Effect of Soil Matric Potential on Phytophthora Root Rot of Alfalfa

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ABSTRACT

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The effect of soil matric potential (ψ_m) on Phytophthora root rot of alfalfa was studied with custom-built ceramic cups on which ψ_m values from 0 to −500 mb were maintained. When seedlings were inoculated with mycelial fragments and oospores, the highest incidence of disease occurred at 0 mb with proportional reduction as ψ_m was reduced. At 0 mb, hyphal tips grew from buried mycelial fragments, zoospores were released from the sporangia formed from germinated oospores, and sporangia formed on infected roots. These effects increased the inoculum density, which subsequently increased the incidence and severity of disease. The optimal ψ_m for production of sporangia from infected roots was 0 mb and from mycelial disks was −100 mb. The progress of disease, following inoculation with mycelial fragments or with oospores, at different matric potentials was plotted against time using the logit transformation values of $\ln 1/(1-x)$ in which x = the porportion of plants killed. The disease increase rates for soil infested with oospores at the ψ_m of 0 mb was 0.24 per unit per day; at −10 mb, 0.06 per unit per day; and at −350 mb, 0.02 per unit per day. In soil infested with mycelial fragments the disease increase rate at 0 mb was 0.36 per unit per day; at −10 mb, 0.04 per unit per day; and at −350 mb, 0.02 per unit per day.

The water status of soil greatly affects the incidence and severity of root rot of alfalfa (*Medicago sativa* L.) caused by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* (Pmm) (11). Excessive soil water increases disease severity (18) and minimal soil water arrests the disease (6). In a previous study (12) we reported that excessive soil water prior to inoculation predisposed alfalfa plants and resulted in increased severity of Phytophthora root rot.

The state of water in soil is commonly described in terms of its potential energy by the term "water potential" (Ψ) (2,9). Sterne et al (24) demonstrated that soil matric potential (ψ_m), one of the components of Ψ, had a stronger influence on disease severity caused by *Phytophthora cinnamomi* than did osmotic potential. High Ψ values increase disease severity caused by *Pythium* spp. or *Phytophthora* spp. (17,22,24,25). There are numerous reports on the effect of Ψ on mycelial growth (1,20,23), germination of oospores (21,22), formation of sporangia (3,4,16), and the dispersal and motility of zoospores (5,13–16). However, few studies have correlated the effects of ψ_m on disease severity and fungal behavior in soil. Stanghellini and Burr reported (22) that the effect of increased Ψ on disease incidence was due to the increase in germination of oospores of *Pythium aphanidermatum*. Sterne et al (25) pointed out that at ψ_m above −0.25 bars in a sandy loam soil, more nutrients were available and the percent germination of chlamydospores of *P. cinnamomi* increased. Addition of glucose and asparagine to soil at −0.25 bars restored the percent germination to that in unamended soil at 0 ψ_m. Disease severity was also reduced at −0.25 bars in sandy loam soil, but not in a clay soil.

The influence of an environmental factor on the progress of disease can be measured by mathematical analysis of the "apparent infection rate" (28). No study has been made in which the effect of the influence of soil water status on the apparent infection rate of Pmm on alfalfa has been quantitatively measured. The objectives of this study were to study the influence of soil ψ_m on incidence and severity of Phytophthora root rot of alfalfa and to correlate this influence with the effect of ψ_m on behavior of different types of inoculum, and to determine quantitatively the influence of soil ψ_m on the severity and on the rate of development of disease (apparent infection rate) under controlled conditions.

MATERIALS AND METHODS

Inoculum. Isolate P1057 (Phytophthora culture collection, Department of Plant Pathology, University of California, Riverside) of *Phytophthora megasperma* f. sp. *medicaginis* Kuan and Erwin (Pmm) (11) was incubated on cleared V-8 juice agar (V8JA) or on cleared liquid V-8 juice medium (V8JM). Cleared V-8

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juice medium (CV8M) was prepared by stirring 5.0 g of $CaCO_3$ into 1,000 ml Campbell V-8 Juice® (Campbell Soup Co., Camden, NJ 08101) and clearing by centrifugation. The supernatant was diluted to 4,000 ml with double-distilled water. Solid media contained 1.5% agar (CV8A). The pH was 6.5 after autoclaving. Mycelial fragments and oospores were used as inoculum. A suspension of mycelial fragments, devoid of oospores, was obtained from mats of 2-day-old cultures grown in 25 ml of CV8M, which had been started from minced mycelium of another 2-day-old culture in CV8M. The mycelial mat from one plate of the 2-day-old culture was washed twice with sterile deionized water and blended in 100 ml of water in a Waring Blendor at low speed for 30 sec, which produced a suspension containing mycelial fragments about 0.5 mm long. The concentration of fragments in the suspension was determined with a 1-ml Hawksley eelworm-counting chamber. The suspension of mycelial fragments was diluted and used immediately as inoculum.

