Production of Sporangia and Release of Zoospores by Phytophthora megasperma in Soil

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ABSTRACT

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Segments of alfalfa radicles colonized by *Phytophthora megasperma* were buried in field soil and subjected to various moisture and temperature conditions in the laboratory. In flooded soil, sporangia were produced at 8-24 C, but not at 28 C; optimum temperatures for production of sporangia were 12-16 C. At 16 C sporangium production was initiated after 4 hr and reached a peak at 12 hr. Maximum production of sporangia occurred in flooded soil, with decreased production in soil at −0.05 and −0.1 bar matric water potential. Very few sporangia were produced at −0.6 bar and none at −2.8 bars. Sporangia germinated indirectly (released zoospores) in flooded soil at 8-24 C; optimum temperature for indirect germination was 16 C. At 16 C indirect germination began 6-8 hr after flooding and was 95% complete after 72 hr. In soil at −0.05 bar both direct (via germ tube) and indirect (via zoospores) germination were observed; sporangia in soil at −0.1 bar germinated directly. Zoospores originating from sporangia, produced at various depths in two flooded soils, were detected in surface water using alfalfa seedlings as bait. Zoospores were able to migrate upward through 65 mm of a sandy loam soil, but rarely moved more than 24 mm upward through a silt loam soil. In the flooded silt loam, probability of zoospores reaching surface water depended on number of sporangia initially present and their depth.

Additional key words: *Medicago sativa*.

Infective propagules, presumably zoospores, of *Phytophthora megasperma* Drechs. and other *Phytophthora* species have been detected in irrigation water by means of baiting techniques (8, 9, 14). However, little work has been published regarding the precise conditions under which *Phytophthora* spp. form sporangia in soil and release zoospores which could reach irrigation water. Ho (6) found that *P. megasperma* var. *sojae* produced sporangia 15 hr after mycelial mats were buried in wet garden soil at 25 C. Sneh and McIntosh (13) determined that mycelial mats of *Phytophthora cactorum* formed numerous sporangia in soil at −0.1 bar and −0.3 bar matric water potential (ψ_m), but formed few or none at −3.0 bars and at temperatures of 10, 15, and 24 C. The optimum temperature for sporangium production by *P. cactorum* in soil at −0.1 bar was 15 C. Mycelial mats of *Phytophthora drechsleri* buried in soil at 23-27 C produced sporangia and released zoospores when ψ_m values were between −0.025 and −0.3 bar (2, 3). Few or no sporangia were produced in flooded soil or in soil drier than −4.0 bars.

The ability of zoospores to act as dispersal units of root pathogens depends upon their ability to move through soil from their point of origin. Mehrotra (10) demonstrated that zoospores could move a limited distance through soil to plant roots. He noted that such migration was largely dependent on movement of water through the soil. However, water percolating downward through soil could not act as a dispersal agent in upward movement of zoospores from root-borne sporangia. The tortuous path through soil pores may limit the distance zoospores could move upward, since contact stimulus has been shown to encourage encystment and thus, loss of motility (7).

In this paper, we examine two areas concerning asexual reproduction of *P. megasperma* in soil: (i) the effect of soil moisture and temperature on production of sporangia and release of zoospores, and (ii) the ability of zoospores to move upward through the soil column to reach surface flood water.

MATERIALS AND METHODS

Phytophthora megasperma, isolated from infected alfalfa (*Medicago sativa* L.) roots collected near Tucson, Arizona, was maintained on V-8 juice agar. Soil used in the experiments was Gila silt loam collected from a site adjacent to a naturally-infested field and stored in 150-liter cans with loosely fitting lids at 20-25 C. Soil was air-dried and sieved through a 2-mm screen prior to use. Moisture-holding characteristics of the soil (Fig. 1) were determined through the use of a tensiometer (consisting

of a Büchner funnel with sintered-glass base plate and a hanging column of water) and a pressure-membrane apparatus (5). To construct the curve in Fig. 1, saturated soil was subjected to various suctions on a tensiometer ($\psi_m \geqslant -0.1$ bar) or pressure-membrane apparatus ($\psi_m < -0.1$ bar), and the amount of water remaining per 100 g of soil after equilibration was determined by drying the soil to constant weight at 105 C and measuring weight of water lost during drying.

