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Duration of zoospore motility of pythium species in situ

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DURATION OF ZOOSPORE MOTILITY OF PYTHIUM SPECIES IN SITU

by

Rex Matthew Quaempts

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT PATHOLOGY

In Partial Fulfillment of the requirements
For the Degree of

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In the Graduate College

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ABSTRACT

Motile zoospores of Pythium dissotocum and P. catenulatum were added to 20 and 60 mesh silica sand and a sandy loam soil to investigate the duration of motility. Both Pythium species remained motile for up to 24 hours in all soil textures tested. However, the duration and percentage of the motile population varied depending upon the soil type and species tested. Survival rates of motile and encysted zoospores of P. dissotocum and P. catenulatum in air dried and saturated sterile silica sand was also tested. P. dissotocum, under air dried conditions, did not survive while P. catenulatum was capable of surviving 4 and 16 days as motile and encysted zoospores, respectively. Both fungi could be recovered at high percentages after 5 weeks under saturated conditions. The survival structure is believed to be in the form of a zoospore cyst.

Chapter 1

INTRODUCTION

The duration of zoospore motility of soil-borne fungi in soil is an area which has been largely ignored. As reviewed by Carlile(4,5), most studies have concentrated on tactic responses, zoospore release, swimming patterns, and the effects of ions and population densities on the length of motility. However, most of these experiments have been in vitro and thus ignored the behavior of zoospores in soil.

Previous experiments conducted on zoospores in soil or "ideal soils" consists of distance traveled by different Phytophthora species (6,12,19), length of survival of zoospores in soil (9,14,15,16,17,21) and the comparison of disease incidence following inoculation with motile and encysted zoospores(11).

Work on duration of zoospore motility in soil is limited. Early reports (3,8,13) concluded that Phytophthora zoospores confined in columns of glass microbeads or silica sand, had reduced durations of motility. This has been interpreted by some researchers (8,10) that zoospore motility plays an insignificant role in dissemination within the soil environment. However, others (1,18,22) reported that contact stimulus did not play a significant role in causing premature encystment under these conditions. In support of this latter conclusion, Mehrotra(15) reported that 7 and 2 % of the initial population of motile zoospores of Ph. megasperma var sojae and Ph.

drechsleri were still motile at 12 and 24 hours, respectively, when confined in columns of soil.

Duration of motility can play a major role in the epidemiology of the zoosporic soil-borne fungi. However, duration of zoospore motility is dependent upon high soil moisture levels and continuous soil pores of the appropiate diameter necessary for migration(1,6,22). Thus the duration of motility in zoosporic soil-borne fungi may be critical in providing adequate time for locating a host.

The objectives of this study are: 1) to investigate the duration of zoospore motility of Pythium spp. in silica sand and soil, 2) to determine the length of survival under wet and dry conditions in sand, and 3) to identify the survival structure.

Chapter 2

MATERIALS AND METHODS

Stock cultures of Pythium dissotocum Drechsler, and P. catenulatum Matthews, originally isolated from roots of spinach and lettuce, respectively, were maintained on 9 cm plates of 10% V-8 juice agar at 20 C. Seven and 5 day old cultures of P. catenulatum and P. dissotocum, respectively, were used in all experiments.

Production and Enumeration of Zoospores

A one third section of agar, removed from a 9-cm-diameter plate containing P. dissotocum or P. catenulatum, was placed in 350 ml of sterile distilled water (SDW) which had been aerated for 3 minutes on high speed with a magnetic stirrer. Motile zoospres in ten, 2.5 ul aliquots were placed on a clean glass slide, observed under the stereoscope, and counted. The average of ten counts was taken and the population estimated. Maximum zoospore production (7-9 X 10 zoospore/ml) occured after 5 and 7.5 hours incubation (agar was removed), respectively, for P. dissotocum and P. catenulatum.

The remainder of the agar was placed into 950 ml of SDW which had been aerated for 3 minutes. The agar sections were removed at the same times as described above. The zoospores were filtered through two layers of millipore pre-filters. The zoospore-free filtrate was used in preparing the sand and soil columns for zoospore infiltration.

Preparation and Characteristics of the Silica Sand and Soil

A coarse and fine silica sand, 20 and 60 mesh respectively, were washed in running water and air dried. Five, 130 g samples of each were measured out and autoclaved for 30 minutes.

In another experiment an unwashed, sandy loam soil from a citrus orchard, located on the mesa at the University of Arizona agricultural station in Yuma, Arizona, was used and treated as above.

The moisture characteristic curve for the three substrates was determined over the ranges of 0 to -50 mbars using a 600 ml Pyrex 10-15 M tensiometer(Fig. 1-3).

Preparation of Columns

Five polypropylene tubes, 30 cm in length X 4 cm in diameter with a removable nozzle, were used to prepare the columns of sand and soil. The nozzle was removed and two layers of gauze were placed over the opening and the nozzle replaced. A ten by one cm piece of tygon tubing was placed over the tip of the nozzle. To prevent evaporation this area was wrapped with parafilm. The tubes were surface sterilized by placing them in a 10% chlorox solution for 5 minutes and allowed to air dry.

A one-hundred and thirty gram aliquant of autoclaved silica sand or soil was added to each column, followed by 100 ml of SDW. The columns were allowed to drain freely. In order to prevent premature zoospore encystment, fifty ml of the zoospore-free filtrate was added to each column and allowed to drain.

Addition and Extraction of Zoospores

The 350 ml zoospore suspension was slightly swirled to distribute the zoospores as evenly as possible. A 50-ml aliquot was measured out, counted and added to each column. This 50 ml suspension was in excess of what the column could retain. The excess was collected, volume measured, and the zoospore population calculated as described above. The population remaining in the column was then derived by subtracting the population of zoospores lost from what was initially added. This technique was repeated for each of five columns.