Oospores were harvested from 1-mo-old cultures grown in CV8M and purified of mycelial fragments (J. S. Baumer and D. C. Erwin, *personal communication*). One petri plate (90-mm diameter) culture was blended in 140 ml of water in a Waring Blendor at high speed for 1 min. The suspension was incubated with the snail enzyme preparation Glusulase® (Endo Laboratories, Inc., Garden City, NY 11350), (1 ml Glusulase in 20 ml of suspension) on a shaker for 24 hr to digest the mycelium. After incubation with Glusulase, the mixture was further purified by several centrifugations and resuspensions. The centrifugation periods were 1 min, 50, 30, 20, 15, and 5 sec. After the last centrifugation for 5 sec, the pellet was resuspended with 20 ml of water containing 1 ml of Glusulase and incubated on a shaker at room temperature for 14–16 hr. The enzyme was removed by three 2-min centrifugations and resuspension in water. The final pellet of oospores was diluted to 10 ml with sterile deionized water and mixed thoroughly. Immediately after being mixed, 20 μl of the suspension was placed on a slide and the oospores were counted microscopically at ×400. The oospore suspension was diluted and used immediately as inoculum.

Control of soil matric potential (ψ_m). The soil ψ_m was controlled by applying tension to a layer of sandy loam soil on custom-made ceramic cups, which served as tension plates (24). The ceramic cup was made by attaching a Plexiglas cylinder (6 cm in height) to a custom-made hollow ceramic disk (8.2 cm in diameter and 1.8 cm thick), which was made from a mixture of clay bodies designated by the company as 3000 and 211 (Butcher Clay Co., 3628 E. Olympic Blvd., Los Angeles, CA 90023) at a ratio of 3:1. The soil was placed as a slurry at a depth of 1 cm inside the ceramic cup on the surface of the porous, ceramic disk. A continuous water column was established through a tube attached to the side of the ceramic cup, leading to a water reservoir. The length of the water column between the porous plate and the surface of a water reservoir was adjusted to give the desired ψ_m. The pressure exerted by a 1.022-cm column of water is equivalent to 1 mb (1,000 mb = 1 bar = 0.99 atm). The function of the apparatus was similar to that of the Büchner funnel tension plates described by Duniway (5). The accuracy of ψ_m control was measured by comparing the water content of soil on the tension plates with soil equilibrated in a pressure-plate apparatus (Fig. 1).

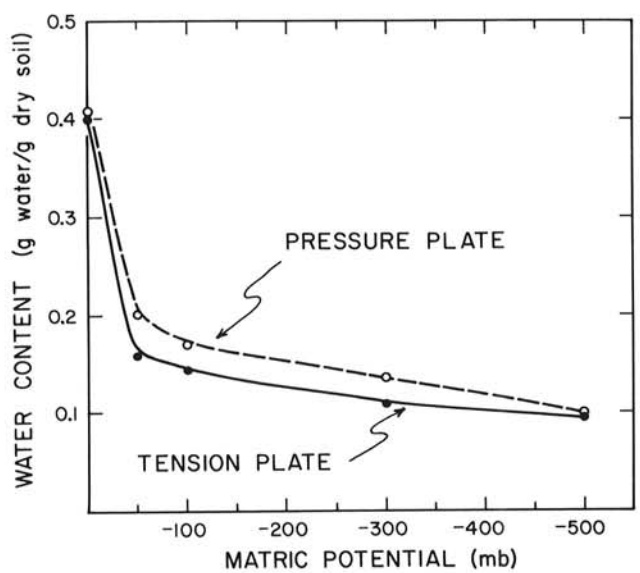

Fig. 1. Relationship between water content and ψ_m for sterilized sandy loam soil. Solid line is for samples taken from the ceramic tension plates used to control ψ_m. Dashed line is for samples equilibrated by use of a pressure plate apparatus.

