PRODUCTION OF SPORANGIA AND RELEASE OF ZOOSPORES BY *PHYTOPHTHORA MEGASPERMA* IN SOIL

by

William Frederick Pfender

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This thesis has been approved on the date shown below:

Richard B. Hine
R. B. HINE
Professor of Plant Pathology

July 16, 1976
Date

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ABSTRACT

Segments of alfalfa radicles colonized by Phytophthora megasperma were buried in field soil and subjected to various soil moisture and temperature conditions. In flooded soil, sporangia were produced at temperatures of 8-24 C, but not at 28 C; optimum temperature for sporangial production was 12-16 C. At 16 C sporangial production was initiated after 4 hours and reached a peak at 12 hours. Maximum sporangial production 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 hours after flooding and was 95% complete after 72 hours. In soil at -0.05 bar both direct and indirect 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 soil, but rarely moved more than 24 mm upward through a silt loam

soil. In flooded silt soil, probability of zoospores reaching surface water depended on number of zoospores initially present and their depth of origin.

INTRODUCTION

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, 15). 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 hours after mycelial mats were buried in wet garden soil at 25 C. Sneh and McIntosh (14) 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 few or none at -3.0 bars, at temperatures of 10, 15, and 24 C. The optimum temperature for sporangial production 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 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 disease pathogens depends upon their ability to move through soil from their point of origin. Mehrotra (10) demonstrated movement of zoospores for a limited distance

through soil to plant roots. He noted that zoospore movement to roots was largely dependent on movement of water through the soil. Water percolating downward through soil, however, could not act as a dispersal agent in upward movement of zoospores from root-borne sporangia. Palzer (12) noted a negative geotactic response in zoospores of Phytophthora cinnamomi. Upward migration of zoospores through soil would be favored by this behavior; however, 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 study, I examine two areas concerning asexual reproduction of P. megasperma in soil: (a) the effect of soil moisture and temperature on production of sporangia and release of zoospores, and (b) the ability of zoospores to move upward through the soil column to reach surface flood water.

MATERIALS AND METHODS

Phytophthora megasperma was isolated from infected alfalfa roots collected near Tucson, Arizona and was maintained on V-8 juice agar. Soil used in the experiments was Gila silt loam collected from a field 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).

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 incubation at 21 C, when the fungus was present in the seedlings only as mycelium and oospores, seedlings were removed from the colonies and the radicles cut to a length of 13 mm. Experiments concerning asexual reproduction of the fungus were conducted by placing the colonized radicles in soil. All treatments consisted of two replicates, and each experiment was repeated at least once.

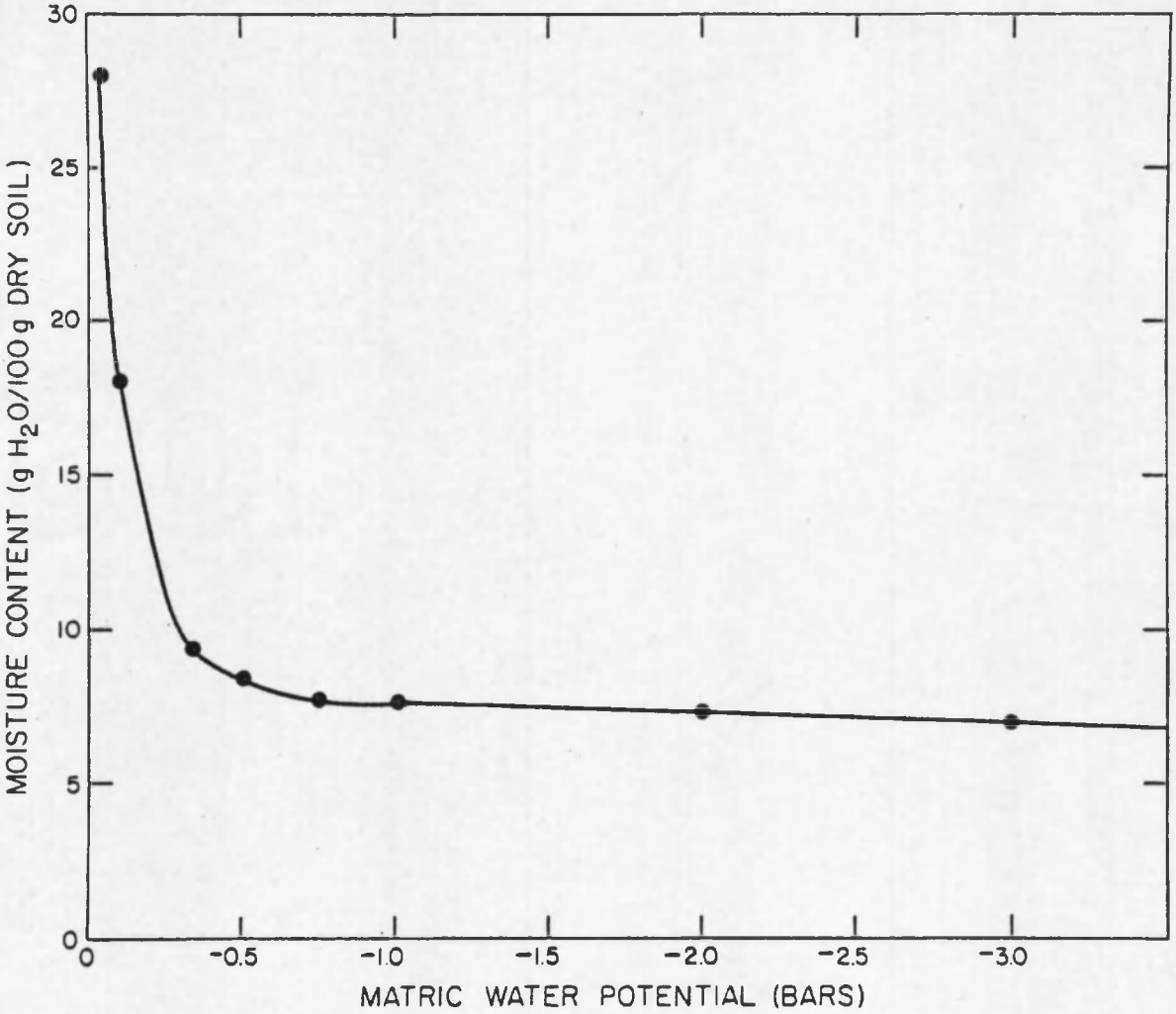

Fig. 1. Moisture characteristics (drying boundary curve) of Gila silt loam.

