Influence of Soil Texture and Temperature on the Motility of Phytophthora cryptogea and P. megasperma Zoospores

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ABSTRACT

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Mycelial disks from agar plate cultures of *Phytophthora cryptogea* and *P. megasperma* incubated in soil at −150 millibars (mb) matric potential (ψ_m) on tension plates formed abundant sporangia in 3–4 days. When soil containing sporangia was wetted to saturation ($\psi_m = 0$) or sporangia from soil were placed in water, equally large numbers of motile zoospores were released. The period of time that *P. cryptogea* zoospores remained motile before encysting in a saturated coarse sand fraction (> 250 μm) of Yolo fine sandy loam was only somewhat less than their period of motility in water. However, the period that they remained motile in a fine sand fraction (38–60 μm) of the same soil was much less than that in the coarse sand or water. The limiting effect of the fine sand on the motile period was not altered when zoospores were released in soil at 12 C rather than 27 C. Compared with zoospores of *P. cryptogea,* those of *P. megasperma* appeared to encyst quickly after release in either of the two soil fractions, in distilled water at 12–27 C temperatures, and in solutions of sucrose or polyethylene glycol 300. Thus, these two species appear to have inherent differences in duration of zoospore motility. The rapid encystment of zoospores in fine-textured soils may contribute to the inability of the spores to swim effectively through such soils.

Zoospores of *Phytophthora* spp. are considered to be important in the epidemiology of diseases caused by the soilborne members of this genus (4). Numerous reports describe the presence and importance of zoospores in irrigation or surface water (4,10,14), and other reports support their role in dissemination and infection processes within the soil matrix (2,5,7,11). However, the movement of zoospores is more restricted in fine-textured soils than in coarse-textured soils (2,5,13). For example, Duniway (2) demonstrated that zoospores of *P. cryptogea* require relatively large, water-filled soil pores for any significant movement through soil. In the absence of large, water-filled pores, because of either the inherent textural qualities of the soil or the draining of water from large pores under slight matric tension, zoospore movement is severely impaired (2).

It is not known whether the impairment of zoospore movement in fine-textured soils results from an inability of the motile zoospores to move efficiently through small water-filled pores or results from a loss of motility due to encystment. However, the length of time that zoospores remain motile is less in soil than in larger, more open volumes of liquid. Hickman (3) reported that, depending on the species and temperature, zoospores may remain motile in liquid as long as several days, while others (7,12) have shown that in soil, zoospores encyst in 4–24 hr. Although other factors could be involved (3), the loss of motility in soil probably results from contact with soil particles which stimulate the zoospores to encyst. Furthermore, an increased frequency of contact with solid surfaces has been reported to cause zoospores to encyst more rapidly (6). In fine-textured soils where zoospores would be confined to small-diameter pores, the resulting increased contact with soil particles could cause a more rapid encystment than in coarse-textured soils and restrict the movement of zoospores through the finer soils. An additional factor that affects zoospore motility is temperature (6). However, the effect of temperature on motility in soil, in the presence of contact stimuli, has not been adequately described.

In this article, we report on the effects of soil texture on the period that *Phytophthora* zoospores remain motile before encystment, independent of their distances of movement through soil (2), and on interactions between soil temperature and texture that may affect the length of the motile period.

MATERIALS AND METHODS

Two previously described (8) isolates of *Phytophthora* were used in this study: an isolate of *P. cryptogea* Pethyb. pathogenic to safflower and an isolate of *P. megasperma* Drechs. pathogenic to alfalfa. Both isolates formed abundant aerial mycelium when cultured in plates containing pea-dextrose agar (8). Disks of aerial mycelium were removed from 7- to 10-day-old cultures and buried 2–4 mm deep in soil as described previously

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(8). The soils used in these experiments were the coarse sand (> 250 μm) and fine sand (38–60 μm) fractions of Yolo fine sandy loam (YFSL) obtained by wet-sieving autoclaved soil. Sieving provided media of different textural qualities from the same parent material. Soil was placed in rings of plastic pipe that had an inside diameter of 13 mm and a height of 8 mm. The rings were open at the top and had a nylon mesh (2-mm openings) support at the bottom. A single mycelial disk was buried in the center of each ring containing soil, and nine rings were evenly spaced on the surface of a 5-mm layer of soil contained on Büchner funnel tension plates (1,2,8). The tension plates then were set to maintain the soil at −150 millibars (mb, 1,000 mb = 1 bar) matric potential (ψ_m), where abundant sporangia formed in 2–4 days (1,8).