Effect of soil moisture on behavior of mycelial fragments and of oospores in soil. Mycelial fragments and oospores were labeled with the fluorescent brightener, diethanol, 4-4′-*bis*[2-diethanol-amino-4-(3-sulfophenylamino)-5-triazinyl-(6)-amino]-stilben-2,2′ isulfonic acid sodium salt (7), and buried in soil at different ψ_m values. Two-day-old mycelial mats were washed twice with water and placed in a solution of the brightener (250 μg/ml) for about 14 hr. Excessive stain was removed by centrifugation three times. Mats were blended for 30 sec in a Waring Blendor at low speed. A suspension of mycelial fragments (0.1 ml) was applied in autoclaved Nitex (Tobler, Ernst & Traber Inc., Elmsford, NY 10523) nylon screen envelopes (1 cm × 1 cm; pore size, 100 μm), which were buried in the soil in ceramic cups at different ψ_m. The behavior of the mycelial fragments was observed by fluorescence microscopy in UV light with a Zeiss photomicroscope III equipped with a UV exciter filter in combination with a UCT 1/UCT 5 filter giving transmission at 365/366 nm. Oospores were labeled with diethanol during the last step of harvesting in which oospores were incubated in Glusulase overnight. The final concentration of diethanol was 500 μg/ml. Diethanol and Glusulase were removed by three centrifugations. The oospore suspension (40 μl) was placed in an autoclaved Nitex nylon screen envelope of pore size 10 ψ_m that was buried in soil in the ceramic cup. The germination of oospores in soil at different ψ_m was determined by fluorescence microscopy.

Inoculation of plants. Coarse sandy loam soil (69% sand, 20% silt, 11% clay) (pH 7.5) was sieved (2-mm pores) and autoclaved for 60 min on two successive days. A suspension of mycelial fragments or oospores was added to dry sterile soil while mixing to provide 350 mycelial fragments per gram of dry soil or 10 oospores per gram of dry soil. Infested soil was packed in a ceramic cup (100 g soil per cup) to a depth of 1 cm and irrigated. Ten alfalfa seedlings (cultivar Moapa 69) (2 to 3 days old) were planted in the soil of each cup. The number of seedlings killed was recorded every day after planting in infested soil for 21 days. Diseased roots were plated on $P_{10}VP$ (27) agar to verify infection. Seedlings grown in uninfested soil at different ψ_m served as controls. Treatments were replicated four times, and experiments were repeated three times.

The water columns attached to the cups were adjusted to provide soil ψ_m values of 0, −10, −50, −100, −200, −350, and −500 mb. The experiments were done in a Sherer Model CEL 512-37 growth chamber (Sherer-Gillett Co., Marshall, MI 49068) (set for a diurnal 12-hr dark period at 18 C and a 12-hr light period, 7.6 nanoeinsteins $cm^{-2} \cdot sec^{-1}$) at 24 C. The height of the water reservoir for each tension cup was adjusted differently to provide the desired ψ_m. Because of limitations due to the height of the growth chamber, and since the length of the column of water required to control ψ_m lower than −200 mb was greater then the height of the growth chamber, the column was set up in a connected series appropriate for these values (J. Duniway, *personal communication*). The tension cups were incubated at the same level under the light banks to eliminate variation due to temperature or light.

Formation of sporangia from infected roots and from mycelial disks. Three-day-old seedlings were planted in soil at different ψ_m and infested with diethanol-labeled mycelial fragments. The number of sporangia formed per root tip (7 cm in length) was determined by fluorescence microscopy. Five-millimeter-diameter mycelial disks without agar were obtained from aerial mycelium, grown on pea-dextrose agar (13) for 7 days. Pea-dextrose agar was prepared by soaking 100 g of dried peas overnight in 800 ml of distilled water, heating to boiling for 10 min, comminuting in a