To maintain constant moisture in each column, the opening of each column was covered with silicone gel and capped with a small petri dish (Fig. 4).

Extraction of the motile zoopore population retained in the columns was conducted at 0, 12, 16, 20 and 24 hours. An individual column was extracted at a given time only once: 100 ml of the zoospore-free filtrate was added to each column and allowed to drain freely. The leachate was collected, the volume measured, and the population of motile zoospores estimated as described above.

The control consisted of 50 ml of the original 350 ml zoospore suspension placed in a beaker and counted at the above sampling times. Each experiment was conducted three times.

Survival of Encysted and Motile Zoospores in Silica Sand

In each experiment, four 9-cm-diameter glass petri plates were filled with 60 gm of 60 mesh silica sand and autoclaved for 20 minutes.

Zoospores were produced and counted as described above, however, the population was only allowed to reach approximately 3200 zoospores per ml (the agar was removed at 3 and 5 1/2 hours, respectively, for P. dissotocum and P. catenulatum). The suspension was passed through 3 layers of cheesecloth to remove hyphal fragments. Then, 10 ml of motile zoospores were pipetted as evenly as possible onto two of the dishes. Additionally, thirty ml of the zoospore suspension was spun at high speed on the magnetic stirrer for 15 minutes to cause encystment of the zoospores. Ten ml of encysted zoospores were placed into the two remaining dishes of silica sand as described above.

Two of the 4 dishes were allowed to remain completely saturated with one containing motile and other encysted zoospores. The remaining two dishes were allowed to air dry at room temperature (20 C). Drying time was 24 hours. At 24 hour intervals during the first week and once a week thereafter, 1 gm of infested silica sand was removed from each dish and plated onto corn meal agar (CMA). The number of colonies formed were then counted after a 24 hour incubation period and the colony origin was observed microscopically. Each experiment was conducted twice.

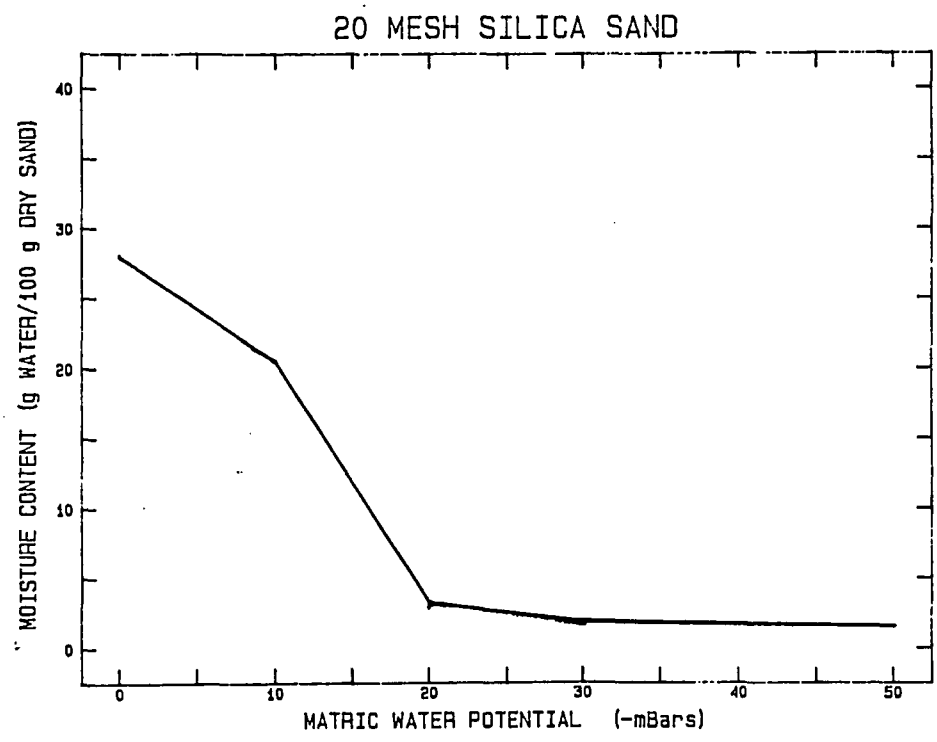

Fig. 1. Moisture characteristic curve of 20 mesh silica sand.

