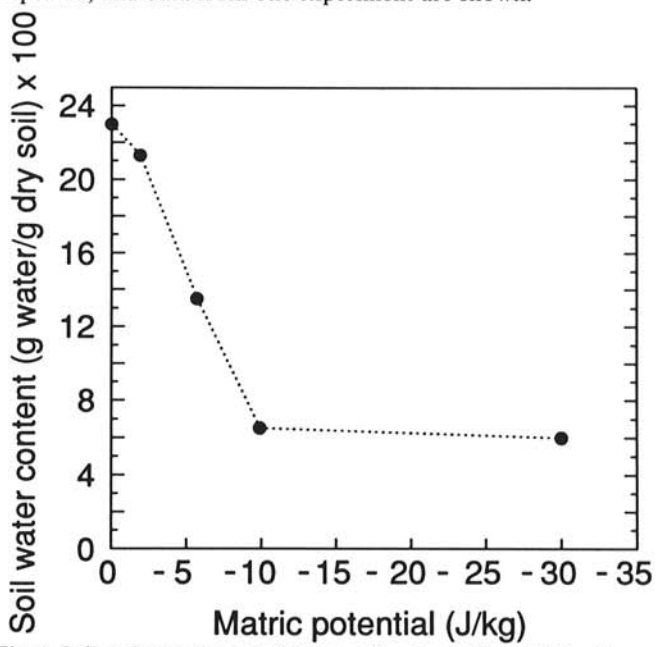

Fig. 1. Soil moisture characteristic curve for sieved (2 mm) Gilead loamy sand (87% sand, 8% silt, 5% clay, pH 5.6).

Number of plants with foliar symptoms of disease was recorded 15 days after plants were transplanted. Foliar symptoms were evaluated and included a watersoaked lesion on the main stem and severe wilting. Plants were removed from funnels, and the root infection was evaluated after entire root systems were surface-disinfested with 10% bleach (0.5% NaOCl), rinsed in sterile distilled water, and plated on PARP medium (14). A plant was considered infected when colonies of *P. capsici* grew from any part of the root system. The percentage of plants in each treatment with infected roots or foliar symptoms was calculated.

Effects of time period before saturation, ψ_m, and duration of saturation on disease. The effects of the time period before saturation and the duration of a single saturation period on infection of pepper seedlings in oospore-infested soil held at a range of ψ_m values were evaluated. Treatments were arranged in a split-plot design, and four replicate tension funnels per treatment were used. Ten pepper seedlings, the minimum number necessary to accurately assess the incidence of disease, were transplanted into 100 g of soil in each funnel. Soil in funnels in mainplot treatments was either maintained at constant saturation (0 J/kg) or adjusted to ψ_m values of −2.5, −5.0, or −10.0 J/kg. Subplot treatments were given either a 2- or 24-h period of saturation after 5 or 10 days at ψ_m values of −2.5, −5.0, or −10.0 J/kg. The ψ_m was not altered in treatments at constant saturation. Soils in funnels were returned to the original ψ_m values after the saturation period. The experiment was conducted two times, and results are shown from one experiment.

Five pepper leaf disks were placed on the surface of free water in each funnel during the saturation period. Leaf disks were also placed in funnels held at constant saturation for either 2 or 24 h after 5 or 10 days at constant saturation. Leaf disks were removed after 2 or 24 h, surface-disinfested in 10% bleach, rinsed in distilled water, and plated on PARP medium. The percentage of leaf disks colonized by *P. capsici* was calculated.

Incidence of plants showing foliar symptoms was recorded daily for 8 days after the saturation period. Entire root systems from individual plants were removed from funnels at the end of the experiment, surface-disinfested, and plated as described above for determining the incidence of root infection.

Statistical analysis. Data were tested for homogeneity of variance before analysis of variance with the Statistical Analysis Systems Version 6.04 (SAS Institute, Inc. Cary, NC) and scatter plots of residual error terms were plotted. We transformed incidence of foliar symptoms recorded over time and incidence of root infections with the arcsine square-root transformation to stabilize variances. Transformed means were back-transformed and are shown on the *y* axis in all figures. Multivariate repeated measures analysis of variance was used for analysis of data recorded over time (9). Tests of the effects of time and time × treatment interactions were performed with Wilks' lambda, which is a likelihood ratio test statistic for multivariate analysis of variance (13). Area under the disease progress curve (AUDPC) was calculated for foliar disease incidence data (22), and sums of squares were partitioned into linear, quadratic, and lack-of-fit components.