Effect of Temperature on Asexual Reproduction

Polypropylene cylinders (4 x 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 loaded cylinder was then placed in a 50-ml beaker containing distilled water at the desired temperature and allowed to become saturated from the bottom. More distilled water was then added to the beaker until the soil surface was covered with 2-4 mm of water. Each beaker was placed in a screw cap jar and incubated at the desired temperature. At various time intervals the colonized radicle segments were recovered after removing the cylinders from the beakers and allowing them to drain for 5-10 minutes. The radicle segments were 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. Empty sporangia (Fig. 2) were interpreted as having released zoospores by indirect germination (2). Only sporangia extending out horizontally from the radicle segments were counted; no attempt was made to count sporangia directly under or above radicle segments.

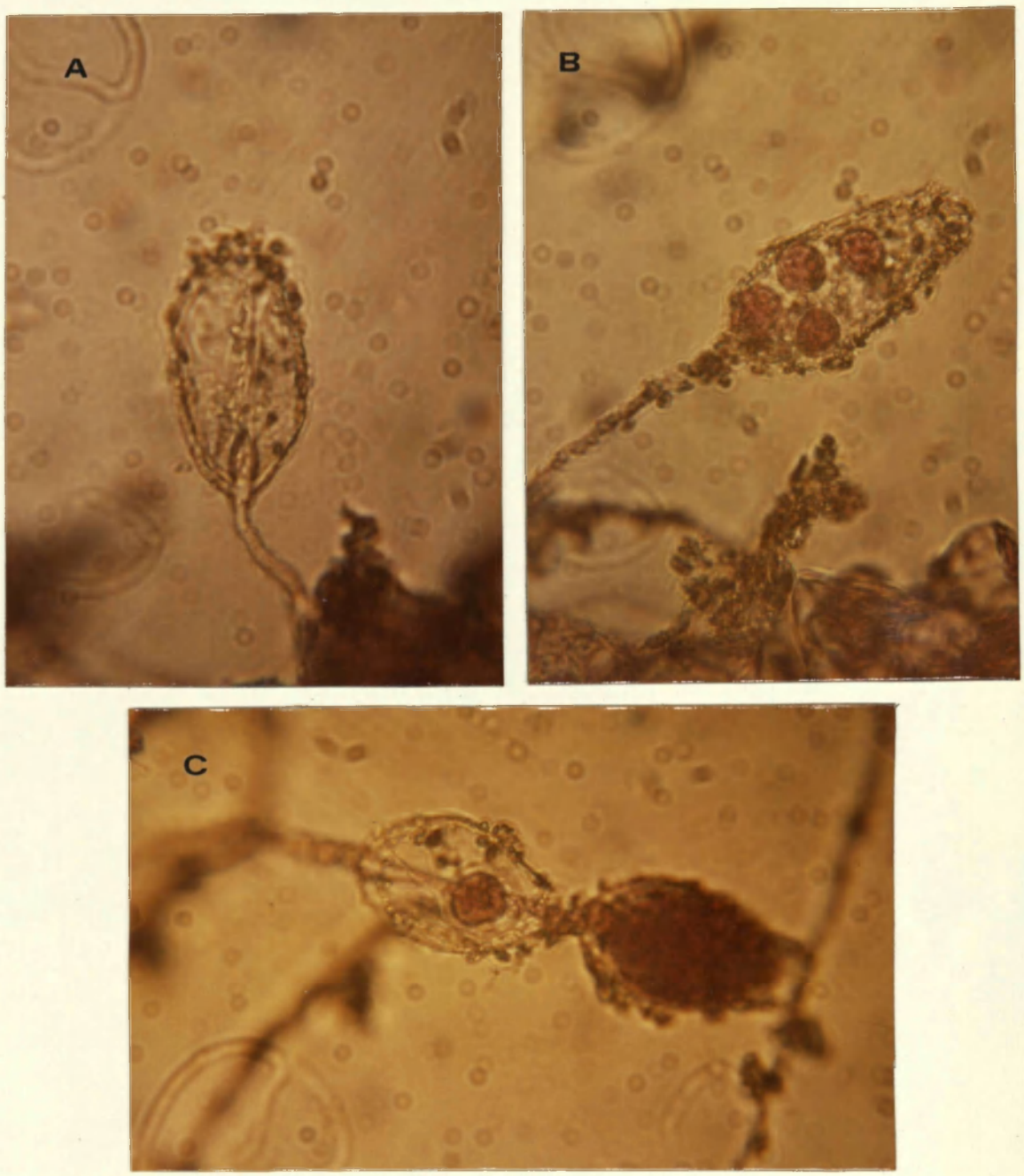

Fig. 2. Photomicrographs of indirectly-germinated sporangia of Phytophthora megasperma, borne on colonized alfalfa radicles in flooded soil at 16 C -- (A) Empty sporangium after zoospore release; (B) sporangium containing several cysts of zoospores which failed to emerge; (C) proliferated sporangium containing one encysted zoospore.