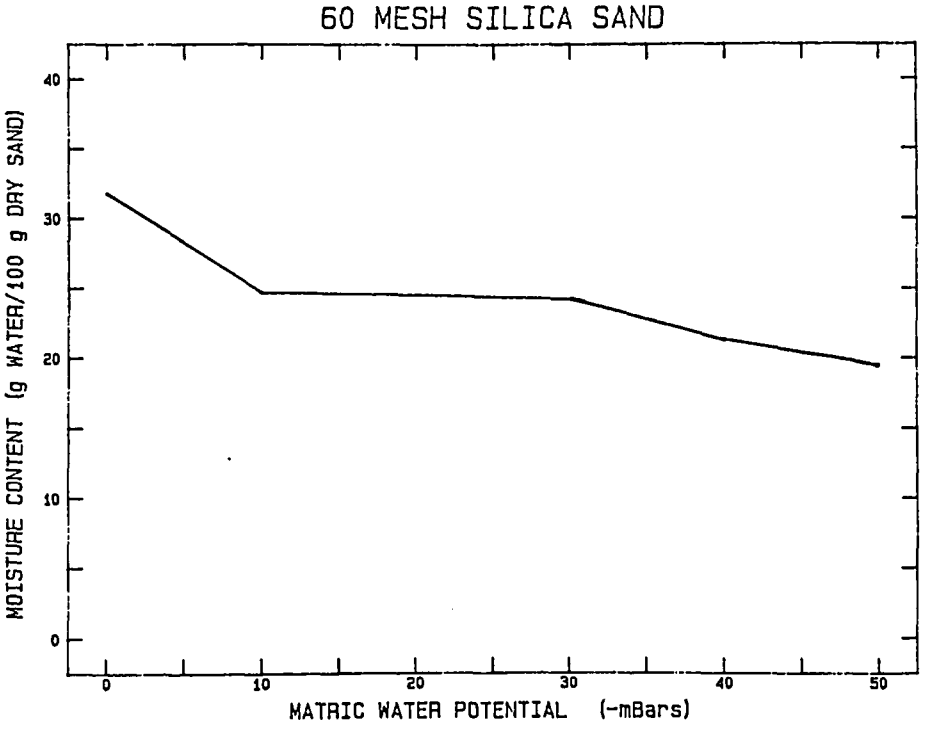

Fig. 2. Moisture characteristic curve of 60 mesh silica sand.

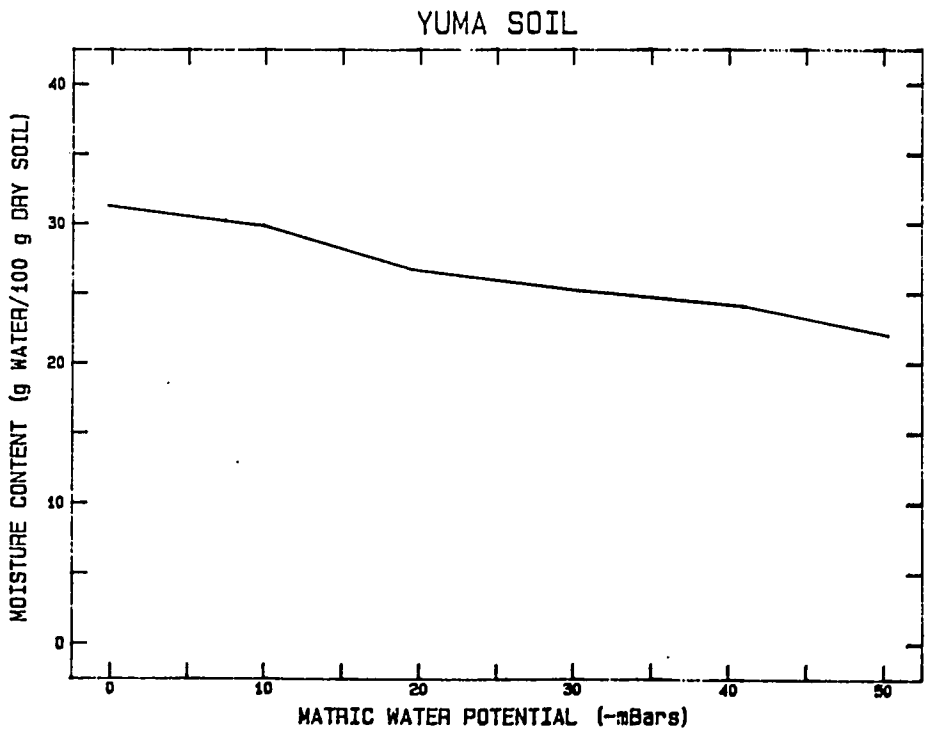

Fig. 3. Moisture characteristic curve of Yuma soil.