RESULTS

Effects of ψ_m and duration of a single saturation period on disease. A saturation period was necessary for infection of pepper seedlings in soil infested with oospores of *P. capsici*. Regardless of presaturation ψ_m values, 33–83.3% of the plants developed foliar symptoms, and 67–92% of the plants had infected roots after a single period of soil saturation of either 2 or 24 h (Table 1). In contrast, the incidence of foliar symptoms and root infection was only 8.3% in plants held in soil at constant saturation (0 J/kg) for 15 days. Foliar symptoms of disease and root infection were not detected in plants held in soil at constant ψ_m values of −2.5, −5.0, or −10.0 J/kg without a saturation period (Table 1). Taproots and hypocotyls of infected plants were extensively colonized by the pathogen, whereas localized infections occurred on lateral roots. Roots appeared healthy in most of the plants held under continuous saturation.

Effects of time period before saturation, ψ_m, and duration of saturation on disease. The incidence of foliar symptoms of disease increased over time after a single saturation period of either 2 or 24 h (Table 2). Rate of disease increase over time was similar when plants held in soil at ψ_m values of −2.5, −5.0, or −10.0 J/kg were given either a 2- or 24-h period of saturation (time × saturation interaction not significant) (Fig. 2; Table 2). Foliar symptoms were not observed until 3 days after saturation and increased with time (Fig. 2).

The time period plants were grown in oospore-infested soil before saturation and the ψ_m had significant effects on disease development (Figs. 3,4). The rate of increase in incidence of foliar symptoms of disease was significantly greater among plants given a saturation period after growth in oospore-infested soil for 10 days than for 5 days at ψ_m values of −2.5, −5.0, and −10.0 J/kg (time × ψ_m × time period before saturation interaction means were significant at $P = 0.01$) (Fig. 3; Table 2). Incidence of foliar symptoms of disease 8 days after the saturation period at ψ_m values of −2.5, −5.0, or −10.0 J/kg were 65.3, 83.7, and 86.7% and 27.8, 34.7, and 40.2% among plants grown in oospore-infested soil for 10 or 5 days, respectively, before saturation (Fig. 3B,A).

Incidence of foliar symptoms of disease over time was lowest among plants grown in soil held at constant saturation (0 J/kg) than among plants grown at ψ_m values of −2.5, −5.0, or −10.0 J/kg for 5 or 10 days before saturation (Fig. 3A,B). Also, the incidence of root infection at harvest was significantly lower in plants held under constant saturation at 0 J/kg than among plants grown in soil at ψ_m values of −2.5, −5.0, or −10.0 J/kg for 5 or 10 days before saturation (ψ_m × time period before saturation interaction effect significant for root infection at $P = 0.05$) (Fig. 4).

Total AUDPCs also increased with decreasing ψ_m values and were higher in plants held at ψ_m values of −2.5, −5.0, or −10.0 J/kg for 10 days than for 5 days before saturation (Fig. 5; Table 3). For plants grown for 10 days before saturation, total AUDPCs at ψ_m values of −2.5, −5.0, and −10.0 J/kg averaged 177%-days, 245%-days, and 329%-days; and for plants grown for 5 days before saturation, total AUDPCs averaged 109%-days, 122%-days, and 144%-days (Fig. 5). The lowest AUDPCs were measured in plants

TABLE 1. Effects of the matric component of soil water potential (ψ_m) and the duration of a single saturation period[a] on infection of pepper seedlings in soil infested with oospores of *Phytophthora capsici*

	Percentage of infection							
	Constant		0 h		2 h		24 h	
ψ_m (J/kg)	Foliar infection[b]	Root infection[c]	Foliar infection	Root infection	Foliar infection	Root infection	Foliar infection	Root infection
0	8.3	8.3	ND[d]	ND	ND	ND	ND	ND
−2.5	ND	ND	0	0	66.7	91.7	33.3	66.7
−5.0	ND	ND	0	0	75.0	83.3	83.3	91.7
−10.0	ND	ND	0	0	75.0	81.8	83.3	91.7

[a] Soil was either constantly saturated or was saturated for 0, 2, or 24 h after 10 days at constant ψ_m values of −2.5, −5.0, or −10.0 J/kg.
[b] Percentage of plants with foliar symptoms of disease determined 5 days after the saturation period.
[c] Percentage of plants with infected roots was determined by plating entire surface-disinfested root systems on selective medium.
[d] ND = not determined.

grown at constant saturation.