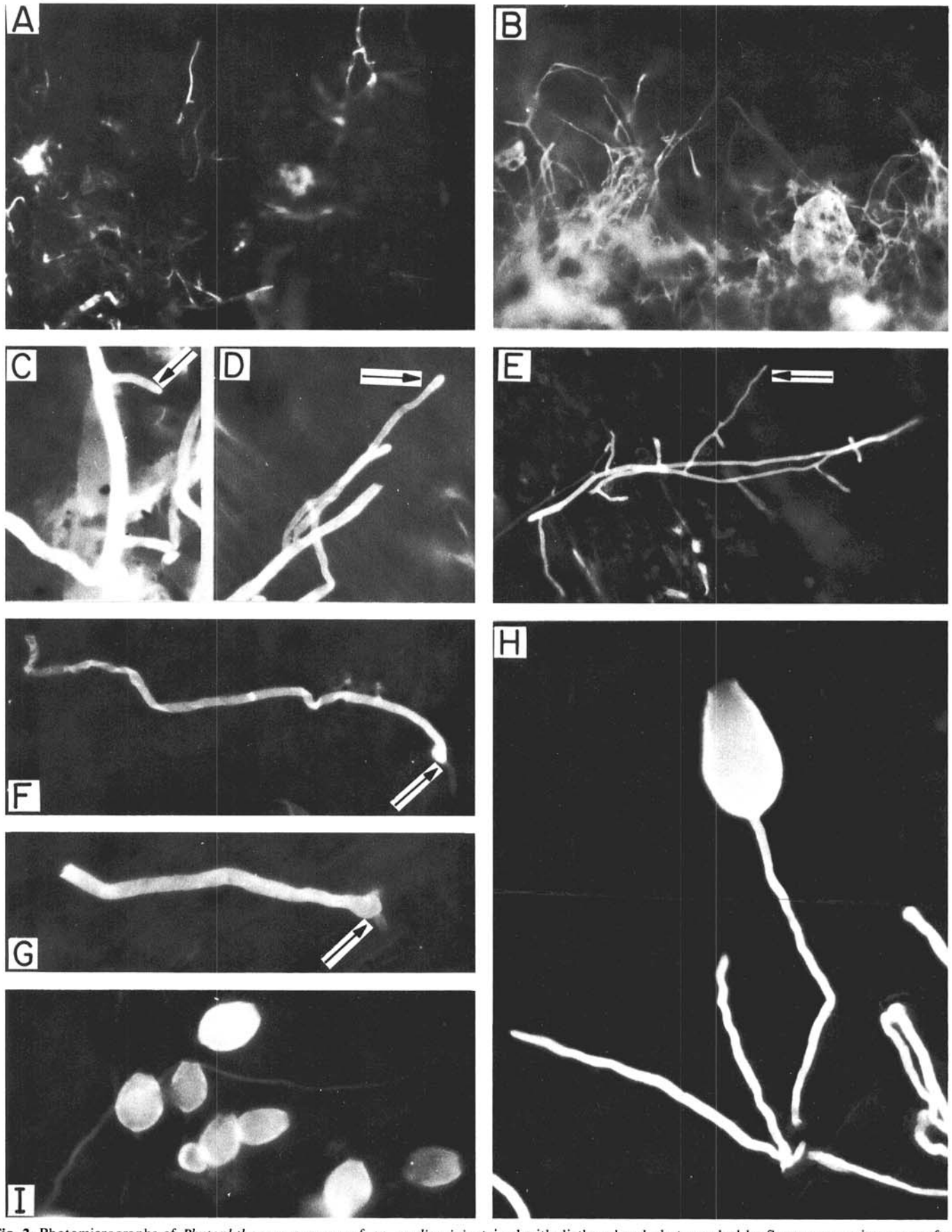

Fig. 2. Photomicrographs of *Phytophthora megasperma* f. sp. *medicaginis* stained with diethanol and photographed by fluorescence microscopy after incubation of mycelial fragments in soil. **A,** Lysis of hyphal fragments 48 hr old at soil ψ_m values lower than −50 mb (×100). **B,** Growth of hyphal fragments after 72 hr in soil when ψ_m were 0, −10, −50 mb (×100). **C to E,** New growth (formation of hyphal tips, arrow) from hyphal fragments 24 hr old at 0 mb (**C and D** = ×400; **E** = ×160). **F and G,** Hyphal fragments >72 hr old at 0 mb that infected alfalfa roots directly. Note the hyphal tips and the penetration peg (arrow) (**F** = ×250; **G** = ×400). **H and I,** Sporangia formed, on infected alfalfa roots 3 days after inoculation at 0 mb (**H** = ×400; **I** = ×160).

Waring Blendor, mixing with 10 g of dextrose and 17 g of Difco agar, adjusting to 1 L and autoclaving. Each mycelial disk was placed in a small Nitex nylon mesh envelope and buried in soil at different ψ_m. The number of sporangia formed at the edge of each mycelial disk was counted after 6 days.