In order to approximate natural conditions, experiments were conducted using host tissue colonized by the fungus. Colonized host tissue was produced by placing 2.5-day-old axenic alfalfa seedlings (cultivar Hayden) on the margins of 10-day-old colonies of *P. megasperma*. After 3 days of incubation at 21 C (when the fungus was present in the seedlings only as mycelium and oospores) seedlings were removed from the colonies, and each radicle was cut to a length of 13 mm. Experiments concerning asexual reproduction of the fungus were carried out by placing the colonized radicles in soil. All treatments consisted of two replicates, and each experiment was repeated at least once.

Effect of temperature on asexual reproduction.—Polypropylene cylinders (4 × 2.6 cm) were covered at one end with coarse nylon cloth (0.2 mm pores) and filled with lightly tamped soil to a depth of 1.6 cm. Five colonized radicle segments and a small nylon marker were placed on the surface and covered with lightly tamped soil to a depth of 2.0 cm. The cylinder was then placed in a 50-ml beaker containing distilled water at the desired temperature and allowed to become saturated from the bottom. Additional distilled water was added to the beaker until the soil surface was covered with 2-4 mm of water. This process of saturating the soil required approximately 5 min. Each beaker was placed in a screw-cap jar and incubated at the desired temperature. At various time intervals, the cylinders were taken from the beakers and allowed to drain for 5-10 min. The radicle segments were then recovered, stained in acid fuchsin (aqueous solution of 0.01% acid fuchsin and 8.5% lactic acid), and examined microscopically to determine the total number of sporangia, and the number of empty sporangia, on each radicle segment. Empty sporangia were interpreted as having released zoospores by indirect germination (2), based on their similarity with empty sporangia in aqueous culture immediately after zoospore release. Only sporangia extending out horizontally from the radicle segments were counted; no attempt was made to count sporangia directly under or above radicle segments.

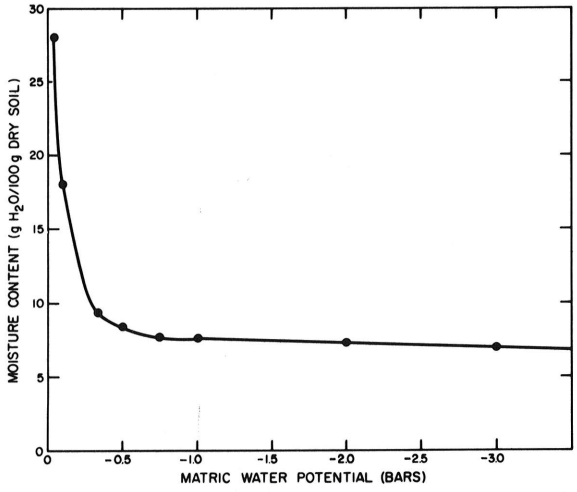

Fig. 1. Moisture characteristics (drying boundary curve) of Gila silt loam.

Effect of matric water potential on asexual reproduction.—The effect of ψ_m on production and germination of sporangia in soil was determined by a modification of the above procedure. "Flooded" treatments were prepared by the method described above. Treatments involving soil at $\psi_m \leqslant -0.6$ bar were prepared using soil that had been air-dried from saturation to the desired level prior to placement in the cylinders. Soil moisture was determined gravimetrically, by drying the soil to a constant weight at 105 C, and related to ψ_m using the curve shown in Fig. 1. Treatments involving soil at ψ_m values of −0.1 and −0.05 bar were prepared by first adjusting soil, which had previously been tamped into the polypropylene cylinders while dry, to the appropriate moisture level on tensiometers. Two cm of soil was then removed from each cylinder, the radicle segments placed on the tamped surface, and the 2.0 cm of soil replaced to cover the radicles. In all nonflooded treatments the cylinders were covered at the bottom with plastic film instead of nylon cloth and were placed directly in a screw-cap jar. All treatments were incubated at 16 C. At intervals, the radicle segments were recovered and examined as described previously. Moisture content of the soil was checked gravimetrically for each replicate at the time of radicle recovery. The change in soil moisture