Leaf disks placed in free water in funnels during the saturation period became infected, suggesting that zoospores were present in the soil water (Table 4). The ψ_m value at which soil was held before saturation had a significant effect on the percentage of leaf disks infected by *P. capsici* (ψ_m main effect significant at $P < 0.01$). A low percentage of the leaf disks placed on soil held under constant saturation became infected, whereas 45–87% of the leaf disks placed on saturated soil that had been held previously at ψ_m values of −2.5, −5.0, or −10.0 J/kg for 5 or 10 days before saturation became infected (Table 4). The ψ_m quadratic effect was significant ($P = 0.01$). However, the ψ_m lack-of-fit effect also was significant for the percentage of leaf disks infected ($P = 0.05$), indicating that a quadratic function did not completely describe the effect of ψ_m on the percentage of leaf disks infected. The time period soil was held at ψ_m values of −2.5, −5.0, or −10.0 J/kg before saturation and the duration of the saturation period did not affect the percentage of leaf disks infected.

Germination of oospores in soil extract was predominantly by production of sporangia. Oospore germination in soil extract was not synchronous but increased from 8 to 47% between 5 and 10 days at 24 C in the dark.

TABLE 2. Repeated measures analysis of variance describing effects of the matric component of soil water potential (ψ_m),[a] time period before saturation,[b] and duration of saturation[c] on the incidence of foliar symptoms of disease in soil infested with oospores of *Phytophthora capsici*

Source of variation for time effects	df	Wilks' λ[d]	P
Time	5	0.06	0.01**
Time × ψ_m	15	0.04	0.70
Time × block × ψ_m[e]	45	0.20	0.07
Time × time period before saturation	5	0.40	0.01**
Time × saturation	5	0.89	0.56
Time × time period before saturation × saturation	5	0.80	0.17
Time × ψ_m × time period before saturation	15	0.30	0.01**
Time × ψ_m × saturation	15	0.59	0.26
Time × ψ_m × time period before saturation × saturation	15	0.77	0.85

[a] Oospore-infested soil in main plot treatments was adjusted to ψ_m values of 0, −2.5, −5.0, or −10.0 J/kg.
[b] The time period before saturation for subplot treatments was 5 or 10 days for treatments at ψ_m values of −2.5, −5.0, or −10.0 J/kg.
[c] Soil in subplot treatments held at ψ_m values of −2.5, −5.0, or −10.0 J/kg was saturated for either 2 or 24 h after 5 or 10 days.
[d] Wilks' λ is a likelihood ratio test statistic for multivariate analysis of variance (9).
[e] The time × ψ_m interaction was tested with the time × block × ψ_m error term. ** Indicates that the Wilks' λ value was significant at $P = 0.01$.

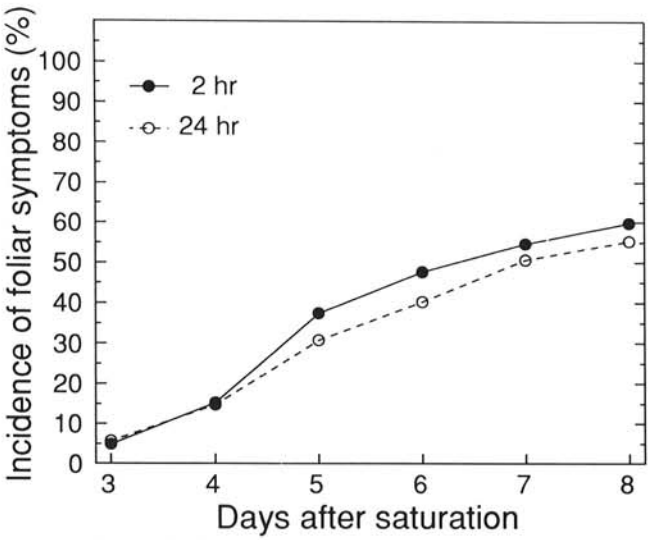

Fig. 2. Incidence of foliar symptoms of disease in pepper seedlings grown in soil infested with oospores of *Phytophthora capsici* at matric potentials of −2.5, −5.0, or −10.0 J/kg before a 2- or 24-h saturation period. Data were transformed with the arcsine square-root transformation, and back-transformed data are shown on the *y* axis. The saturation main effect means are shown at each time and were not significant. Each point represents means from 240 plants.