Effect of Matric Water Potential on Asexual Reproduction

The effect of matric water potential 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 $\psi_m \leq -0.6$ bar were prepared using soil which had been air-dried from saturation to the desired level. 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 $\psi_m = -0.1$ and -0.05 bar were prepared by first adjusting soil, contained in the polypropylene cylinders, 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.

Zoospore Mobility Studies

Zoospores originating from sporangia produced at various depths in flooded soil were detected in the surface flood water using alfalfa seedlings as bait. In this study two soils were used: (a) the Gila silt loam described previously, and (b) a 1:1 (v:v) mixture of this silt loam with coarse silica sand (#20 mesh). A comparison of pore size composition in the two soils is shown in Table 1.

Table 1. Comparison of pore space composition in the two soils used in zoospore mobility study.

	Fraction of pore space			
	As % of total soil volume		As % of pore volume	
Pores with neck diameters of	Gila silt loam	Silt loam/sand[a]	Gila silt loam	Silt loam/sand
> 0 µm	46.3[b]	36.7	100	100
> 60 µm	15.0	15.9	32.0	38.8
> 120 µm	6.0	7.7	13.0	21.0
> 190 µm	3.2	3.7	6.9	10.0
> 294 µm	2.7	3.0	5.8	8.2

[a] 1:1 (v:v) mixture of Gila silt loam and coarse silica sand.

[b] Values determined volumetrically using tensiometers.

The apparatus for this study consisted of polypropylene cylinders (2.6 x 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. 3). 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 was then 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 was then removed from the water, dried off, and the bottom dipped in melted parrafin to seal the rubber stopper and cork. The top of the cylinder was covered with nylon screen (170 µm pores). The whole assembly was then 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 hours after flooding

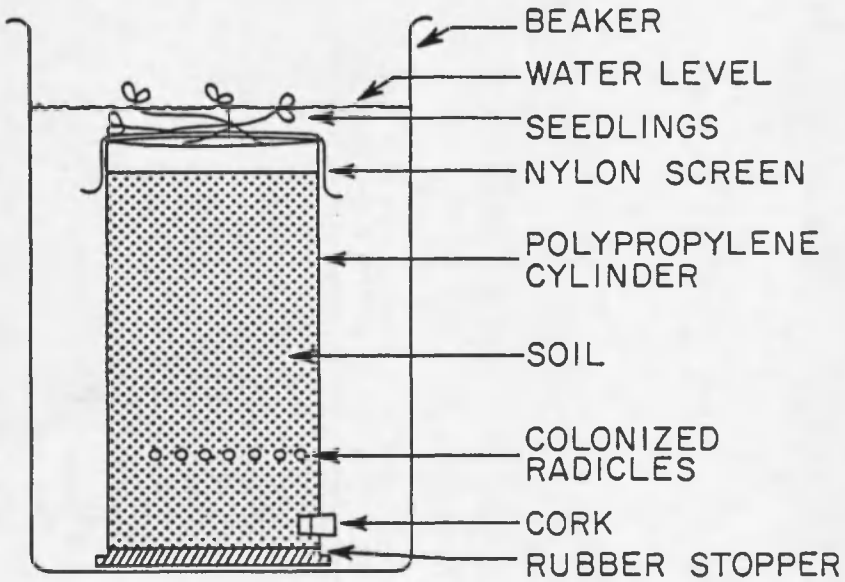

Fig. 3. Apparatus used in zoospore mobility study -- Ability of zoospores to swim upward through soil from their point of origin on colonized radicles to surface water was indicated by infection of seedlings.

and incubated 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 relative inoculum level which indicated the relative number of zoospores produced at the given depth of each particular replicate.

Check treatments were included to demonstrate that (a) infection of bait seedlings would not occur from Phytophthora species indigenous to the soil, and (b) there were no openings at the bottom of the apparatus through which zoospores could escape, thereby reaching surface water without travelling upward through the soil column. These checks consisted of, respectively, (a) treatments similar to those described in the previous paragraph, but with the colonized radicles omitted, and (b) treatments similar to those described in the previous paragraph, but with a sealed rubber stopper substituted for the 170-µm nylon screen at the top.

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 bait seedlings. This assumption is supported by two considerations. Preliminary

experiments with zoospore suspensions showed that zoospores would swim into a capillary tube containing alfalfa seedling extract, encyst on the tube's inner wall, and germinate within several hours. If the tubes containing germinated zoospore cysts were broken and placed on MVP agar (16), Phytophthora colonies were produced. When capillary tube traps were placed in the water above the 170-µm screen of the apparatus in Fig. 3, cysts appeared on the tube's inner wall at about the same time that infection of bait seedlings occurred (6-8 hours after flooding). Recovery of Phytophthora from these traps was difficult, due to the large number of contaminants in the system, but was successful on one occasion. The second consideration pointing to zoospores as the infective propagule in this system is the fact that the propagule could move relatively long distances through the soil in less than 24 hours, a feat possible only for a motile propagule of this slow-growing fungus.

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 bait seedlings placed in the water above the screen to serve as bait.

RESULTS

Effect of Temperature on Asexual Reproduction

Table 2 shows the results of one experiment concerning the effect of temperature on production of sporangia in flooded soil. Both full (ungerminated) and empty (indirectly germinated) sporangia were included in counts of sporangia present. Repetitions of the experiment gave similar results in terms of relative numbers of sporangia produced at various temperatures, though the absolute numbers differed. Sporangial production was greatest at temperatures of 12 and 16 C, with no sporangia produced at temperatures of 28 C or higher (Fig. 4). The lower number of sporangia observed at 48 hours, as compared to the number observed at 24 hours, was a consistent feature of the results and is probably due to lysis of the empty sporangia shells after zoospore release. Indirect germination of sporangia was greater at 48 hours than at 24 hours at all temperatures, and was greater at 16 C than at any other temperature tested (Fig. 5).