TABLE 1. Comparison of pore space composition in the two soils used in zoospore mobility study with *Phytophthora megasperma*

Suction applied (cm H_2O)	Theoretical diameters of pore necks (μm)	Pore space[a]	
		Gila silt loam (%)	Silt loam/sand[b] (%)
0.0	> 0	100	100
24.5	>120	13.0	21.0
15.5	>190	6.9	10.0
10.0	>294	5.8	8.2

[a]Values were determined by placing a known volume of saturated soil on a tensiometer and measuring volume of water released at various suction levels.

[b]Mixture of Gila silt loam and coarse [0.97-mm (20-mesh)] silica sand.

level between the beginning of the experiment and the time of radicle recovery was minimal. Reported values of ψ_m include the following ranges of variation: -0.05 ± 0.01 bar, -0.10 ± 0.01 bar, -0.60 ± 0.03 bar, -2.8 ± 0.1 bar.

Zoospore mobility studies.—Zoospores originating from sporangia produced at various depths in flooded soil were detected in the surface flood water by using alfalfa seedlings as bait. In this study two soils were used: the Gila silt loam described previously, and a 1:1 (v:v) mixture of this silt loam with coarse [0.97 mm (=20-mesh)] silica sand. The silt loam/sand mixture was prepared in small amounts as needed for each trial. A comparison of pore size composition in the two soils is shown in Table 1. Data in Table 1 were obtained by placing a known volume of saturated soil on a tensiometer fitted with a burette, and measuring the volume of water released from the soil at various suction levels. Diameters of the soil pores which drain at any given suction can be calculated with the aid of the capillary rise equation. Thus the volume of water released at a given suction level is a direct measure of the volume of pores of the calculated diameter in the soil (5, pages 76-82).

The basic unit for the zoospore mobility study consisted of a polypropylene cylinder (2.6×5.5 or 7.5 cm) plugged at the bottom with a rubber stopper and having a small hole drilled through the wall near the bottom (Fig. 2). Soil was placed in each cylinder and tamped according to a uniform procedure. A variable number of colonized radicle segments was placed on the surface, and more soil added to cover the segments to the desired depth (4-36 mm in the shorter cylinders and 65 mm in the longer cylinders). A 5-mm space was left between the soil surface and the top of the cylinder. The cylinder then was placed in distilled water which entered via the small hole in the cylinder wall to saturate the soil from the bottom. After the water had flooded the soil, leaving standing water over the surface, a small cork was inserted to plug the hole in the cylinder wall. The cylinder then was removed from the water, dried, and the bottom dipped in melted paraffin to seal the rubber stopper and cork. The top of the cylinder was covered with nylon screen (170-μm pores). The whole assembly then was placed in a beaker and distilled water added to cover the 170-μm screen with 2-4 mm of water. The shorter cylinders were placed in 100-ml beakers; the longer cylinders were placed in 400-ml beakers. Four, 3-day-old alfalfa seedlings were placed in the water above the screen to serve as bait and the system was incubated at 16 C. Seedlings were removed 24 and 48 hr after flooding and incubated in sterile distilled water at 16 C. Infected seedlings bore sporangia within 5 days. At the time of removal of the last seedlings from the system, the colonized radicle segments were recovered from the cylinders and examined microscopically to determine the number of indirectly-germinated (empty) sporangia present. This number was used as an index of inoculum level which indicated the relative number of zoopores produced at the given depth of each particular replicate.