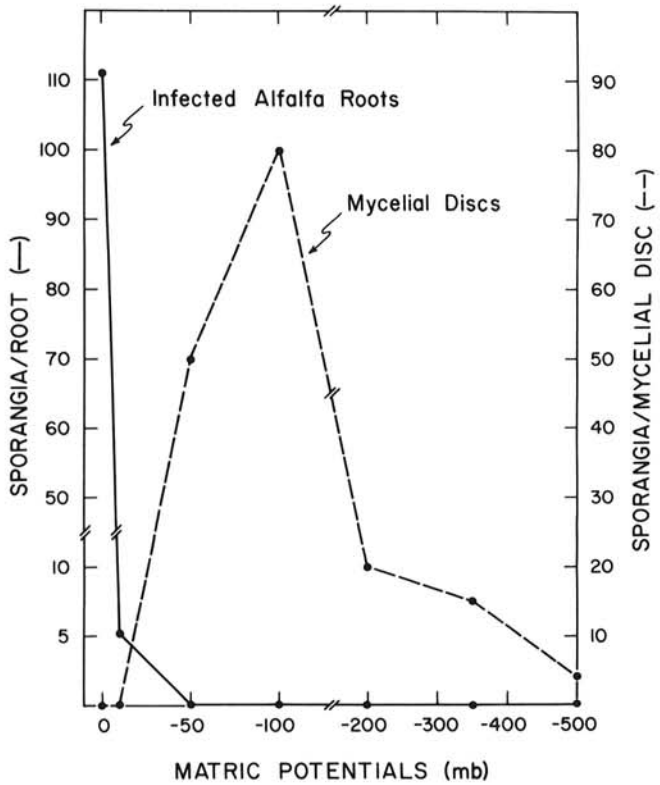

Fig. 3. Production of sporangia on infected alfalfa roots and on mycelial disks of *Phytophthora megasperma* f. sp. *medicaginis* at different soil ψ_m. Roots were from diseased alfalfa seedlings (3 days old) that had been planted in mycelium-infested soil at different ψ_m. Mycelial disks stripped from pea-dextrose agar were placed in nylon cloth envelopes and buried in soil. The soil ψ_m was varied by use of ceramic tension cups connected to columns of water at different heights.

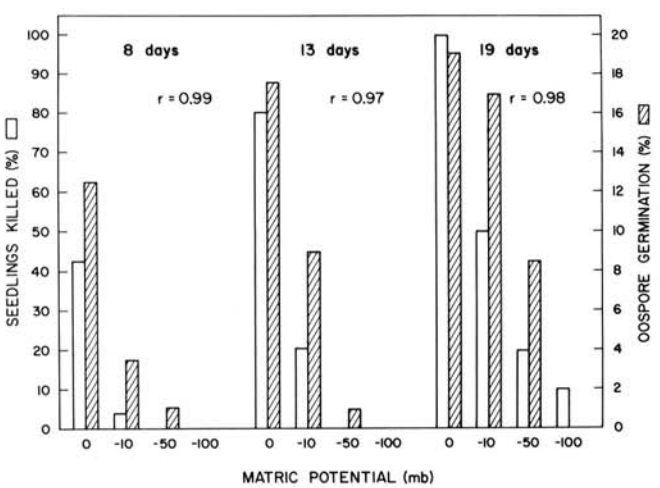

Fig. 4. Effect of soil ψ_m on incidence of Phytophthora root rot of alfalfa and on germination of oospores of *Phytophthora megasperma* f. sp. *medicaginis*. Alfalfa seedlings (3 days old) were planted in soil infested with oospores (10 oospores per gram of dry soil) stained with diethanol at different ψ_m. Oospore germination was observed by fluorescence microscopy and was detected by presence of sporangia from the oospores. Data are means of 12 replications of disease incidence and six replications of oospore germination.

RESULTS

Effect of ψ_m on the incidence of Phytophthora root rot in plants growing in soil infested with mycelial fragments. No disease occurred in plants grown in uninfested soils regardless of ψ_m. The percentage of plants killed in infested soil increased as the ψ_m approached 0 mb. At eight days after planting in infested soil the average percentage of plants killed at each ψ_m was: 0 mb, 62%; −10 mb, 30%; −50 mb, 28%; −100 mb, 20%; −200 mb, 14%; and −350 to −500 mb, 8%. The greatest incidence of plants killed occurred at 0 mb. Even the slight reduction of ψ_m to −10 mb significantly reduced the incidence of plants killed.

Behavior of mycelial fragments in soil at different ψ_m. When mycelial fragments were buried in soil at different ψ_m and observed at intervals from 12 hr to 8 days, no sporangia or zoospores were found. At 0 and −10 mb, hyphal tips were initiated from mycelial fragments 24 hr after they were buried. Branching of hyphal tips occurred 48 hr later (Fig. 2C–E) and after 72 hr the mycelium was densely branched (Fig. 2B). At −50 mb, formation of hyphal tips occurred at 24 hr, but mycelium lysed 48 hr later. At soil ψ_m lower than −100 mb, hyphae did not grow but lysed within 48 hr (Fig. 2A). Pegs from hyphal tips penetrated the root surface directly at 0 mb (Fig. 2F and G).