To investigate zoospore motility after sporangia formed, the tension plates were set to $\psi_m = 0$, using the surface of the reservoir and the level of the mycelial disks in soil as reference points (2), and the soil surface was wetted with distilled water to bring it immediately to saturation. This provided optimal conditions for zoospore release (8) and allowed zoospores to be released into the soil matrix with no *in vitro* handling or manipulation. The plastic rings confined zoospores in a small volume of soil that could be sampled easily. At intervals following saturation, three rings containing soil were removed from each treatment and their contents suspended individually in 10 ml of distilled water. The numbers of actively motile zoospores in the samples were estimated by placing an aliquot of the soil suspension on a counting grid as described previously (8). In addition to the number of motile zoospores, the total number of zoospores in each sample also was estimated by spotting 20 μliter of the soil suspension onto the surface of a selective agar medium (1), and germinated cysts were counted after an 18-hr incubation (8). Soil suspensions were sampled and counted rapidly to assure that only the zoospores released in soil prior to sampling were counted and that motile zoospores did not encyst during the counting procedure (8). Unless otherwise specified, zoospores were released into soil held constant at 22–24 C.

RESULTS

The number of *P. cryptogea* zoospores that remained actively motile after release was greatly affected by soil texture (Fig. 1-A,B). Two hr after the coarse sand fraction of YFSL was saturated, all the zoospores that had been released were still motile (Fig. 1-A). After 4 hr at

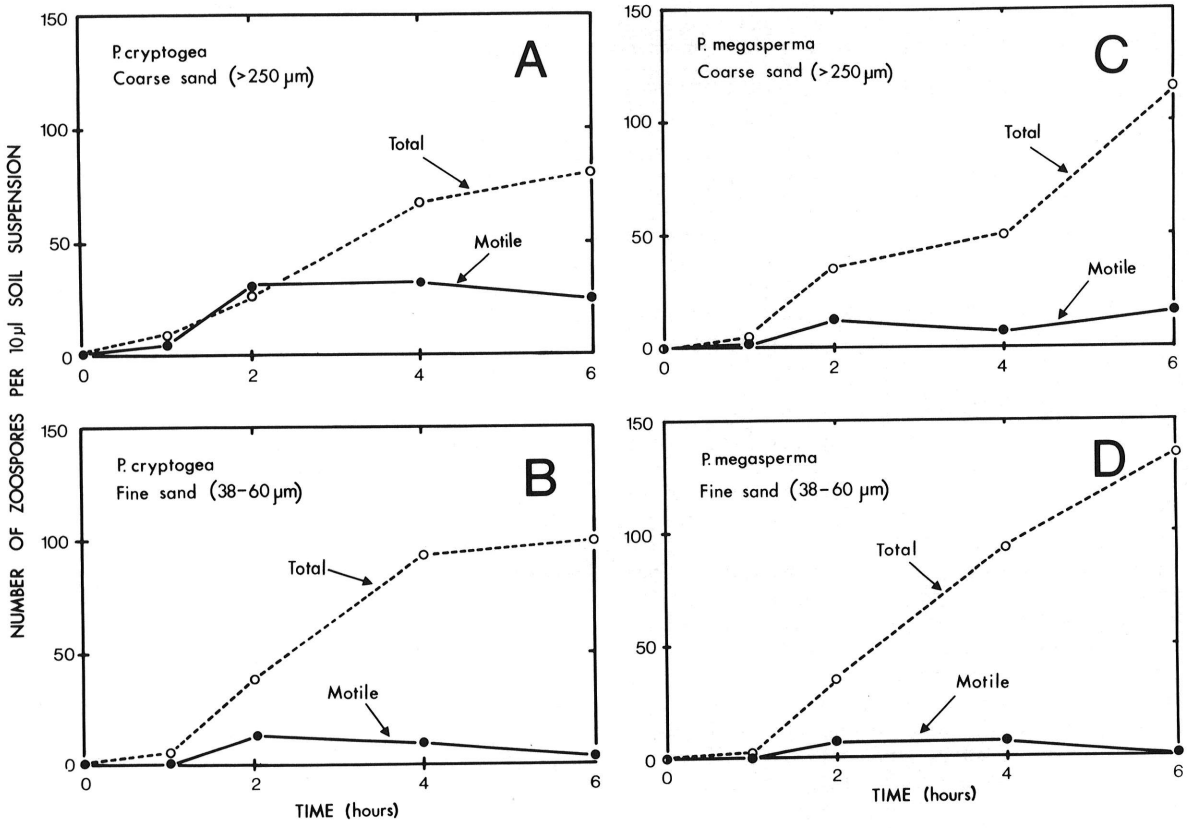

Fig. 1-(A to D). Total numbers of *Phytophthora cryptogea* and *P. megasperma* zoospores released, and the numbers of zoospores that were motile at various times after sieved fractions of Yolo fine sandy loam were saturated. Sporangia formed on mycelial disks in the soils at −150 mb matric potential before they were wetted to saturation at the start of the experiment. Soils were maintained saturated throughout the 6-hr period.

saturation, 50% of the zoospores released were motile, and after 6 hr, 30% were motile. In contrast, 2 hr after the fine sand was saturated, only 25% of the zoospores released were motile. This decreased to 10% after 4 hr, and almost none remained motile after 6 hr at saturation (Fig. 1-B). Zoospores of *P. megasperma,* on the other hand, appeared to lose motility quickly after release in either soil fraction (Fig. 1-C,D).