Since the soils used in these experiments were nonsterile, infected radicles were omitted in check treatments to determine if the soil contained indigenous *Phytophthora* species that could infect the alfalfa seedlings used as bait. As additional checks, representative cylinders that contained infected radicles were sealed at their tops with rubber stoppers to determine if there were openings at the bottoms of the cylinders through which zoospores could escape, thereby reaching surface water without traveling upward through the soil column.

The sensitivity of the baiting technique for detecting zoospores was determined by setting up the system as described previously, but omitting the colonized radicle segments. A known number of zoospores was introduced under the 170-μm screen of each cylinder before placement in the beaker, and four seedlings placed in the water above the screen to serve as bait.

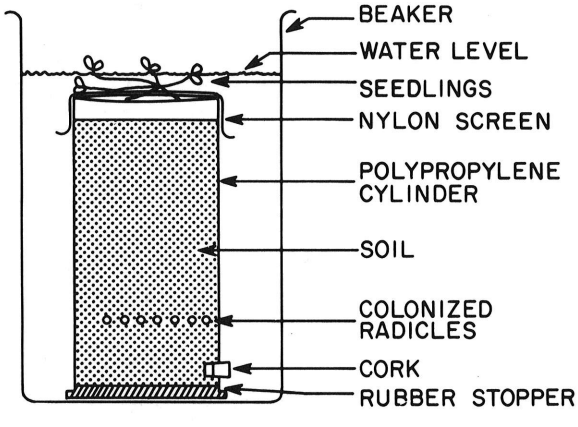

Fig. 2. Apparatus used in zoospore mobility study. Dimensions are described in the text. Ability of *Phytophthora megasperma* zoospores to swim upward through soil from their point of origin on colonized alfalfa radicles to surface water was indicated by infection of seedlings. Seedlings were removed from the beaker at 24 and 48 hr and incubated in distilled water at 16 C, where infected seedlings bore sporangia within 5 days.

RESULTS

Effect of temperature on asexual reproduction.—Fig. 3-A shows the results of one experiment concerning the effect of temperature on production of sporangia in flooded soil. Both full (nongerminated) and empty (indirectly germinated) sporangia were included in sporangia counts. Repetitions of the experiment gave similar results in terms of relative numbers of sporangia produced at various temperatures, though the absolute numbers differed. Sporangium production was greatest at 12 and 16 C, and none was produced at 28 C or higher. The fewer sporangia at 48 hr compared to 24 hr, was a consistent observation. This decrease was attributed to lysis of the walls of empty sporangia after zoospore release, based on the observation that sporangial walls in various states of degradation were present on the radicles as time progressed. Indirect germination of sporangia was greater at 48 hr than at 24 hr at all temperatures, and was greater at 16 C than at any other temperature tested (Fig. 3-B).