DISCUSSION

A saturation period was required for infection of pepper seedlings in soil infested with oospores of *P. capsici*. Plants did not develop symptoms or become infected when held for 15 days at constant ψ_m values of −2.5, −5.0, or −10.0 J/kg without a saturation period (Table 1). High levels of infection only occurred when a saturation period of 2 or 24 h was imposed. *P. capsici* did not infect plants grown in oospore-infested soil at ψ_m values that were lower than those that favor zoospore release from sporangia (2). Similarly, Shew found that tobacco planted in soil naturally infested with chlamydospores of *P. p. nicotianae* or colonized root tissue did not become infected at soil ψ_m values lower than those that favored zoospore release (23). In contrast, Bowers and Mitchell (3) obtained low levels of disease caused by *P. capsici* in pepper in oospore-infested soil held at a constant ψ_m value of −2.5 J/kg for 37–40 days. They suggested that oospores germinated and formed sporangia and that limited

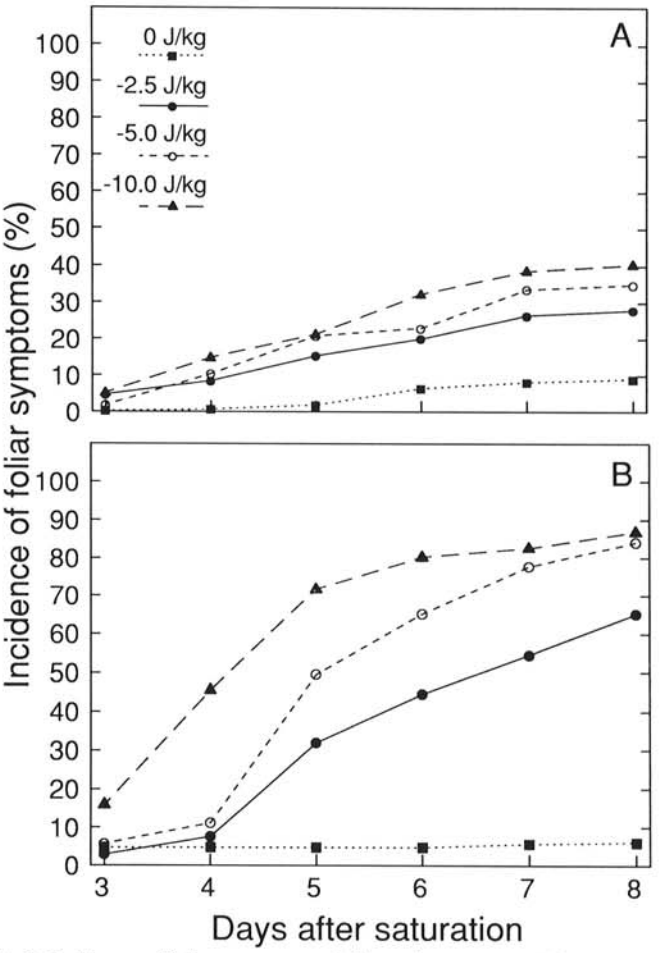

Fig. 3. Incidence of foliar symptoms of disease in pepper seedlings grown in soil infested with oospores of *Phytophthora capsici*. The matric component of soil water potential (ψ_m) was maintained at 0, −2.5, −5.0, or −10.0 J/kg before saturation of soils in treatments at ψ_m values of less than 0 J/kg after **A,** 5 days or **B,** 10 days. Data were transformed with the arcsine square-root transformation, and back-transformed data are shown on the *y* axis. The time × ψ_m × time period before saturation interaction means are shown and were significant at $P = 0.01$. Each point represents means from 80 plants.

zoospore movement occurred at ψ_m values that were suboptimal for zoospore release (3). Soil saturation was not a requirement for infection of alfalfa in soil infested with oospores of the homothallic species *P. m. medicaginis*, which germinated and formed sporangia at ψ_m values of −1.0 and −5.0 J/kg but germinated directly and formed germ tubes at a soil ψ_m value of −10.0 J/kg (15). In our work, the absence of disease in plants held in soil at constant ψ_m values of −2.5, −5.0, or −10.0 J/kg without a saturation period suggests that either germination of oospores did not occur or oospores that germinated by production of germ tubes or sporangia did not cause infection. The latter hypothesis seems probable, because zoospore release by *P. capsici* occurred with a brief 2-h saturation period after prior incubation of soil at ψ_m values of −2.5, −5.0, or −10.0 J/kg.