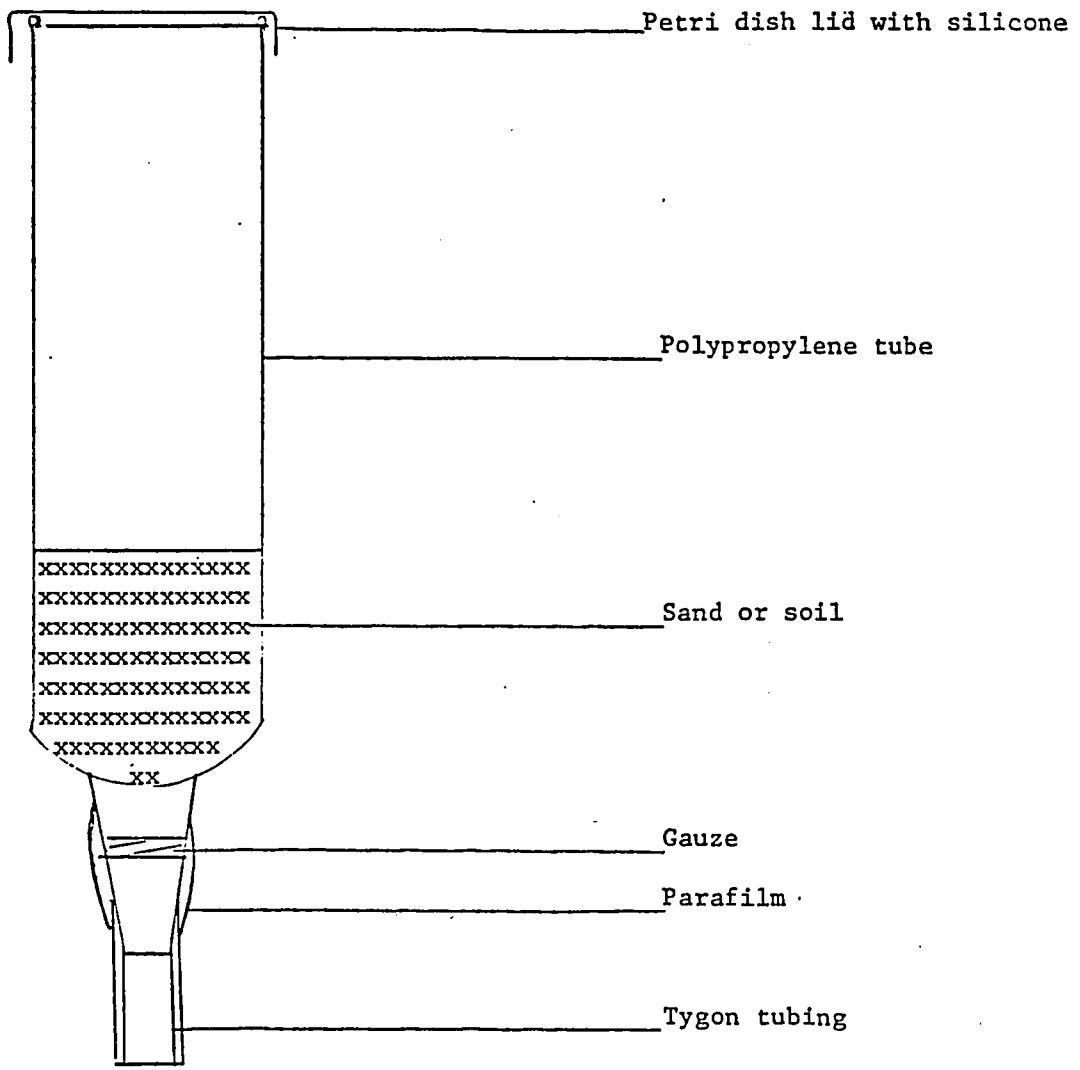

Fig. 4. Schematic representation of a soil column used for studying the duration of motility of Pythium zoospores.

CHAPTER 3

RESULTS

Motility in 20 Mesh Silica Sand

Eighteen to fifty-six percent of the zoospore population of P. catenulatum retained in the column were still motile at 16 hours (fig 6). Thirty to forty percent of the population of P. dissotocum were still motile at 16 hours (fig. 9).

The efficiency of recovering the zoospores (time zero), ranged from 55-100% (fig. 6) and 67-97% (fig. 9) for P. catenulatum, and P. dissotocum, respectively.

Motility in 60 Mesh Silica Sand

Four to eleven percent of the initial population of P. catenulatum retained in the columns were still motile at 16 hrs (fig. 7). Nine to thirty-four percent of the initial population of zoospores of P. dissotocum extracted at 16 hrs were still motile (fig. 10).

The efficiency of extraction ranged from 35-40% (fig. 7), and 35-84% (fig. 10), respectively, for P. catenulatum and P. dissotocum.

The motility of the zoospores of either fungus in the controls decreased linearly over the 24 hour period (figs. 5 and 8). Five to twenty percent of P. catenulatum zoospores, and 0-18% of P. dissotocum zoospores were still motile after a 24 hour incubation period.

Motility in Yuma Soil

Zoospores of neither fungus could be efficiently extracted with from the Yuma soil with the described technique. However, zoospores were observed under the stereoscope in all extraction solutions during the 0-24 hour sampling times.

Survival of Zoospores in Sand

Pythium dissotocum was not recovered from infested soil which was allowed to air dry for 24 hours(table 1). In contrast, one percent of the motile population of P. catenulatum was recovered at 4 days and up to 16 days for the encysted population (table 1).

In the saturated sand, high populations for both fungi were recovered after 5 weeks in all experiments. On the average, 75 and 57% of the encysted and motile population, respectively, for P. dissotocum were recovered. Sixty-four and 97% of the encysted and motile zoospores, respectively, for P. catenulatum were recovered (table 2). Colony origin was traced to propugales with the dimensions of zoospore cysts (fig. 11).

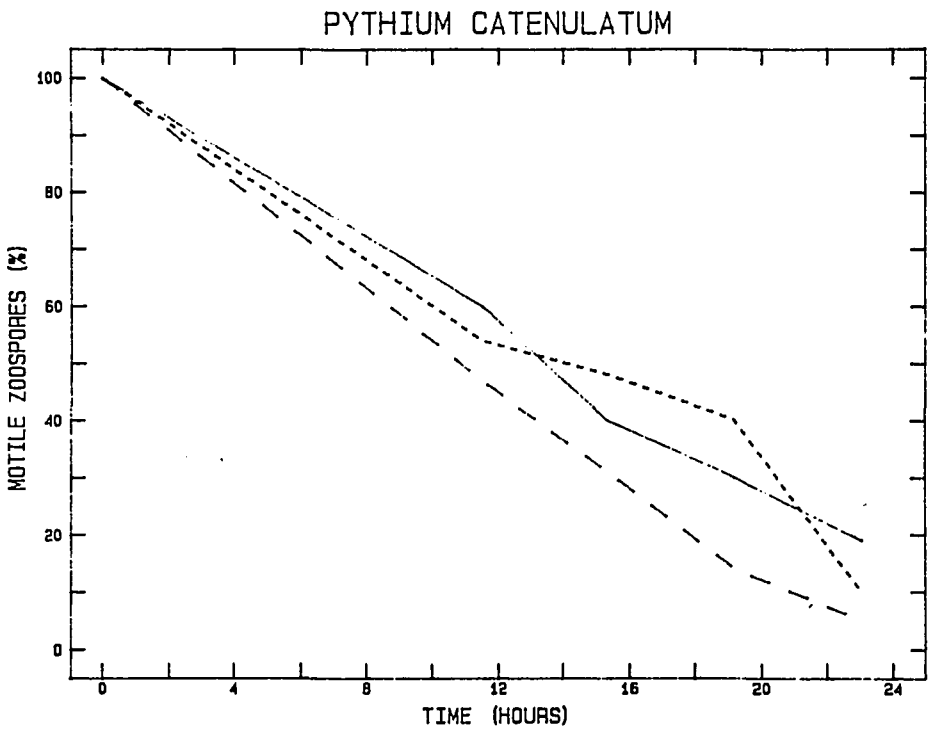

Fig. 5. Duration of zoospore motility of P. catenulatum in sterile distilled water contained in a beaker. Time represents the number of hours after the agar was removed. Results of 3 independent experiments.