Effect of soil ψ_m on sporangium production. Six days after 3-day-old seedlings were planted in infested soil, sporangia formed on infected root tips (Fig. 2H and I). At 0 mb, 111 ± 36 sporangia formed per infected root (7 cm long), but at −10 mb there were less than 10 sporangia per root. At a soil ψ_m lower than −50 mb, no sporangia formed on infected alfalfa roots (Fig. 3).

When mycelial disks, instead of infected alfalfa roots, were placed in soil, the optimum ψ_m for sporangium production was −100 mb (Fig. 3). No sporangia were found at 0 mb. At −50 mb, fewer sporangia were formed from mycelial disks. The number of sporangia was decreased at soil ψ_m below −100 mb (Fig. 3).

Effect of soil ψ_m on incidence of disease on plants in soil infested with oospores. When the inoculum density was kept constant (10 oospores per gram of dry soil), the incidence of plant death increased with increasing ψ_m (Figs. 4 and 5). Thirteen days after planting in oospore-infested soil, the disease incidence at 0 mb was 78% and was 18% at −10 mb (Fig. 5). No disease occurred at −50

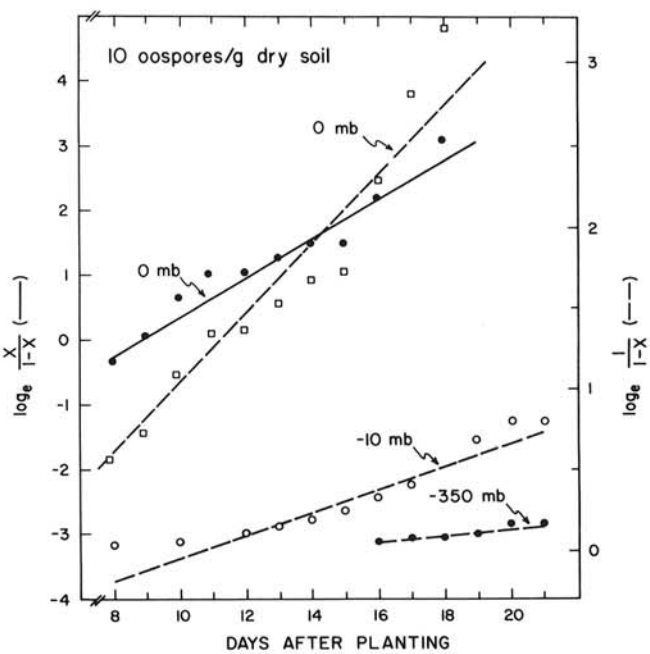

Fig. 5. Regression lines for the increase in mortality ($\ln 1/(1-x)$ in which x = percentage of seedlings killed) with time after planting in soil infested with 10 oospores per gram of soil at different ψ_m. The transformation x to $\ln 1/(1-x)$ (broken line) has been considered typical of single cycle diseases (28). Data for 0 mb were also transformed to $\ln x/(1-x)$ (solid line) considered to be typical of multiple cycle diseases (28).

mb or at lower ψ_m.

Germination of oospores in soil at different ψ_m. Eight days after oospores were buried in soil, about 12% germinated via sporangia formation and subsequent release of zoospores at 0, −10, −50 mb, but not at lower ψ_m. After 19 days, 19% germinated at 0 mb, 17% at 50 mb, and 8% at −50 mb and 0% at −100 mb (Fig. 4). There was a significant correlation ($r = 0.97, 0.98, 0.99$) between the effect of ψ_m on incidence of plant death and the effect of ψ_m on germination of oospores after different times of incubation (Fig. 4).

Effect of soil ψ_m on the disease increase rate in soil infested with hyphal fragments or oospores. When hyphal fragments were used as inoculum, the disease incidence (percentage of seedlings killed) increased with time at different ψ_m (Fig. 6). When the percentage of mortality of seedlings versus time was plotted arithmetically, the disease progress curve was sigmoid at 0 mb. The exponential stage occurred 6 days after planting in infested soil. At 0 mb a higher incidence of disease occurred earlier than at soil ψ_m values less than 0 mb. Eventually after 16 days, 100% of the seedlings died at 0 mb. In contrast, even at prolonged incubation times, at −10 mb, the percentage of seedlings killed did not exceed 60% and at −50 to −500 mb was about 30%.

Soil ψ_m influenced the disease increase rate (Table 1) determined by linear regression analyses of time after planting in the infested soil versus the logit transformation ($\ln 1/(1-x)$ in which x = percentage of plants killed) of the incidence of plant death (28). Before 6 days (after planting) the disease increase rate for soil at 0 mb was 0.04 per unit per day, which was the same as that for soil at and lower than −10 mb. However after 6 days the rate in soil at 0 mb increased markedly to 0.36 per unit per day, while in soil at −10 mb to −200 mb the rate remained at 0.04 per unit per day. In soil at −350 and −500 mb the rate was only 0.02 per unit per day. Thus, a reduction of the soil ψ_m was significantly correlated with a reduction in the disease increase rate.