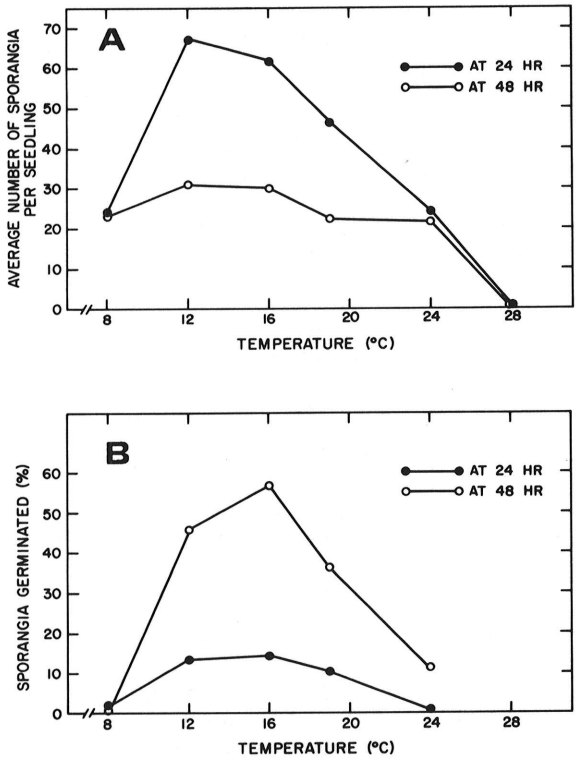

Fig. 3-(A,B). Effect of temperature on asexual reproduction by *Phytophthora megasperma* in flooded soil. Radicles of 2.5-day-old alfalfa seedlings, which had been infected by placing them on colonies of *P. megasperma* for 3 days at 21 C, were buried 2.0 cm deep in Gila silt loam. Values for each time and temperature are averages of two replicates. **A)** Sporangial production in flooded soil as a function of temperature. **B)** Indirect germination (zoospore release) of sporangia in flooded soil as a function of temperature, where percentage sporangia germinated = number of empty sporangia observed/total number of sporangia observed.

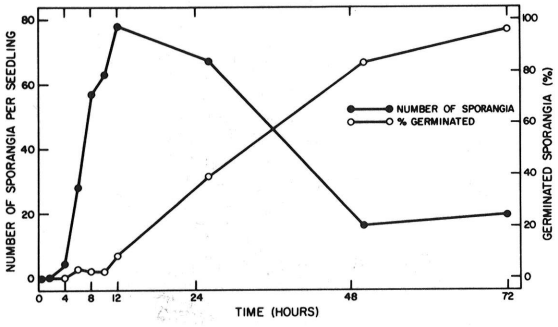

Fig. 4. Production and indirect germination of sporangia of *Phytophthora megasperma* in flooded soil at 16 C as a function of time. Radicles of 2.5-day-old alfalfa seedlings, which had been infected by placing them on colonies of *P. megasperma* for 3 days at 21 C, were buried 2.0 cm deep in Gila silt loam. Percentage germinated sporangia = number of empty sporangia/total number of sporangia. Values are averages of two replicates.

Sporangial production began about 4 hr after the colonized radicle segments were buried in flooded soil at 16 C, and reached a peak at 12 hr (Fig. 4). Sporangia that had germinated indirectly were first observed 6-8 hr after flooding and comprised about 95% of the total number of sporangia present by 72 hr. The total number of sporangia observed on the buried radicle segments was highest 12 hr after flooding; the lower numbers observed at later intervals were the result of increasing numbers of germinated sporangia which were subject to lysis in the soil.

Effect of matric water potential on asexual reproduction.—Greatest sporangium production occurred in flooded soil (0.0 bar), with less and slower production in soil at $\psi_m = -0.05$ bar and -0.10 bar (Fig. 5). Very few sporangia were produced at -0.6 bar, and none at -2.8 bars, within 12 days.

Whereas 95% of the sporangia had germinated indirectly after 3 days in flooded soil, less than 10% of the sporangia in soil at $\psi_m = -0.05$ bar had germinated indirectly after 12 days, and no empty sporangia were observed in soil at -0.10 bar or -0.6 bar. Some of the sporangia produced in soil at $\psi_m = -0.05$ bar and -0.10 bar germinated directly (via germ tube) after 6 days in soil. Hyphal swellings frequently were observed in mycelium of the fungus originating from radicle segments after 1-2 days in soil at $\psi_m = -0.6$ bar and -2.8 bars. Although no sporangia were produced in soil after 12 days at -2.8 bars, the mycelium was still viable as demonstrated by the production of numerous sporangia when the radicle segments were recovered and incubated in distilled water at 16 C.