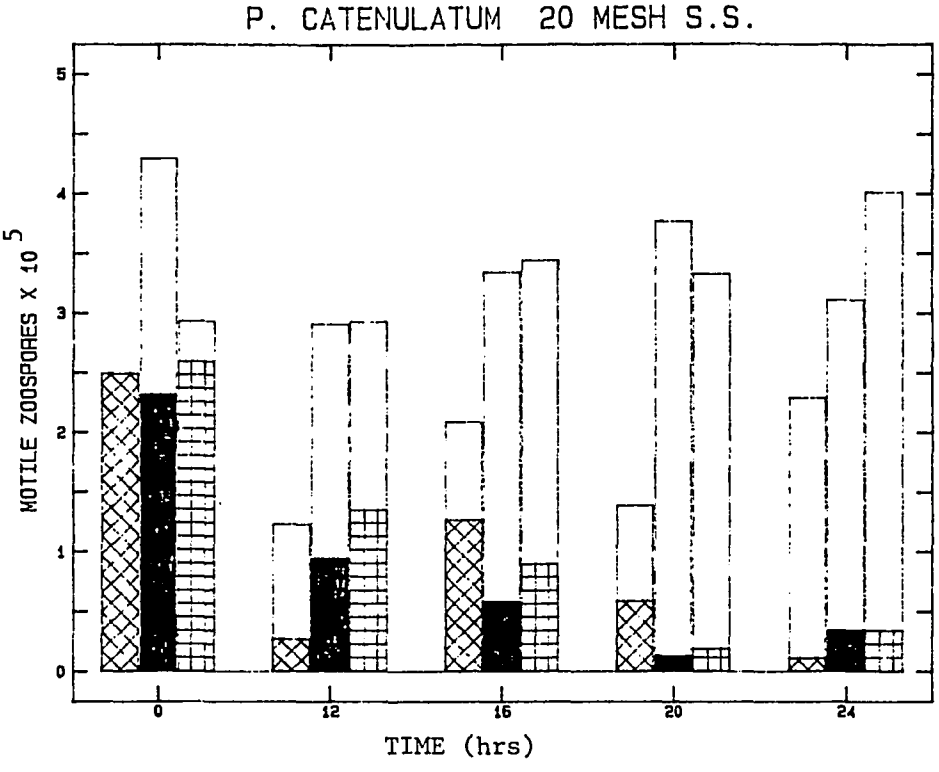

Fig. 6. Duration of zoospore motility of P. catenulatum in 20 mesh silica sand at -8 mbars matric potential. Open face bar represents the population initially within column. Shaded bar represents the population of motile zoospores extracted at the given time interval. Results of 3 independent experiments.

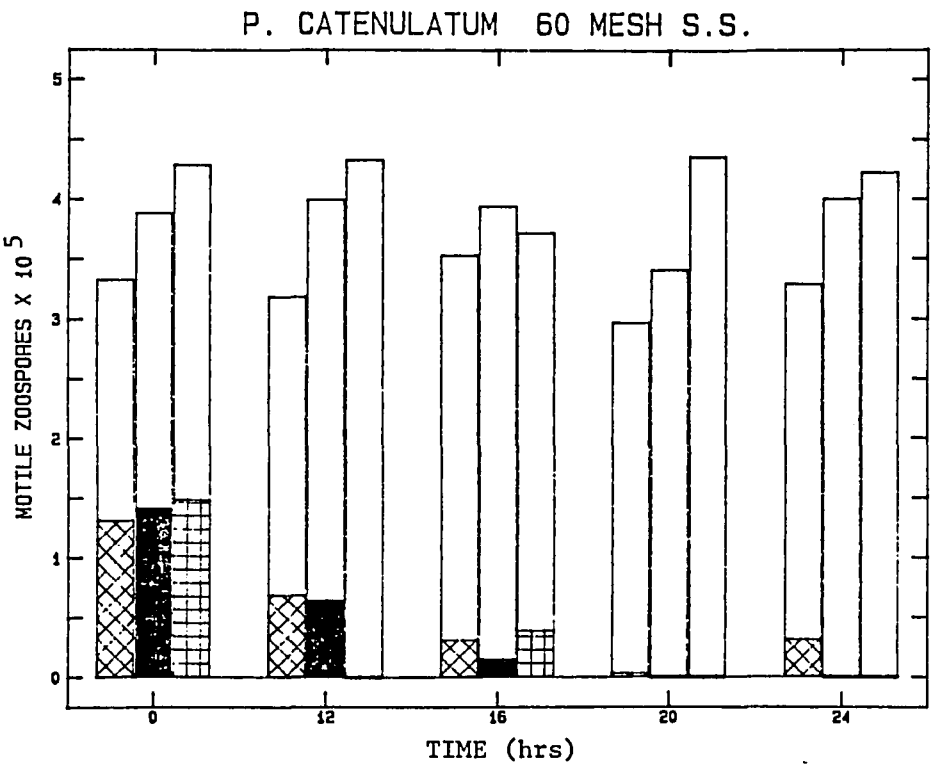

Fig 7. Duration of zoospore motility of P. catenulatum in 60 mesh silica sand at -8 mbars matric potential. Open face bar represents the population initially within column. Shaded bar represents the population of motile zoospores extracted at given time interval. Results of 3 independent experiments.