Sporangial production began about 4 hours after the colonized radicle segments were buried in flooded soil at 16 C, and reached a peak at 12 hours (Fig. 6). Indirectly germinated sporangia were observed 6-8 hours after flooding and comprised about 95% of the total number of sporangia

Table 2. Effect of temperature on production and indirect germination of sporangia of Phytophthora megasperma in flooded soil.

Temperature	Time	Average number of radicles[a] bearing 0 sporangia	1-9 sporangia	10-20 sporangia	> 20 sporangia	Avg. number of sporangia per radicle	% indirect germination
8 C	24 hr	0.0[b]	0.5	1.5	3.0	24.6[b]	2[b]
	48 hr	0.0	1.5	1.0	2.5	23.5	1
12 C	24 hr	0.0	0.0	0.0	5.0	67.0	13
	48 hr	0.0	1.0	0.5	3.5	30.8	46
16 C	24 hr	0.0	0.0	0.5	4.5	62.0	14
	48 hr	0.0	0.5	2.0	2.5	29.9	57
19 C	24 hr	0.0	0.5	0.0	4.5	46.2	10
	48 hr	0.0	2.0	0.5	2.5	22.4	36
24 C	24 hr	0.0	1.0	1.5	2.5	24.5	1
	48 hr	0.0	2.0	0.5	2.5	22.4	12
28 C	24 hr	5.0	0.0	0.0	0.0	0	--
	48 hr	5.0	0.0	0.0	0.0	0	--

[a]Based on a total of 5 radicles per replicate at each time and temperature.

[b]All values are averages of two replicates.

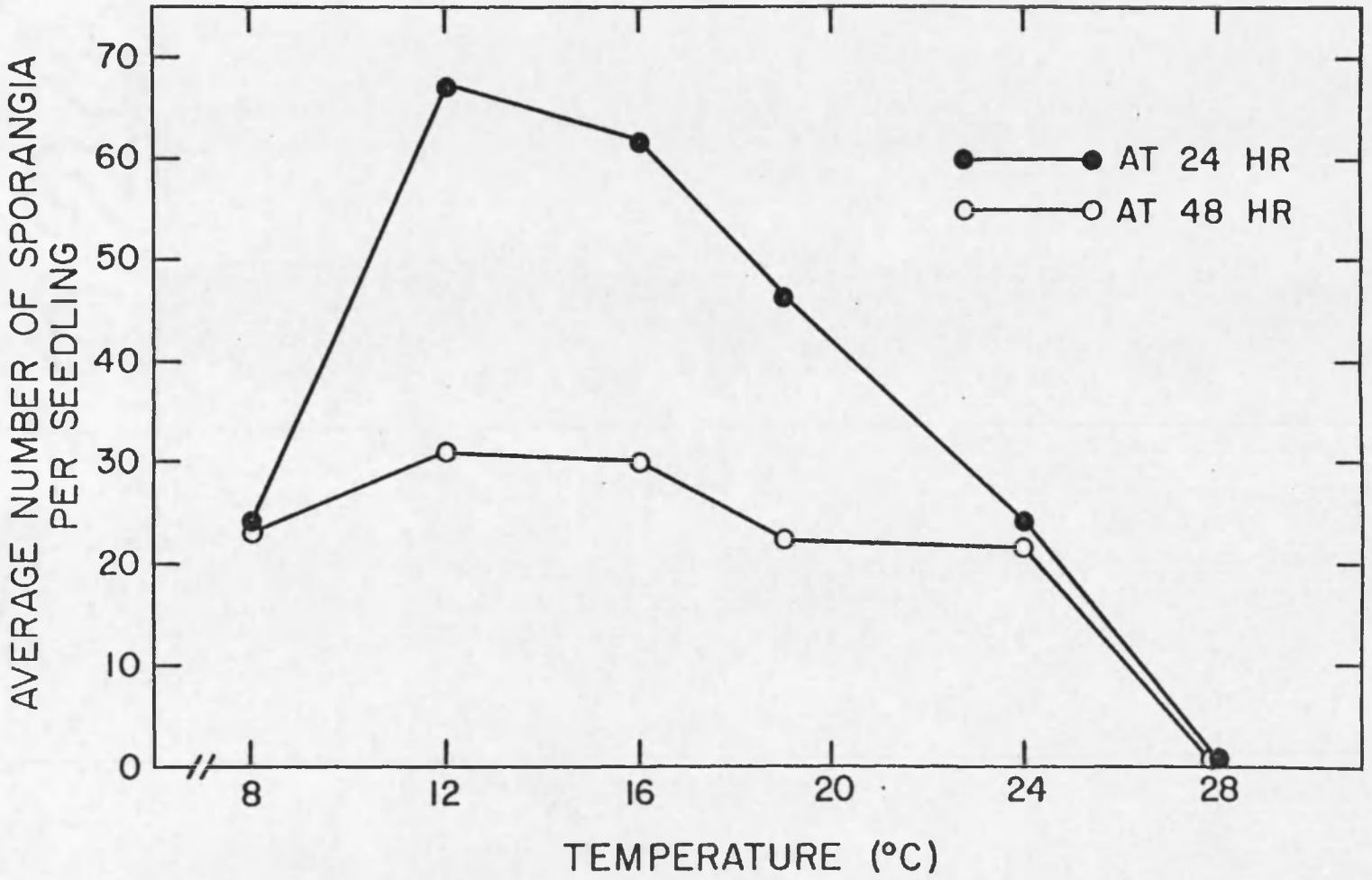

Fig. 4. Effect of temperature on sporangia production by Phytophthora megasperma in flooded soil.