Zoospore mobility studies.—Experiments concerning the sensitivity of the baiting technique for determining presence of zoospores showed that a minimum of 10-20

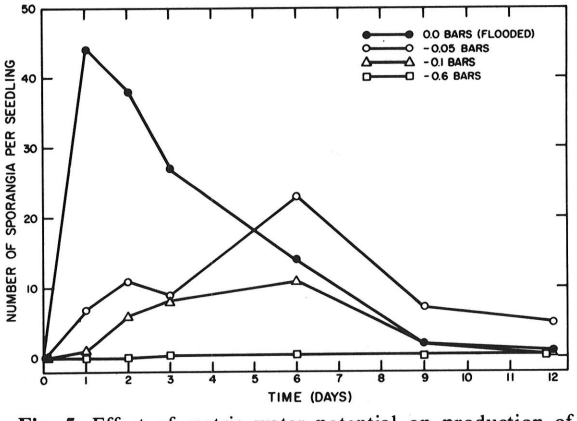

Fig. 5. Effect of matric water potential on production of sporangia by *Phytophthora megasperma* in soil at 16 C. Radicles of 2.5-day-old alfalfa seedlings, which had been infected by placing them on colonies of *P. megasperma* for 3 days at 21 C, were buried 2.0 cm deep in Gila silt loam. Soil moisture was adjusted to desired matric water potential (ψ_m), prior to addition of colonized radicles, using tensiometers for $\psi_m = -0.05$ and -0.1 bar, and by drying from saturation to predetermined water content (by weight) for $\psi_m = -0.6$ bar. Values are averages of two experiments with two replicates per treatment in each experiment.

zoospores were required for seedling infection in the 100-ml beaker system, whereas 20-40 zoospores were necessary in the 400-ml beaker system.

Seedlings used as bait in the check treatments were never colonized, indicating that colonization of the seedlings in other treatments was due to infection by zoospores that originated from the added inoculum and swarm upward through the soil column to surface water. water.

In the great majority of the zoospore mobility trials the same results were obtained with bait seedlings removed at 24 and 48 hr; however, in five of the 87 trials, seedlings removed at 48 hr were colonized whereas those removed at 24 hr were not.

We found that zoospores could move upward through 65 mm of the silt loam/sand mixture, but that they rarely moved more than 24 mm upward through the silt loam (Table 2). Ability of zoospores to move upward through all depths tested using the silt loam/sand mixture generally was unaffected by the number of zoospores initially present. In treatments using silt loam, however, the probability of zoospores reaching surface water generally depended on the number of zoospores initially present and their depth of origin. For example, 10-50 indirectly-germinated sporangia usually were sufficient to cause infection of bait seedlings if the radicles bearing the sporangia were buried 4 mm deep, but usually were insufficient if they were ≥ 8 mm deep. When the colonized radicles were 24 mm deep in silt loam, infection of bait seedlings was less probable at an inoculum level of < 50 than at > 50 indirectly-germinated sporangia.

The validity of the described method for determining the distance zoospores can move through a soil column rests on the assumption that zoospores, and no other propagules, are responsible for infection of the bait seedlings. This assumption is supported by two considerations. Preliminary experiments with zoospore suspensions showed that zoospores will swim into a capillary tube containing alfalfa seedling extract, encyst on the inner wall of the tube, and germinate within several hours. If the tubes containing germinated zoospore cysts are broken and placed on Myprozine-Vancomycin-Pimaricin agar (15), *Phytophthora* colonies are produced. When capillary tube traps were placed in the water above the 170-μm screen of the apparatus in Fig. 2, cysts appeared on the inner wall of the tube at about the same time that infection of bait seedlings occurred (6-8 hr after flooding). Recovery of *Phytophthora* from these traps was difficult, owing to the large number of contaminants in the system, but was successful on one occasion. That zoospores are the infective propagules in this system also is indicated by the fact that the propagules could move relatively long distances through the soil in less than 24 hr, a feat possible only for a motile propagule of this slow-growing fungus whose mycelium generally extended less than 2 mm out from a colonized radicle after several days in soil.

DISCUSSION

Phytophthora megasperma growing in alfalfa radicles produced sporangia in soil at temperatures ranging from 8 to 24 C, and at ψ_m values of 0.0 to -0.6 bar at 16 C. Flooded soil at 16 C was most favorable for production of sporangia and release of zoospores.