High levels of disease developed rapidly after a single, brief 2-h period of saturation in soil held previously at ψ_m values of −2.5, −5.0, or −10.0 J/kg (Figs. 2,3). In contrast, Bowers and Mitchell demonstrated that repeated periods of soil saturation were required for high levels of infection of pepper in soil infested with oospores of *P. capsici* (3). Repeated floodings may have been necessary for obtaining sufficient pathogen-root contact in their experiments, because inoculum was mixed in an infested soil layer and not throughout the soil volume, the initial inoculum densities were lower in their studies than in our work, and a different pair of isolates was used to produce oospores (3).

An increase in the duration of the soil saturation period from 2 to 24 h did not result in a subsequent increase in foliar symptoms of disease (Fig. 2). Oospores of *P. capsici* may have germinated and formed sporangia in soil at ψ_m values of −2.5, −5.0, or −10.0 J/kg, and zoospores may have been released with a brief saturation period. Although we did not evaluate the mode of oospore germination in soil microscopically, leaf disks placed in free water on the surface of saturated soil were infected during the 2-h saturation period, indicating that zoospores were present in the soil water. Similarly, a saturation period of only 0.5 h was sufficient for zoospore release and infection of tobacco in soil naturally infested with *P. p. nicotianae* (23). We have demonstrated in previous work that several days were required for a

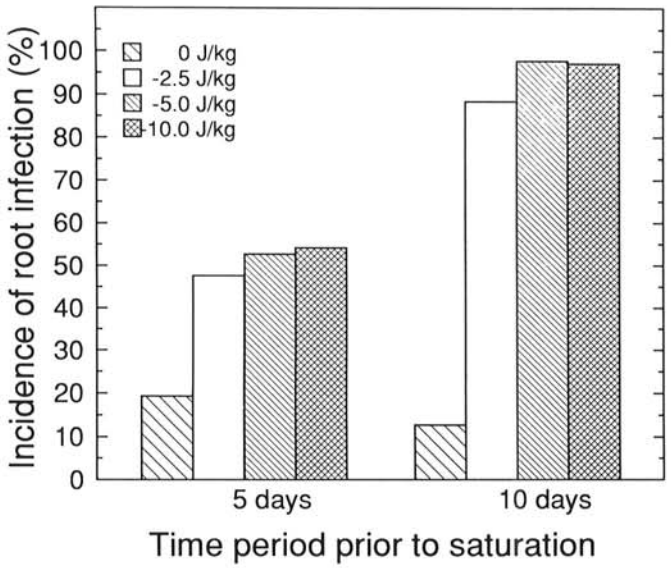

Fig. 4. Incidence of root infection in pepper seedlings grown in soil infested with oospores of *Phytophthora capsici*. The matric component of soil water potential (ψ_m) was maintained at 0, −2.5, −5.0, or −10.0 J/kg for 5 or 10 days before saturation of soils in treatments with ψ_m values of less than 0 J/kg for 2 or 24 h. The ψ_m × time period before saturation interaction means are shown and were significant at $P = 0.05$. Each bar represents means from 80 plants. For comparison of means within and between clusters, $LSD_{0.05} = 19.5$.