To determine whether the rapid loss of motility by *P. megasperma* zoospores was a result of greater sensitivity to contact stimuli in soil or merely demonstrated an inherent difference in swimming behavior between these species, mycelial disks of both species were removed from soil after sporangia had formed and placed in 10 ml of distilled water at 22–24 C for zoospore release. At intervals of 2, 4, 6, and 12 hr after the disks were placed in water, the water was gently mixed and aliquots examined for the presence of actively motile zoospores. The total numbers of zoospores present also were determined with selective agar medium. The first zoospores were released approximately 60 min after the sporangia were placed in water. Microscopic observations determined that all zoospores were motile when initially released from sporangia. However, 2 hr after the sporangia were placed in water, only 75% of the zoospores of *P. cryptogea* that had been released were still motile (Fig. 2). This dropped to 50% after 6 hr or 12 hr in water. In contrast to *P. cryptogea,* only 15% of the zoospores of *P. megasperma* appeared motile 2 hr after the sporangia were placed in water, and the percentage of motile spores dropped to less than 5% after 12 hr. In further experiments with zoospores of *P. megasperma,* motility was not enhanced in water adjusted to various temperatures ranging from 12–27 C or in solutions of sucrose (0.1 m) or polyethylene glycol 300 (25 g/Kg H_2O).

Sporangia of *P. cryptogea* were used in additional experiments to examine the effects of temperatures near the upper and lower limits for zoospore release (9) on the duration of zoospore motility in soil. Plastic rings of soil were removed from tension plates after sporangia had formed at −150 mb ψ_m and were placed in petri plates of soil in incubators at 12 or 27 C. The soil was wetted to complete saturation after temperature equilibrium, and the numbers of motile zoospores and the total numbers of zoospores present in soil were determined periodically. Although the duration of zoospore motility was somewhat longer in coarse sand at 12 C than at 27 C, the lower temperature did not overcome the limiting effect of the fine sand on zoospore motility (Fig. 3).

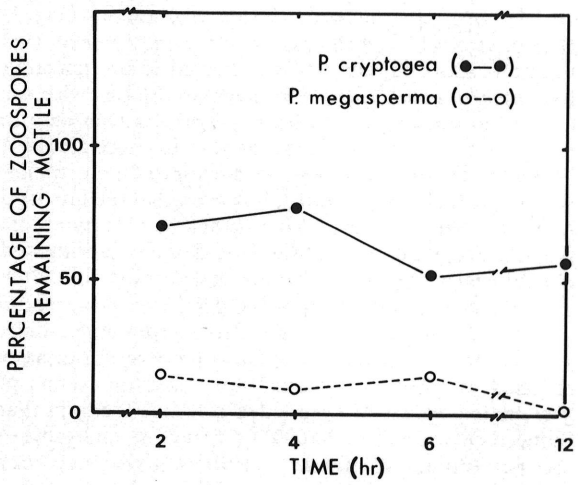

Fig. 2. Percentage of *Phytophthora cryptogea* and *P. megasperma* zoospores that were motile at various times after sporangia were placed in water. Sporangia formed on mycelial disks in soil at −150 mb matric potential before being placed in water and required 1 hr in water to begin release of zoospores. Maximum release was completed within 4 hr, and all zoospores were initially motile when released from sporangia.