When soil was infested with 10 oospores per gram of dry soil, the incidence of disease was also reduced by decreasing the ψ_m of soil (Fig. 5). The incidence of disease after 8 days was much higher at 0 mb than at the lower ψ_m values. Disease progressed most rapidly at 0 mb. At 0 mb, 100% of the seedlings were killed, whereas at −10 mb, 60% were killed and at −50 to −500 mb, less than 20% were killed. At −50 to −500 mb, the plants were not killed until 16 days after planting. When disease incidence values were transformed, the rate of disease increase at 0 mb was about four times higher than at the lower soil ψ_m (Table 1). Reduction of soil ψ_m not only reduced the incidence, but also delayed the development of Phytophthora root rot.

The relative transformation in $\ln x/(1-x)$ at 0 mb gave a straighter disease increase rate line than the absolute transformation $\ln 1/(1-x)$. This suggested that at 0 mb inoculum increased as in multiple infection diseases. At $\psi_m < 0$ the absolute transformation $\ln 1/(1-x)$ gave the straightest disease increase rate line, which suggested that at less than 0 mb the inoculum density did not increase (28) (Figs. 5 and 6).

DISCUSSION

Results reported here reemphasize the importance of soil moisture in the epidemiology of Phytophthora root rot of alfalfa. The high incidence of plant death in soil at ψ_m values of 0 mb and reduction of disease by a decrease in the ψ_m of soil (Fig. 4) agrees with the field observation that root rot of alfalfa is always more severe in soil with a high water content (18).

The behavior of hyphal fragments in soil explained in part the greater incidence of plant death in flooded soil. Germ tubes from hyphal fragments penetrated the alfalfa root directly without the production of sporangia and release of zoospores (Fig. 2F and G). The data suggest that at 0 mb more nutrients were available due to leakage and diffusion from roots. We previously reported (12) that a large amount of nutrients leaked from roots of alfalfa plants following the saturation of soil for 1–4 days. Since a wet soil is interlaced with water-filled pores, diffusion of nutrients may be facilitated (29). Increased percentage of germination of chlamydospores of *P. cinnamomi* was correlated with increased incidence of infection of avocado roots by Sterne et al (25). They also showed that more chlamydospores germinated when greater amount of nutrients diffused from roots at higher soil ψ_m. In our study, when soil ψ_m was reduced, it appeared that less nutrients and free water were available and the mycelium lysed (Fig. 2A).

Our finding that no sporangia or zoospores were formed on buried hyphal fragments even at 0 mb is not in agreement with observations reported by other investigators utilizing mycelial disks in soil (3,4,14). This difference can be explained because the nutrient content of hyphal fragments is less than in mycelial disks and the physical damage to hyphal fragments places a greater stress on the inoculum. Although it is not likely that hyphal fragments exist for an extended period in natural field soil, this study demonstrates that direct infection of roots can occur by hyphae.

Since oospores could be the most important survival structures of Pmm, any factor(s) that would affect the germination of oospores would probably contribute to the infection of alfalfa roots. The high correlation between germination of oospores and

TABLE 1. The influence of soil matric potential on the disease increase rate of Phytophthora root rot of alfalfa

Soil matric potential (mb)	Disease increase rate (/unit/day)[a]	
	Oospores[b]	Mycelial fragments[b]
0	0.24	0.360
−10	0.06	0.040
−50	0.050	0.040
−100	0.030	0.040
−200	0.020	0.040
−350	0.020	0.020
−500	0.02	0.020

[a] Disease increase rates were slopes of linear regression lines generated when $\ln 1/(1-x)$ was plotted against time (28); x = the proportion of plants dead.
[b] Soil was infested with either oospores (10 oospores per gram of dry soil) or with mycelial fragments (350 mycelial fragments per gram of dry soil). Rate of disease increase for the first 6 days after planting was 0.04