Pratt and Mitchell (12) found their baiting technique for *P. megasperma* in flooded samples of Wisconsin soils to be more effective at 15 and 20 C than at 25 C, and ineffective at 30 C. These results are consistent with our finding that production of sporangia and release of zoospores by *P. megasperma* in flooded soil was greater at 16 C than at 24 C, and absent at 28 C or above.

Our results for the effect of ψ_m on sporangial production are consistent with those of Sneh and McIntosh (13), who found production of sporangia by *P. cactorum* in soil at -0.1 and -0.3 bar, but not at -3.0 bars. In contrast, our results differ significantly from those for *P. drechsleri* (3) which produced numerous sporangia in soil at -2.1 bars to -3.5 bars, but few to none in flooded soil. Also *P. drechsleri* was shown to release zoospores in soil at ψ_m as low as -0.3 bar, whereas we found no indirect germination of *P. megasperma*

TABLE 2. Ability of zoospores of *Phytophthora megasperma* to swim upward through flooded soil at 16 C, relative to depth of zoospore origin and number of zoospores present

Inoculum level[c]	Frequency of successful zoospore movement[a] to surface from: Depth in Gila silt loam						Depth in silt loam/sand mixture[b]				
	4 mm	8 mm	16 mm	24 mm	36 mm	65 mm	8 mm	16 mm	24 mm	36 mm	65 mm
10-50	4/5[d]	1/5	0/5	2/7	1/5	0/2	3/3	1/2	0/4	2/2	2/2
50-100	2/2	3/3	3/3	3/3	0/2	0/1	1/1	3/3	...	3/3	...
100-150	1/1	...	1/2	1/2	...	...	...	1/1	1/2	...	3/3
150-200	...	...	...	...	...	...	1/1	1/1	2/2	1/1	1/1
200-250	...	...	...	...	0/1	0/1	1/1	1/1	1/1	1/1	1/1

[a]Zoospores were detected in surface water by their colonization of floating alfalfa seedlings used as bait.

[b]1:1 (v:v) mixture of Gila silt loam and coarse [0.97-mm (20-mesh)] silica sand.

[c]Radicles of 2.5-day-old alfalfa, which had been colonized by incubation on colonies of *P. megasperma* for 3 days at 21 C, were buried at various depths in flooded soil. Inoculum level is the number of indirectly-germinated sporangia observed on the colonized radicles in a given trial 48 hr after burial, and constitutes a measure of the number of zoospores originating at the specified depth.

[d]Frequency of successful zoospore movement to surface water from point of origin at radicle-borne sporangia is expressed as the number of trials in which bait seedlings were colonized divided by the total number of trials involving the indicated depth of origin and range of relative inoculum level.

sporangia in soil at ψ_m −0.1 bar. As Duniway noted (3), different species of *Phytophthora* may have inherently different water requirements for production of sporangia in soil. However, differences in the content of solutes in the soil water, both ionic and gaseous, also may be important. In Duniway's experiments the soil had been flooded for at least 12 hr prior to burial of *P. drechsleri*, which probably resulted in lowered oxygen levels due to microbial respiration combined with the low rate of oxygen diffusion through water. Since we flooded the soil immediately after *P. megasperma* had been buried, and made no effort to purge the water of dissolved oxygen, soil oxygen levels were probably lower in the experiments with *P. drechsleri* (3) than in our experiments with *P. megasperma*. The inhibiting effect of low oxygen levels on sporangium production by *Phytophthora* spp. has been noted (11). Further, a fungus colonizing host substrate in soil (as used in our experiments) may behave differently than if buried as a mycelial mat in soil [as used by Duniway (3)].