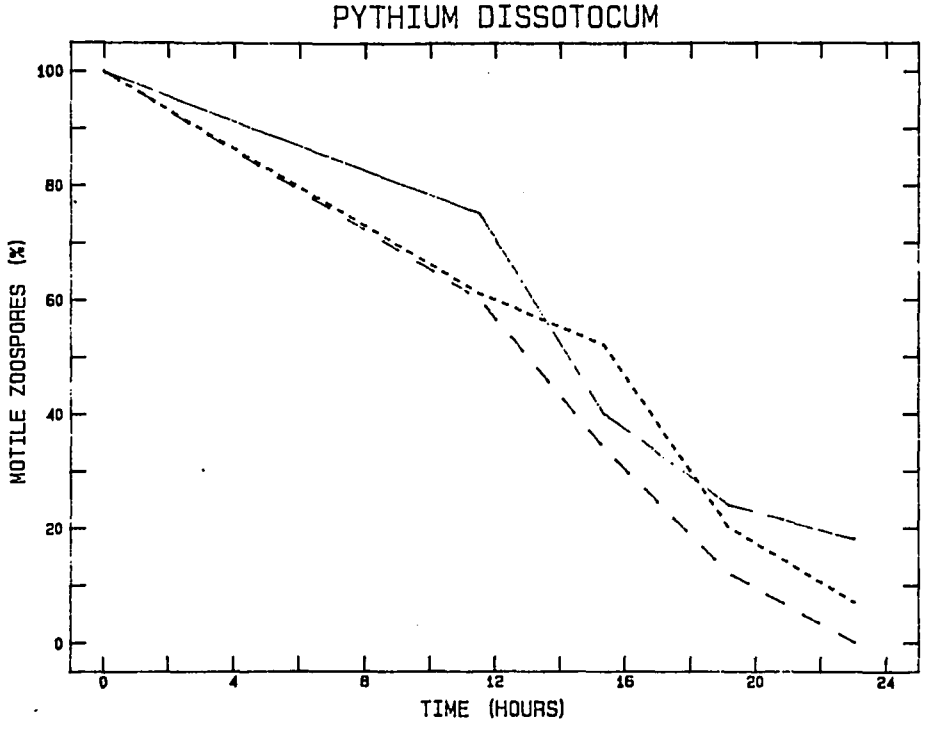

Fig. 8. Duration of zoospore motility of P. dissotocum in sterile distilled water contained in a beaker. Time represents the number of hours after the agra was removed. Results of 3 independent experiments.

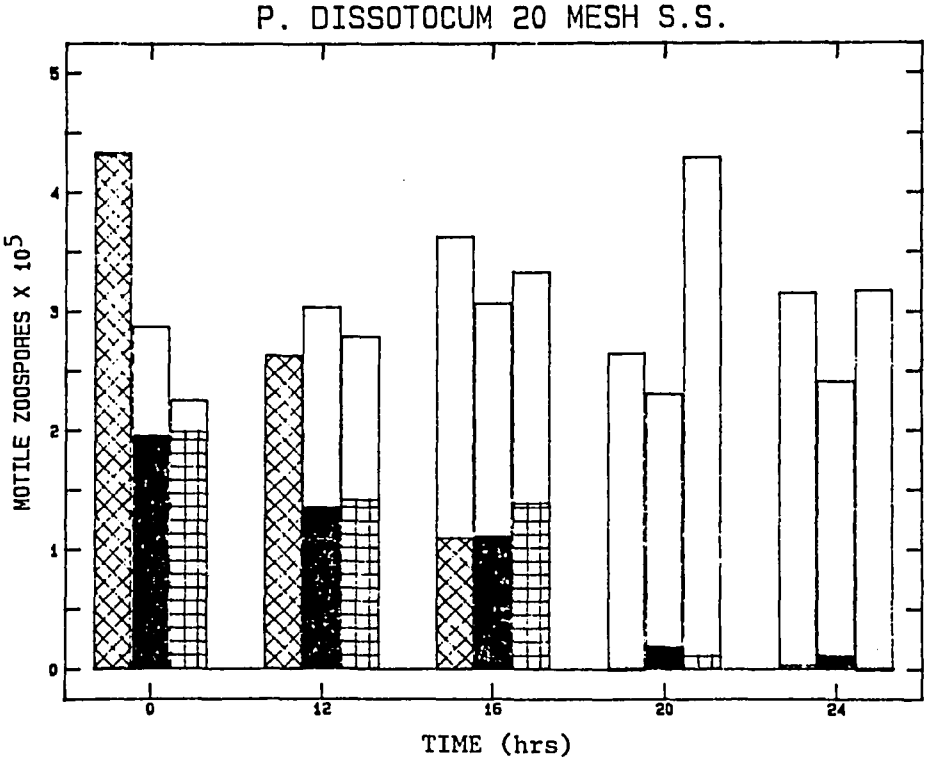

Fig. 9. Duration of zoospore motility of P. dissotocum in 20 mesh silica sand at -8 mbars matric potential. Open face bar represents the population initially within the column. Shaded bar represents the population of motile zoospores extracted at the given time interval. Results of 3 independent experiments.