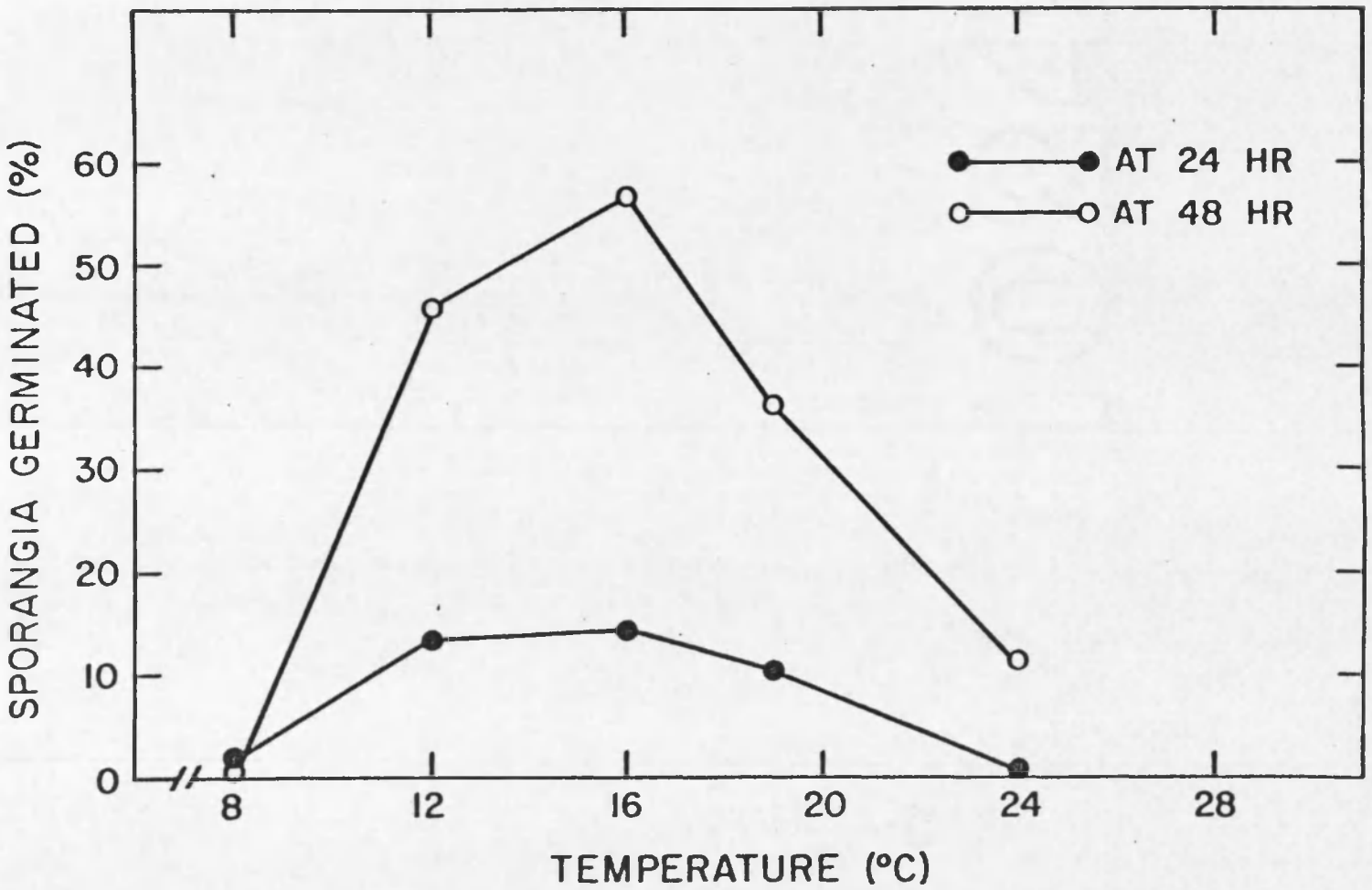

Fig. 5. Effect of temperature on indirect germination (via zoospores) of sporangia of Phytophthora megasperma in flooded soil -- Data are averages of two replicates.