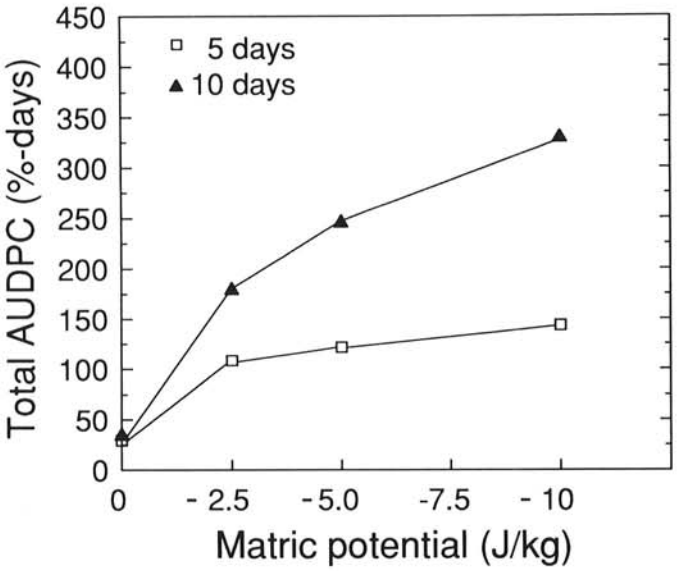

Fig. 5. Effect of the matric component of soil water potential (ψ_m) on the total area under the disease progress curve (AUDPC) for pepper seedlings grown in soil infested with oospores of *Phytophthora capsici* for 5 or 10 days before a saturation period. The ψ_m × time period before saturation interaction means are shown and were significant at $P = 0.01$. Points for 5 or 10 days represent the mean AUDPCs shown in Figure 3.

TABLE 3. Analysis of variance describing the effects of the matric component of soil water potential (ψ_m),[a] time period before saturation,[b] and duration of saturation[c] on the area under the disease progress curve in soil infested with oospores of *Phytophthora capsici*

Source of variation	df	Mean square	$P>F$
ψ_m	3	120,679	0.01**
Block × ψ_m[d]	9	5,144	0.34
Time period before saturation	1	144,875	0.01**
Saturation	1	2,437	0.46
Time period before saturation × saturation	1	12,516	0.10
ψ_m × time period before saturation	3	23,768	0.01**
ψ_m × saturation	3	10,176	0.09
ψ_m × time period before saturation × saturation	3	1,800	0.75

[a] Oospore-infested soil in main plot treatments was adjusted to ψ_m values of 0, −2.5, −5.0, or −10.0 J/kg.
[b] The time period before saturation for subplot treatments was 5 or 10 days for treatments at ψ_m values of −2.5, −5.0, or −10.0 J/kg.
[c] Soil in subplot treatments held at ψ_m values of −2.5, −5.0, or −10.0 J/kg was saturated for either 2 or 24 h after 5 or 10 days.
[d] The time × ψ_m interaction was tested with the time × block × ψ_m error term. ** Indicates that F value significant at $P = 0.01$.

TABLE 4. Effects of the matric component of soil water potential (ψ_m), time period before saturation, and duration of saturation[a] on the percentage of pepper leaf disk baits infected[b] in soil infested with oospores of *Phytophthora capsici*

	Percentage of leaf disks infected			
	5 days		10 days	
ψ_m (J/kg)[c]	2 h	24 h	2 h	24 h
0	5.0	5.0	5.0	0
−2.5	45.0	66.7	70.0	70.0
−5.0	55.0	86.7	75.0	75.0
−10.0	70.0	60.0	70.0	85.0

[a] Soil was either constantly saturated or was saturated for 2 or 24 h after 5 or 10 days at ψ_m values of −2.5, −5.0, or −10.0 J/kg.
[b] The percentage of leaf disks infected was determined by plating surface-disinfested pepper leaf disks on selective medium immediately after the saturation period.
[c] The ψ_m main effect was significant at $P < 0.01$, and the ψ_m quadratic effects were significant at $P < 0.01$.

low percentage of oospores of *P. capsici* to germinate in vitro and produce sporangia in soil or root extract (11). In addition, oospore germination was asynchronous and predominantly by production of sporangia in soil extract. Therefore, a 2-h period of saturation was probably insufficient for oospores to germinate by formation of sporangia, but was probably sufficient for release of zoospores from sporangia previously formed at lower ψ_m values.