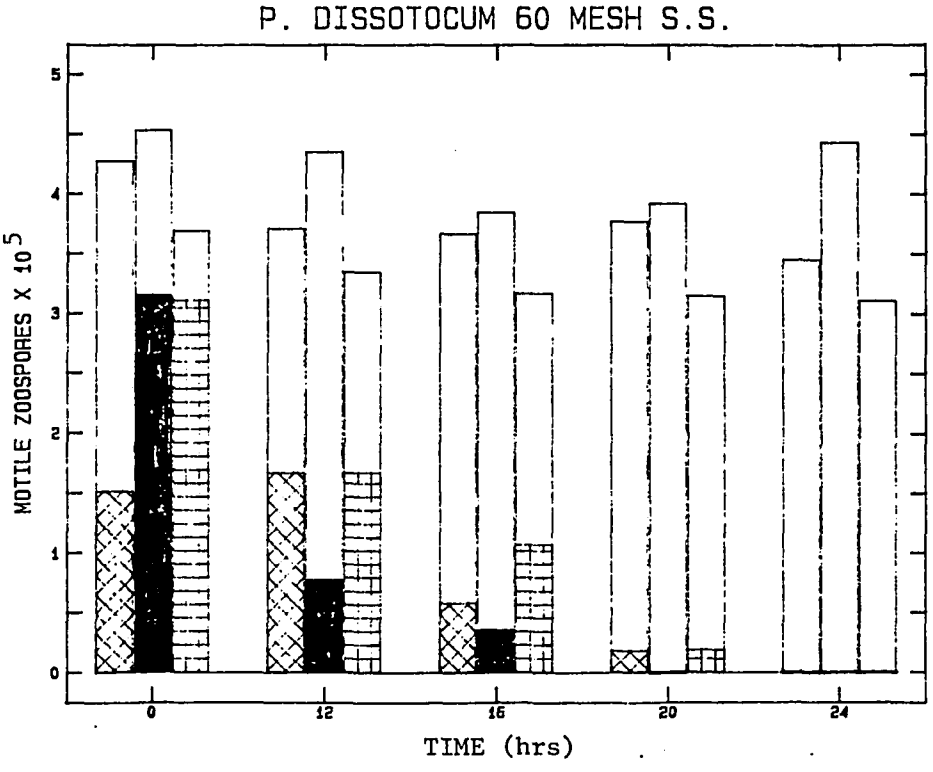

Fig. 10. Duration of zoospore motility of P. dissotocum in 60 mesh silica sand at -8 mbars matric potential. Open face bar represents the population initially within the column. Shaded bar represents the population of motile zoospores extracted at the given time interval. Results of 3 independent experiments.

Table 1. Percent survival of motile and encysted zoospores of P. dissotocum and P. catenulatum in air dried 60 mesh silica sand. Results of two independent experiments.[a,b]

	P. dissotocum				P. catenulatum			
	Encysted		Motile		Encysted		Motile	
Day	1	2	1	2	1	2	1	2
1	0	0	0	0	2.2	5.0	0	<1
2	0	0	0	0	2.3	6.0	1.0	<1
3	0	0	0	0	2.3	<1	0	0
4	-	-	-	-	2.3	0	0	<1
5	-	-	-	-	2.3	<1	0	0
6	-	-	-	-	1.0	0	0	0
7	-	-	-	-	2.0	<1	0	0
14	-	-	-	-	1.0	0	-	-
15	-	-	-	-	1.0	-	-	-
16	-	-	-	-	1.0	-	-	-

a Initial population was 500± 20 zoospores/gram sand.
b sand was allowed to air dry for 24 hours before sampling was initiated.

Table 2. Percent survival of motile and encysted zoospores of P. dissotocum and P. catenulatum in saturated 60 mesh silica sand. Results of two independent experiments.[a]

	P. dissotocum				P. catenulatum			
	Encysted		Motile		Encysted		Motile	
Week	1	2	1	2	1	2	1	2
1	tmtc[b]	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc
2	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc
3	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc	tmtc
4	80	76	100	76	80	16	100	46
5	67	86	24	90	94	34	100	94

a Initial population was 500± 20 zoospores/gram sand.
b tmtc represents to many colonies to count on the plates.