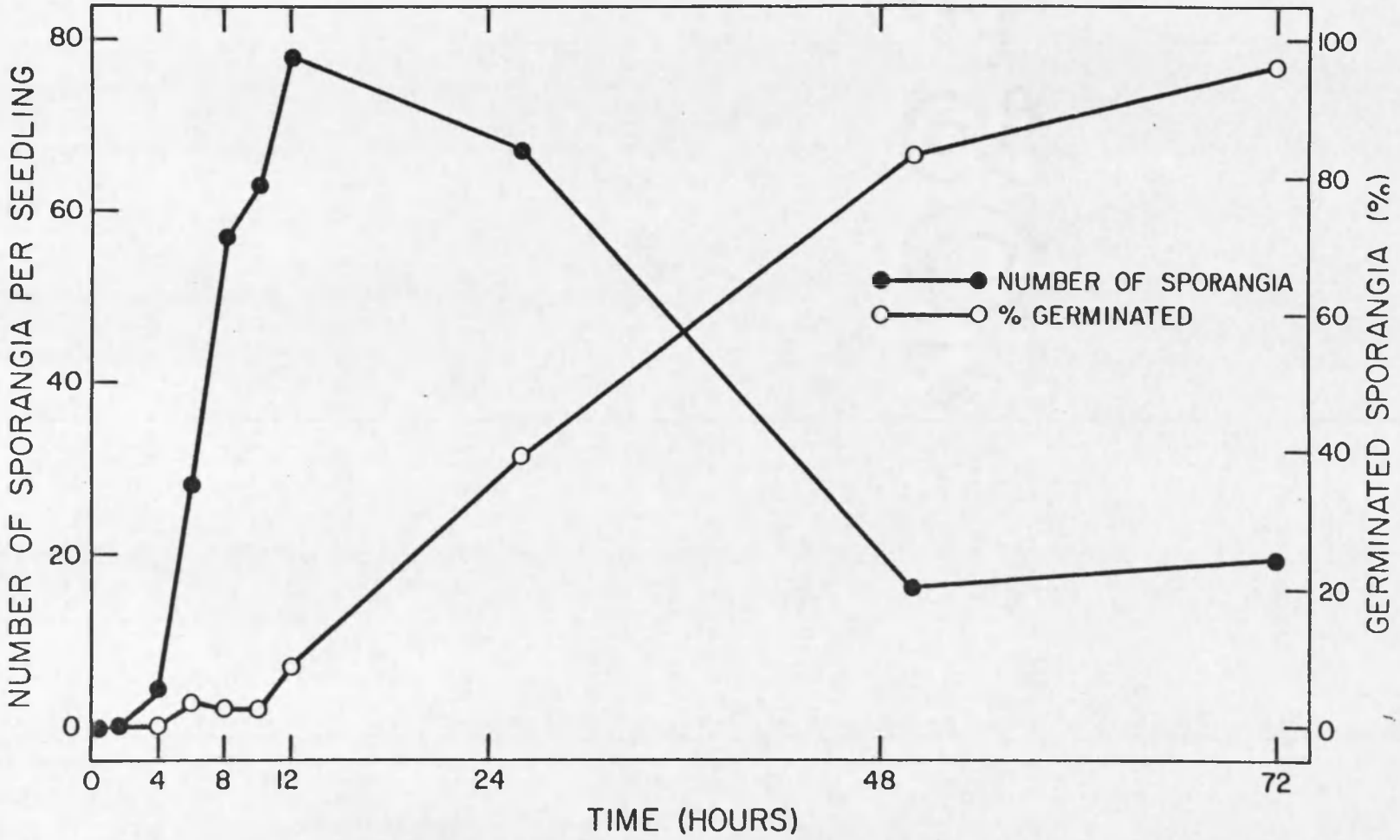

Fig. 6. Production and indirect germination of sporangia of Phytophthora megasperma in flooded soil at 16 C as a function of time -- Data are averages of two replicates.

present by 72 hours. The total number of sporangia observed on the buried radicle segments was highest 12 hours 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

The results of all experiments concerning the effect of matric water potential on production of sporangia in soil at 16 C are averaged in Table 3. Greatest sporangial production occurred in flooded soil (0.0 bar), with less and slower production in soil at ψ_m = -0.05 bar and -0.10 bar (Fig. 7). Very few sporangia were produced at -0.6 bar, and none at -2.8 bar, within 12 days.

While 95% of the sporangia had germinated indirectly after 3 days in flooded soil, less than 10% of the sporangia in soil at ψ_m = -0.05 bar had germinated indirectly after 12 days, and no empty sporangia were observed in soil at -0.10 bar or -0.5 bar. Some of the sporangia produced in soil at ψ_m = -0.05 bar and -0.10 bar germinated directly (via germ tube) after 6 days in soil. Hyphal swellings were frequently observed in mycelium of the fungus after 1-2 days in soil at ψ_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

Table 3. Effect of matric water potential on production of sporangia by *Phytophthora megasperma* in soil at 16 C.

ψ_m (bars)		Time (days) 1	2	3	6	9	12
0.0	Radicles with >20 sporangia[a]	4.0[b]	3.5	1.5	1.5	0.0	0.0
	Sporangia per radicle	44[b]	38	27	14	2	1
-0.05	Radicles with >20 sporangia	<0.5	0.0	0.0	2.5	0.5	0.0
	Sporangia per radicle	7	11	9	23	9	5
-0.10	Radicles with >20 sporangia	0.0	0.5	<0.5	0.5	0.0	0.0
	Sporangia per radicle	1	6	8	11	2	1
-0.6	Radicles with >20 sporangia	0.0	0.0	0.0	0.0	0.0	0.0
	Sporangia per radicle	0.0	0.0	<1	<1	<1	<1
-2.8	Radicles with >20 sporangia	0.0	0.0	0.0	0.0	0.0	0.0
	Sporangia per radicle	0	0	0	0	0	0

[a]Based on a total of 5 radicles at each time interval.

[b]Values are averages of 2 experiments with 2 replications per experiment.