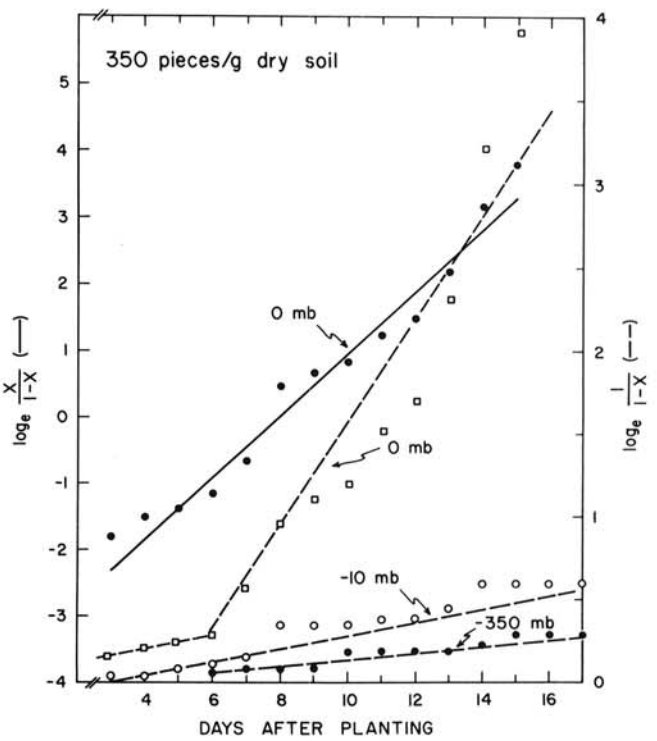

Fig. 6. Regression lines for the increase in mortality ($\ln 1/(1-x)$ in which x = percentage of seedlings killed) with time after planting in soil infested with 350 mycelial fragments per gram of soil at different ψ_m. The transformation of x to $\ln 1/(1-x)$ (broken line) has been considered typical of single cycle diseases (28). Data for 0 mb were also transformed to $\ln x/(1-x)$ (solid line) considered to be typical of multiple cycle diseases (28).

incidence of disease at different soil ψ_m ($r = 0.97$) supports this hypothesis. With a constant number of oospores in soil, germination via formation of sporangia and release of zoospores (indirect germination) would increase the inoculum density more than direct germination of oospores (via germ tube and mycelial growth). At higher ψ_m (0, −10, −50 mb) oospores germinated indirectly and produced sporangia, but at lower soil ψ_m, oospores germinated directly, but did not produce sporangia. This observation could explain why the incidence of disease was greatest at higher ψ_m. In addition to the production of sporangia and zoospores from oospores at 0 mb, the increased chemotactic effect of root exudates in saturated soil (12) would provide still another factor that could favor a higher incidence of disease at 0 mb. Stanghellini and Burr (21,22) also pointed out the importance of increased nutrients and soil moisture on the germination of oospores of *Pythium aphanidermatum*.

Among the numerous reports on the effect of soil ψ_m on the formation of sporangia by *Phytophthora* (2,3,8,13,14,16,26), there are different explanations for the relationship of sporangia production and soil ψ_m (8). When washed mycelium (8) or mycelial disks (2,3,13,14) were used, the optimal ψ_m was between −10 to −100 mb. When infected radicles (16) and infected leaf disks (26) were used, the optimum was 0 mb. The difference has been attributed to inter- and intraspecific differences in sensitivity to aeration and/or moisture requirements. Gisi et al (8) compared data from different authors and suggested that the apparent discrepancy in values for optimal ψ_m was due to use of mycelial mats as compared to infected tissue as inoculum. In this study, the optimal ψ_m for sporangium formation on infected roots was 0 mb and −100 mb for mycelial disks. Apparently, infected roots provide a substrate for the fungus that differs from mycelial mats in nutrients, water status, and microbial interaction. Although a study with mycelial mats may provide information on the precise effect of control of ψ_m on the physiology of *Phytophthora*, research with infected roots may provide data more applicable to field conditions and to epidemiology.

Transformation models are useful in quantitation of epidemics (10,19,30,31). The soil water status had a significant effect on the disease increase rate and time of onset of disease. At 0 mb, the break in the curve (transformation of x to $\ln 1/[1-x]$ at 6 days after planting (Fig. 6) coincided with the production of sporangia from infected roots. It appears that root diseases caused by *Phytophthora* could behave as multiple cycle diseases.

Both oospores and infected roots, could serve as inoculum. At 0 mb, oospores germinated via formation of sporangia and release of zoospores, and sporangia formed from infected roots. These two effects plus a predisposition effect caused by flooding soil (12) and the better growth of hyphal tips in wet soil contribute to an explanation why diseases induced by *Phytophthora* are more severe in soil with excessive moisture. With the same amount of inoculum, varying the soil ψ_m affected the rate of disease increase (Figs. 5 and 6). Thus, in the epidemiology of Phytophthora root rot, soil moisture may be much more important than the initial inoculum density, which is characteristic of multiple cycle diseases (28).

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