Finally, the possibility exists that temperature and soil moisture interact in their effect on sporulation, with sporangium production occurring at low temperatures in flooded soil, but inhibited by high temperatures in flooded soil. It should be noted that neither *P. drechsleri* nor *P. megasperma* produced significant numbers of sporangia in flooded soil at temperatures ranging from 23-28 C. Since Duniway's work with *P. drechsleri* did not include treatments in flooded soil at temperatures < 23 C, and our work with *P. megasperma* did not include treatments in drier soil at 23-27 C, a further comparison of data on temperature and soil moisture effects on sporulation in these two fungi is not possible. However, the data of Sneh and McIntosh (13) concerning the behavior of *P. cactorum* in soil indicate an interaction of soil moisture and temperature on sporangium production; after 8 days in soil, sporangium production was more than twice as great at −0.1 bar as it was at −0.3 bar when the temperature was 15 C (the optimum temperature for sporangium production in this species), but was only slightly greater at −0.1 bar than at −0.3 bar when the temperature was 29 C. Such an effect of temperature and soil moisture on sporulation could be mediated by lowered oxygen levels, since increasing temperatures would increase oxygen consumption by soil microorganisms while decreasing the solubility of oxygen in water.

After release from sporangia in flooded soil, zoospores of *P. megasperma* were found to swim upward through the soil column and infect healthy host tissue in surface water. Zoospores in numbers sufficient to infect host tissue were able to migrate upward through at least 65 mm of the silt loam/sand mixture, but they rarely moved more than 24 mm upward through the Gila silt loam. The probability of successfully reaching and infecting host tissue in surface water was enhanced by increasing the number of zoospores present at a given depth in Gila silt loam soil; the probability of infection, given a specified quantity of zoospores, decreased with increasing depth of zoospore origin in this soil type.

Allen and Newhook (1) found that zoospores of *P. cinnamomi* swim in a helical path with an amplitude of 26-70 μm, thus requiring a cylindrical space approximately 50-140 μm in diameter for unobstructed locomotion. They further noted that collision of zoospores with solid surfaces produces a disorienting effect which markedly restricts their active movement, and that such restriction makes zoospore movement through pores < 190 μm in diameter improbable. The difference in maximum upward migration of zoospores between our two soil types may be related to these spatial requirements of swimming zoospores. The silt loam/sand mixture contains a slightly higher proportion of large pores than does the Gila silt loam (Table 1). Thus, of the total water-filled volume in the two flooded soils, pores > 120 μm and > 190 μm in diameter are, respectively, 61.5% and 44.9% more frequent in the silt loam/sand mixture than in the silt loam. In addition, the arrangement of pores may be such that there are fewer continuous pathways of large pores in the Gila silt loam than in the silt loam/sand mixture. The consequent increased occurrence of contact stimulus, as well as the greater time required to find a navigable path, would favor encystment of the zoospores within the silt loam column. These considerations may be relevant to Ho's (6) observation that zoospore cysts were found on roots of plants in sand, but not on roots of plants in soil, after zoospore suspensions were applied to the surfaces of the two media. It should be pointed out that biological and physical differences between the two soils used in our study, other than pore-size composition, cannot be ruled out as possible influences on zoospore mobility.

Our study indicates that mycelium of *P. megasperma* growing in host tissue can produce sporangia and release zoospores in flooded soil within 6-8 hr of flooding at temperatures near 16 C. Such conditions are not uncommon in spring and fall for flood-irrigated alfalfa on heavy soils, where irrigation water may remain on the surface as long as 30 hr. The zoospores released in soil can act as agents of long-distance dispersal if they reach surface water. Our results indicate, however, that the distance zoospores can move upward through flooded soil is limited, and is restricted in fine-textured soils. Gray and Hine (4) determined that *P. megasperma* lesions on mature alfalfa taproots in Arizona occur at depths of 1-40 cm, with the majority occurring 3-20 cm below the soil surface. Given the limited range of zoospores in soil, only those lesions near the soil surface would be an important source of secondary inoculum which could contaminate irrigation water, unless soil cracks offered a clear path to surface water from greater depths in the soil.

LITERATURE CITED

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