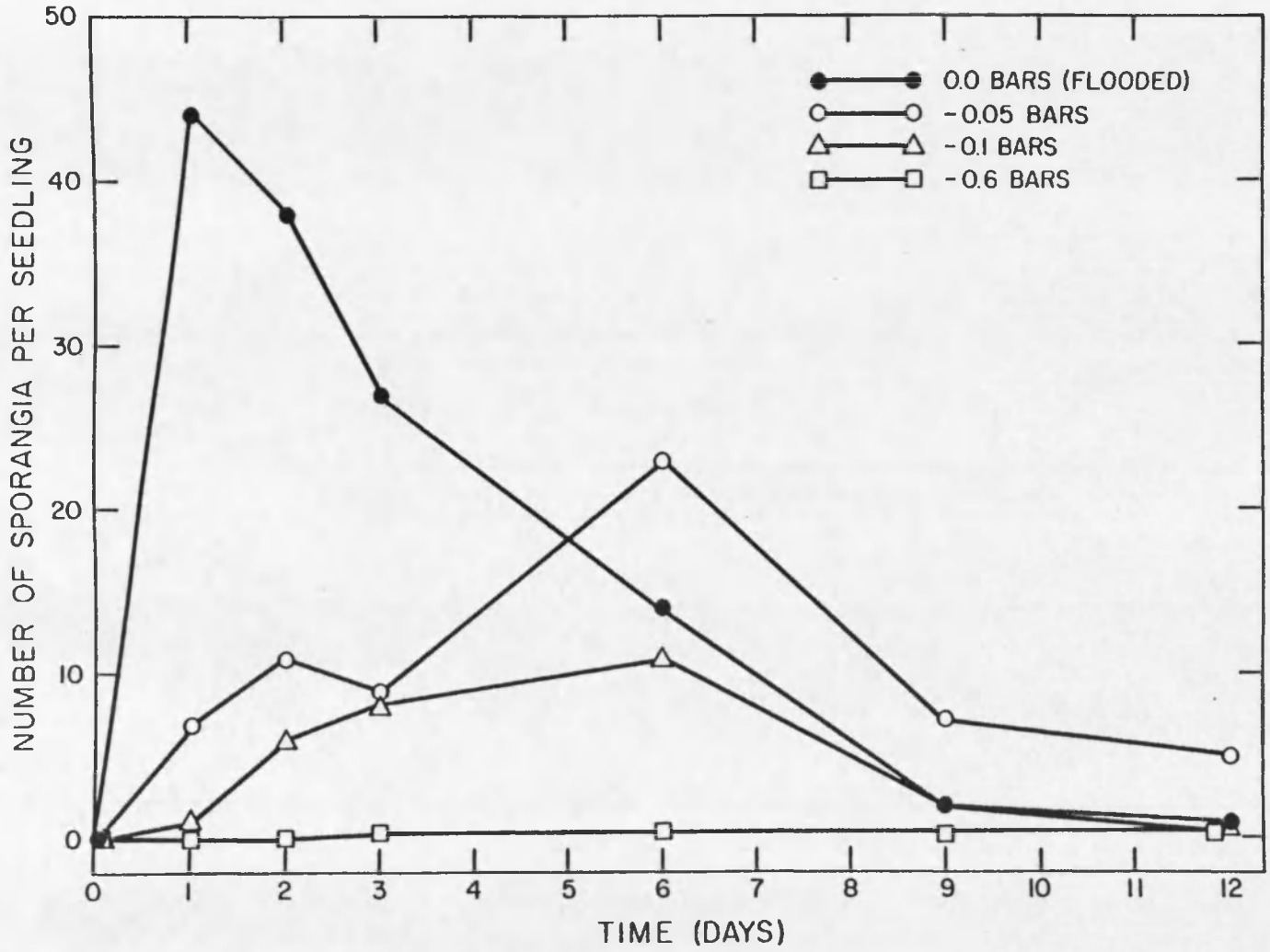

Fig. 7. Effect of matric water potential on production of sporangia by Phytophthora megasperma in soil at 16 C -- Data are averages of two experiments with two replications per experiment.

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 10-20 zoospores were required to infect the seedlings in the 100-ml beaker system, while 20-40 zoospores were necessary to infect the seedlings in the 400-ml beaker system. Zoospores in numbers lower than these critical ranges did not colonize the seedlings, while numbers within these ranges or greater were able to colonize them.

Using the described baiting technique, it was determined that zoospores could move upward through 65 mm of the silt loam/sand mixture (Fig. 8A), but they could not move upward through 65 mm of the silt loam (Fig. 8B). Zoospores routinely reached surface water through 36 mm of the silt loam/sand mixture, but rarely reached surface water through 36 mm of the silt loam. In treatments using silt loam, the probability of zoospores reaching surface water increased with increasing number of zoospores initially present at depths of 4, 8, 16, and 24 mm. For example, a relative inoculum level of 25 (25 indirectly germinated sporangia) was sufficient to cause infection of bait seedlings if the radicles bearing the sporangia were