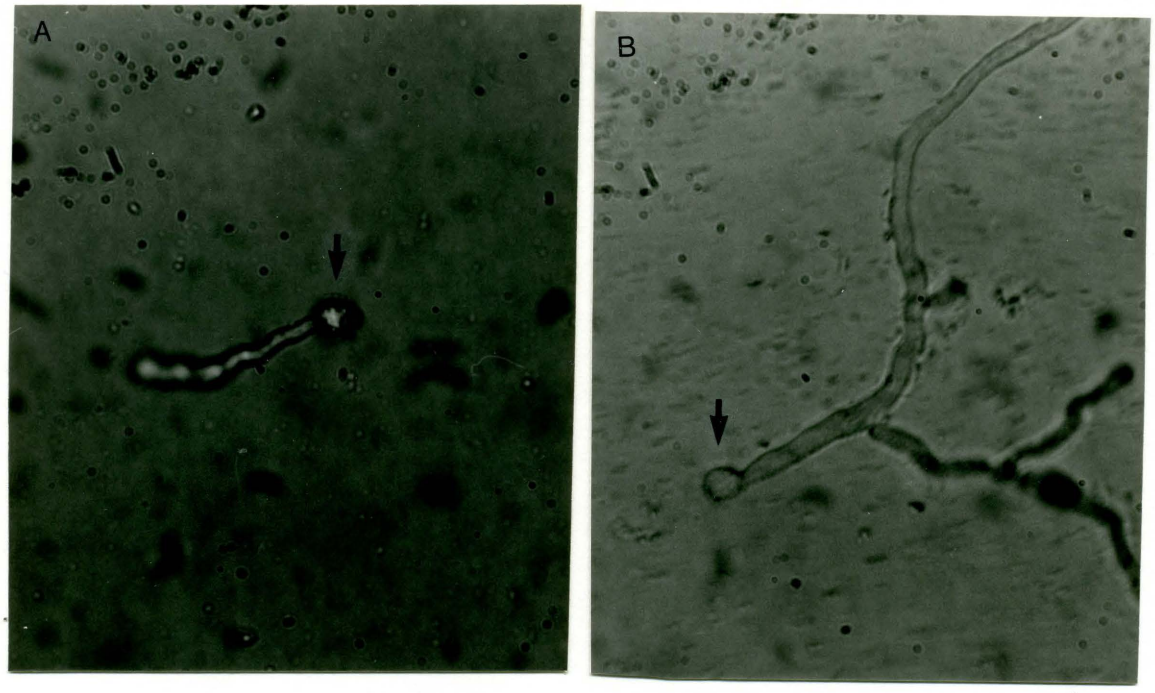

Fig. 11. Germinated cysts (arrows) of a. P. catenulatum , and b. P. dissotocum isolated from silica sand and incubated on corn meal agar for 10 hours. Size of cysts approximately 10-12 microns.

Chapter 4

DISCUSSION

The results of our study indicate that zoospores of P. catenulatum and P. dissotocum have the ability to remain motile for up to 24 and 20 hours, respectively, when confined to water filled pores in a soil-like environment. Although, the motile zoospore population declined with an increase in incubation time, a high percentage were still motile at 16 hours for both fungi. For example, in the 20 mesh silica sand experiment using P. dissotocum, 30, 34, and 40% (average 35±5%) of the zoospores extracted from the sand column were still motile while 30, 40 and 55% (average 41±11%) of the zoospores in the controls (zoospores in SDW) were still motile. Similar results in the 20 mesh silica sand and SDW were also recorded for P. catenulatum. In the 60 mesh silica sand, there was a reduction in the duration of motility for both fungi compared to the 20 mesh sand; however, there was also a reduction in the efficiency of zoospore extraction (time zero) from the 60 mesh sand which may account for the difference. We conclude from our results that there is little or no effect of confinement on the duration of zoospore motility with the Pythium spp. tested.

Our results support and extend the findings of others (1,18,22), who confined zoospores of P. cinnamomi to different sizes of glass

microbeads and found no effects on the duration of motility. In contrast, other researchers(3,8,13) reported a decrease in motility times for zoospores of other Phytophthora spp. confined in columns of sand or glass microbeads. They concluded that the increase in collision with the sand particles caused premature encystment.

The apparent discrepancies concerning the results on the duration of zoospore motility in columns of sand may be due to differences in methodology employed by various researchers. For example, we allowed our columns to drain under the influence of gravity (-8 mbars), while others(1,2,3,13,15,18,22) maintained their columns at 100% saturation. Additionally, genetically determined variations among the genera and species employed in these types of studies may account for the differences. For example, Benjiman (2) results showed varying durations of zoospore motility in glass microbeads for different Phytophthora spp.

Our work is the only report on the duration of zoospore motility of Pythium spp. in silica sand and comparison with other work is difficult. In fact, most studies(1,2,3,13,18,22) on the duration of zoospore motility of Phytophthora spp. were conducted only up to an 8 hour period, whereas ours lasted for 24 hours. However, Mehrotra(15), reported that 7 and 2% of the zoospores of P. megasperma var sojae and P. dreschleri were motile at 12 and 24 hours, respectively, while in soil.

A higher percentage of zoospores persisted longer under saturated conditions than under air dried conditions in the infested silica sand (tables 1 and 2). In contrast, others(9,14,15,16,17,21) infested a nonsterile soil with zoospores and reported a decrease in the population of zoospores over time under saturated conditions. They concluded the decrease was due to lysis of the propagules by soil microflora. Our experiment was conducted under sterile conditions and therefore was not influenced by these microorganisms. Our results indicate that under air dried conditions, P. dissotocum was not recoverable after 24 hours while P. catenulatum was recoverable for up to 16 days (table 1). In contrast, both fungi were recoverable at very high populations even after 5 weeks under saturated conditions (table 2). The survival structure under these conditions is believed to be in the form of cysts. In the vast majority of colonies observed from CMA plated with infested sand, we saw structures resembling germinated cysts (fig. 11). In support of this conclusion, MacDonald and Duniway(14), using a fluorescent antibody staining technique, observed growth of germ tubes from cysts of Phytophthora zoospores after plating on selective media. In contrast, Tsao(20) reported that zoospores cysts of Phytophthora were able to germinate and form microsporangia or hyphae. However, we did not observe any of these structures.

The length of zoospore motility and survival in the soil may play a role in the pathogenesis of zoosporic producing fungi. Prolonged motility of a zoospore can aid the pathogen in several ways. One of

the important factors facing the zoospore is the ability to locate the appropriate soil pore channels which lead to the host root. The longer a zoospore remains motile, the more potential pathways a zoospore can navigate in order to find a host. Additionally, for chemotaxis to occur, it is important to consider the length of time for the root exudate gradient to be re-established after an irrigation. Thus time is crucial for autonomous movement of the zoospore to its host. Zoospores that do not reach a host and subsequently encyst will increase the inoculum density of the fungus. If they can survive for extended periods of times under high soil moisture conditions, they could germinate and infect newly formed roots.

The biology of fungal zoospores needs further studies under field conditions. The sand and soil used in these experiments were devoid of microflora and of consistent texture. The behavior of zoosporic soil-borne fungi will be greatly influenced by the microflora, root exudates, and soil structure of the rhizosphere. Thus the use of sterile, reconstituted sand and soil make it difficult to predict zoospore behavior but our results may indicate their optimum potential.

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