A low incidence of disease in plants grown in oospore-infested soil under constant saturation indicates that oospores of *P. capsici* were capable of germinating and infecting plants under saturated conditions (Figs. 3,4; Table 1). However, oospore germination may have been reduced at a constant ψ_m of 0 J/kg, because the incidences of foliar symptoms and root infection were lower in saturated soil than in soil held at ψ_m values of −2.5, −5.0, or −10.0 J/kg before saturation (Table 1; Figs. 3,4). In contrast, highest levels of disease in alfalfa occurred when oospores of *P. m. medicaginis* were incubated in soil held at constant saturation (15). In addition, sporangia formation on alfalfa radicles infected with *P. megasperma* were greater in saturated soil than in soil held at ψ_m values of −5.0 or −10.0 J/kg (15,18). Low soil aeration may have reduced the level of oospore germination in soil under constant saturation in our study; however, we did not measure the soil oxygen concentration to confirm this. In this and previous work, oospores of *P. capsici* germinated in small volumes of soil extract in petri dishes under saturated conditions, but it is unlikely that oxygen was limiting in these experiments (11). Parasitism of oospores by microorganisms in the microwaved soil may have contributed to the low incidence of disease in saturated soil, but this was not investigated. Oospores of *P. m. sojae* and *P. cactorum* were parasitized by oomycetes, chytridiomycetes, and bacteria in saturated soil (24).

Oospores might germinate more rapidly when exposed to saturated conditions after 5–10 days before incubation at lower ψ_m values. Bowers and Mitchell suggested that oospores germinated and formed sporangia and released zoospores during repeated 24-h periods of soil saturation, however, the mode of oospore germination in soil was not evaluated in their research (3). Bernhardt and Grogan (2) demonstrated that mycelial mats of *P. capsici* formed sporangia more rapidly under saturated conditions when previously incubated at −20.0 or −30.0 J/kg. Wilcox and Mircetich (27) demonstrated that no sporangia were produced by *P. cambivora* on colonized leaf disks at a constant ψ_m value of −2.5 J/kg, but sporangia formation was abundant within 3–6 h, when leaf disks were subsequently placed under saturated conditions. Further experiments that quantify germination of oospores of *P. capsici* in soil by using fluorescence microscopy will be necessary for a critical examination of the effects of soil physical factors on the mode and timing of germination.

Our experimental design did not allow us to separate the effects of the time period before saturation on the host from effects on the pathogen. Our data indicate that the rate of increase in the incidence of foliar symptoms of disease and final incidence of root infection were greater in plants grown in oospore-infested soil at ψ_m values of −2.5, −5.0, or −10.0 J/kg for 10 days than for 5 days before saturation. The greater incidences of foliar symptoms and root infection with a longer time period before saturation of soil could have been attributable to an increase in root growth and/or an increase in oospore germination. Oospore inoculum probably contacted more roots after 10 than after 5 days in soil. In addition, germination of oospores by production of sporangia in sterile soil extract in this study increased from 8 to 47% after incubation for 5 or 10 days, respectively, and a similar increase in germination may have occurred in soil. Germination of oospores of *P. m. medicaginis* also increased in soil over time (15).

Disease progress and subsequent AUDPCs were low at constant saturation and increased with decreasing ψ_m values. This may have been attributable in part to ψ_m effects on root growth. Plants appeared to develop larger root systems as the ψ_m value was decreased from 0 to −10.0 J/kg (M. J. Hord and J. B. Ristaino, *unpublished data*). A larger root system could increase the probability of root contact with inoculum in the soil and thus result in more infections and more rapid symptom expression. However, in a field situation, a larger root system may enable a plant to compensate for infection (21).

The final incidence of foliar symptoms and root infection was high and similar in plants held in oospore-infested soil at ψ_m values of −2.5, −5.0, and −10.0 J/kg after a single saturation period. The effect of these levels of ψ_m on the incidence of root infection may have been obscured by the density of inoculum used in our work. Inoculum densities of 0–24 propagules of *P. capsici* per gram of soil have been reported in naturally infested fields during the growing season (17), but little quantitative information is available on the relationship between initial densities of oospore inoculum present in naturally infested fields and subsequent disease. Because low levels of oospores germinate on selective media, quantification of inoculum densities by dilution-plating of naturally infested soil has been difficult (17).

Results of our study demonstrate that after a period of incubation at ψ_m values of −2.5, −5.0, or −10.0 J/kg, a single brief period of soil saturation was sufficient to cause high levels of infection in pepper seedlings grown in soil infested with oospores of *P. capsici*. Continuous periods of soil saturation were not conducive to disease. Zoospores released during brief periods of soil saturation are the primary infective propagules in oospore-infested soils. Further studies that quantify the effects of soil physical factors on the timing and mode of oospore germination in soil are needed; then, realistic measurements of oospore inoculum density and disease relationships can be made in fields with natural inoculum.

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