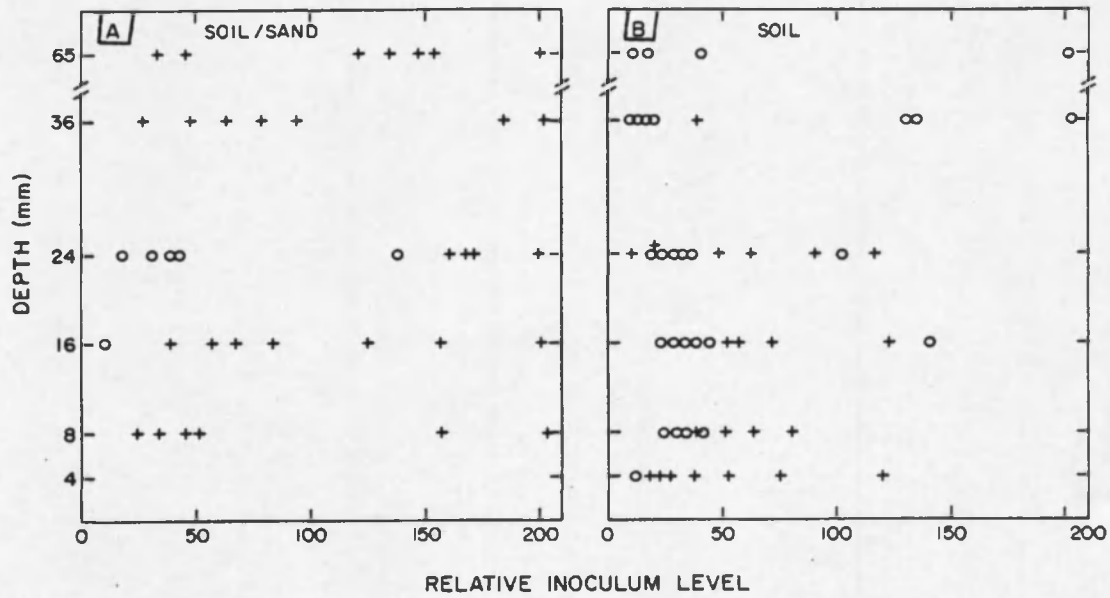

Fig. 8. Ability of zoospores of Phytophthora megasperma to swim upward through varying depths of (A) Gila silt loam/sand (1:1, v:v) mixture, and (B) Gila silt loam -- Alfalfa radicles colonized by Phytophthora megasperma were buried at a given depth in flooded silt loam or silt loam/sand mixture, and healthy seedlings were placed in the surface water above the soil or soil/sand column to serve as bait. Each symbol (+ or 0) in the figure represents a separate trial in which a certain relative inoculum level was produced on colonized radicles buried at the specific depth. Trials in which zoospores reached surface water (demonstrated by colonization of bait seedlings) are represented by a "+"; trials in which zoospores failed to reach surface water (bait seedlings not colonized) are represented by a "0."

Relative Inoculum Level is the number of indirectly germinated sporangia observed on the colonized radicles in a given trial, and constitutes a measure of the number of zoospores originating at the specified depth in a given trial.

buried 4 mm deep, but not if they were 8 or 16 mm deep. When the colonized radicles were 24 mm deep in the silt loam, infection of bait seedlings was less probable at a relative inoculum level of <50 than at relative inoculum level of >50.

DISCUSSION

Phytophthora megasperma growing in alfalfa radicles was found to produce sporangia in soil at temperatures ranging from 4 to 24 C, and at ψ_m values of 0.0 to -0.6 bar at 16 C. Flooded soil at 16 C was the environment most favorable for production of sporangia and release of zoospores.

My data concerning the effect of temperature on asexual reproduction by Phytophthora megasperma may be relevant to observations made by Pratt and Mitchell (13), who used a baiting technique to detect P. megasperma in flooded samples of Wisconsin soils. They found the baiting technique to be more effective at 16 and 20 C than at 25 C, and ineffective at 30 C.

My results concerning the effect of ψ_m on sporangial production are consistent with results of Sneh and McIntosh (14), who found production of sporangia by P. cactorum in soil at -0.1 and -0.3 bar, but not at -3.0 bars. My results differ significantly from those found by Duniway (3) for P. drechsleri in that P. drechsleri produced numerous sporangia in soil at -2.1 bar 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 I found no indirect germination of P. megasperma sporangia in soil at

$\psi_m \leq -0.1$ bar. Several explanations may be suggested for these differences. As Duniway (3) noted, different species of Phytophthora may have inherently different water requirements for production of sporangia in soil. However, differences in environmental factors may also account for the varying responses reported. The inhibiting effect of low oxygen levels on sporangial production by Phytophthora species has been noted (11). In Duniway's (3) experiments the soil had been flooded for at least 12 hours prior to burying P. drechsleri in it, whereas I flooded soil immediately after P. megasperma had been buried. Therefore, oxygen levels in the soil may have been lower in the experiments with P. drechsleri than in the experiments with P. megasperma. It is also possible that water requirements for sporulation are influenced by the energy source available to the fungus. Thus, a fungus colonizing host substrate in soil may have different behavior from the same fungus buried as a mycelial mat in soil. Finally, there may be an interacting effect of temperature and soil moisture on sporulation, with sporangial 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 my work with P. megasperma did not include treatments in drier soil at 23-27 C, a further comparison of temperature and soil moisture effects on sporulation in these two fungi is not possible. However, the data of Sneh and McIntosh (14) concerning the behavior of P. cactorum in soil indicate an interaction of soil moisture and temperature effects on sporangial production in this species. They found that after 8 days in soil, sporangial 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 sporangial 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 oxygen levels, since increasing temperatures would increase oxygen consumption by 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 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 increased by increasing the number of

zoospores present at a given depth in soil; the probability of infection, given a specified quantity of zoospores, decreased with increasing depth of zoospore origin.

Allen and Newhook (1) found that zoospores of Phytophthora cinnamomi swim in a helical path with an amplitude of 26-70 μm, thus requiring a cylindrical space 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 an improbable event. The difference I observed, regarding maximum upward migration distance of zoospores, between the two soil types used may be related to these spatial requirements of swimming zoospores. Comparison of pore space composition of the two soils (Table 1) shows that the silt loam/sand mixture contains a slightly higher proportion of large pores than does the Gila silt loam. The difference is small when considered as a fraction of the total soil volume, but increases when considered as a fraction of the space which is water-filled under flooded conditions. Thus, of the total water-filled volume in the two flooded soils, pores > 120 μm and > 190 μm in diameter are respectively 62% and 45% more frequent in the silt loam/sand mixture than in the silt loam. The number of unobstructed paths from the zoospores' point of origin to the surface water would be

lower in the silt loam than in the silt loam/sand mixture due to the lower absolute volume of pores > 190 μm in diameter as well as their lower frequency in relation to total water-filled space. 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 my study, other than pore size composition, can not be ruled out as possible influences on zoospore mobility.

My study indicates that P. megasperma mycelium growing in host tissue can produce sporangia and release zoospores in flooded soil within 6-8 hours 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 hours. Zoospores originating from sporangia in soil can act as agents of long-distance dispersal if they

reach surface water. My results indicate, however, that the distance zoospores can move upward through flooded soil is limited, and is restricted by soils of fine texture. 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 zoospores' limited range 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.

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