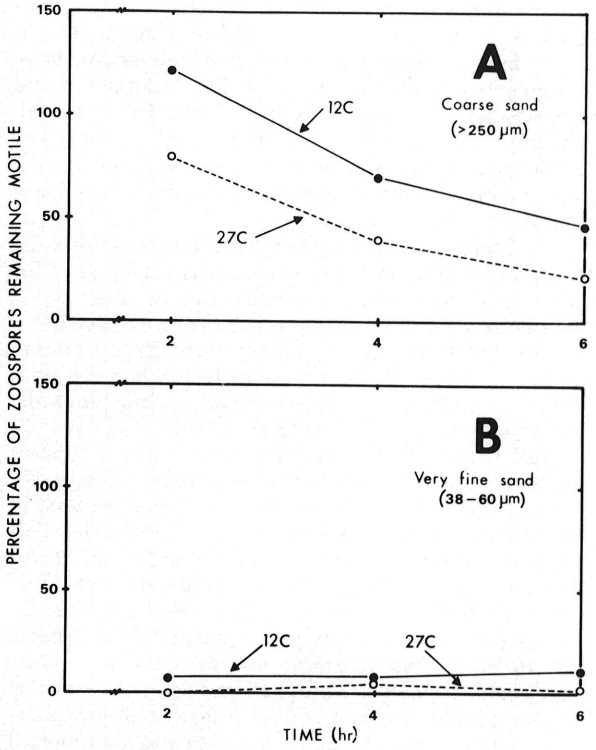

Fig. 3-(A,B). Percentage of *Phytophthora cryptogea* zoospores that were motile at various times after the **A)** coarse and **B)** very fine sand fractions of Yolo fine sandy loam were wetted to saturation at 12 and 27 C. Sporangia formed on mycelial disks in the soils at −150 mb matric potential and 22–24 C before they were wetted to saturation at the start of the experiment.

DISCUSSION

In our experiments, zoospores were released directly into soil from sporangia formed in soil, and under conditions considered optimal for both the *Phytophthora* species used (8). The soil was saturated throughout the course of the observations and soil texture remained as the main variable that limited the duration of zoospore motility. The magnitude of textural limitations on the motile period of zoospores is indicated by the observation that 50% of *P. cryptogea* zoospores released in water, in the absence of soil particles, were motile after 6 hr (Fig. 2). During the same interval, only 30% were motile in the coarse sand fraction of YFSL (Fig. 1-A), and

almost none were motile in the fine sand fraction (Fig. 1-B). Zoospores of *P. megasperma* var. *sojae* were reported to encyst more rapidly when subjected to an increased frequency of contact with solid surfaces (6). Likewise, the loss of zoospore motility by *P. cryptogea* seen here in response to soil texture may be due to more frequent contact with soil particles in the correspondingly smaller soil pores. It should be noted, however, that the fine sand fraction used in these experiments had a minimum particle size of 38 μm and was devoid of the finer silt and clay particles that would be present in most soils. In a native soil, therefore, the motile period of *P cryptogea* zoospores might be even less than represented here. Furthermore, estimates of the total numbers of zoospores released in these experiments are based on counts of germinated zoospore cysts on a selective medium that induced between 70 and 100% germination, and some of the percentages of motile zoospores given here may actually overestimate their potential for motility.

Compared with zoospores of *P. cryptogea,* those of *P. megasperma* appeared to have a short motile period under all conditions tested. Because some zoospore release occurred throughout the course of most of the experiments (Fig. 1), the duration of zoospore motility cannot be determined accurately from these data. However, most zoospores of *P. megasperma* remained motile for less than 2 hr in water (Fig. 2) and probably for a much shorter period in soil. This estimate is based on the observation that only a small percentage of the zoospores present in soil at the end of each 2-hr sampling period were motile, despite a continuing release of large increments of zoospores (Fig. 1-C,D). Although we did not visually observe zoospore discharge under all circumstances in which it can occur, we observed discharge under a variety of conditions and found that the vast majority of zoospores are motile when they leave sporangia.

The ability of relatively low temperatures to prolong zoospore motility in liquid systems has been known for some time (4,6). The same effect can be observed in coarse-textured soils that are inherently conducive to zoospore motility (Fig. 3) and movement (2). Soil texture appears to be such a determinining factor, however, that lower temperatures do not significantly extend the motile period of zoospores in fine-textured soils.

Duniway (2) showed that the movement of zoospores was much less in loam soils than in a coarse soil mix. We found that the motile period of zoospores is reduced in fine-textured compared with coarse-textured soils. This shortened motile period may be an important factor limiting the movement of zoospores through fine-textured soils or soils under small matric tensions (2). Compared with zoospores of *P. cryptogea* (2), zoospores of *Olpidium brassicae* are not as limited in their movement through soil by small matric suctions or fine soil texture (15). Zoospores of *O. brassicae,* however, are smaller than *Phytophthora* zoospores and are reported not to encyst as rapidly in response to contact stimuli (15).

On the basis of these results and those of Duniway (2) and Pfender (13), we believe zoospore movement in free surface water is probably of greater significance in the epidemiology of Phytophthora diseases than is movement through the soil matrix. In soil, however, zoospores may not have to move far to encounter a host root, and unidirectional movement in response to a chemotactic stimulus may make their movement efficient despite a brief period of motility. The data (Fig. 1 and 2) also show that species of *Phytophthora* may differ in their capacity for zoospore motility, and this could yield differences in their ability to move through soil. Because of the inherent differences observed in this study, it is not possible to know whether our isolates represent either the upper or lower limit of zoospore motility among the species of *Phytophthora*.

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